
Genomikai praktikum

Kiadás 0.0.1

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1. fejezet

Kapilláris/Sanger szekvenálás

1.1 Bash

<https://linuxconfig.org/bash-scripting-tutorial-for-beginners>

```
%bash  
cp ../bioinfo/tutor/prakt20180227.ipynb ./
```

1.2 R

1.2.1 Magyar internetes jegyzetek:

Abari Kálmán (2013): http://psycho.unideb.hu/munkatarsak/abari_kalman/szamitastechnika_II/bevezetes_az_R_be_2008_04.pdf

Solymosi Norbert (2005): <https://cran.r-project.org/doc/contrib/Solymosi-Rjegyzet.pdf>

Tóth Dénes (2016): https://tdeen.es.gitbooks.io/rintro_ma/content/

1.2.2 Magyar könyvek:

Dinya Elek, Solymosi Norbert (2016): <https://www.medicina-kiado.hu/kiadvanyaink/szak-es-tankonyvek/biofizika/biometria-a-klinikumban-2-/>

Reiczigel Jenő, Harnos Andrea, Solymosi Norbert (2007): <http://biostatkonyv.hu/>

Az R-rendszer kis-, és nagybetűérzékeny, így például az R és az r különböző objektumokat jelent. Általában minden alfabétikus szimbólum használható a kódban használt nevekben, ezek mellett a . és az _ is néhány megkötéssel. A nevek vagy .-tal vagy betűvel kezdődhetnek, ha .-tal kezdődik egy név, a második karakter nem lehet szám. Habár ékezetes betűket is használhatunk a kódokban, amennyiben hordozható kódot szeretnénk írni, akkor lehetőség szerint az objektumnevekben érdemes mellőzni az ékezetes betűket. A megjegyzéseket #-el jelöljük, az ezt követő karakterek a sor végéig megjegyzésként értelmeződnek.

Az alaputasítások kifejezések vagy értékadások lehetnek. Ha egy kifejezést értékadás nélkül adunk meg mint utasítást, akkor az R kiértékeli és megjeleníti az értékét, ami egyben el is vész.

```
1 + 2
```

Egy értékadás esetén ugyancsak kiértékeli a kifejezést, de az értékét automatikus megjelenítés nélkül eltárolja egy objektumban. Az értékadás általános szintaxisa objektum = kifejezés. Az S- és R-nyelvben eredetileg az értékadás jele a <- volt, sok esetben ma is ezt használják. Mivel ez a megoldás két billentyű-leütést igényel és eredményét tekintve nem különbözik az = jel alkalmazásához képest, a továbbiakban nem használjuk.

```
a = 1 + 2
a
```

Itt már két utasítást használtunk, az első sor elvégzi az összeadást és az értékadást, a második sor pedig kiírja az a objektumban tárolt értéket. Fontos megjegyezni, hogy amennyiben egy változónak új értéket adunk, akkor annak a korábbi értéke törlődik és felülíródik az újjal.

```
a = 5
a
```

Függvények

Az R-ben létrehozott és kezelt egységeket objektumoknak nevezzük. Ezek lehetnek változók, tömbök, karakterláncok, függvények vagy ezek komplex struktúrái. Az R-rendszeren belül az objektumokon operátorokkal és függvényekkel végezhetünk különböző műveleteket. A függvények a függvény.neve(arg1, arg2, ...) szintaxis szerint épülnek fel. A függvény.neve határozza meg a függvény nevét, amivel azonosítja a rendszer a meghívandó eljárás(oka)t. A zárójelek közé foglalt értékek a függvény argumentumai, amelyekkel a függvény futását állítjuk be. Általában a függvények nem minden argumentumának kell értéket adnunk, mivel a függvény rendelkezik alapértelmezett értékekkel.

Objektumok

Az R-en belül mind az adatstruktúrák, mind pedig a függvények objektumként jelennek meg. Az egyes objektumok típusára vonatkozó információkat a typeof(x)-függvény segítségével olvashatjuk ki.

```
typeof(a)
```

```
a = 'szöveg'
typeof(a)
```

Adattároló objektumok

1.2.3 Vektorok

A vektorokat alkotják numerikus, szöveges, komplex vagy logikai adattípusok. Ugyanazon vektoron belül csak egyféle típus használható.

A vector()-függvénnyel létrehozhatunk különböző típusú „üres” vektorokat, numerikus vektort.

```
a = vector(mode = "numeric", length = 5)
a
```

```
a = vector(mode = "logical", length = 5)
a
```

```
a = vector(mode = "character", length = 5)
a
```

Ugyanezt érhetjük el egyetlen argumentum, a length megadásával, ha a numeric()-, a logical()- vagy a character()-függvényeket használjuk.

```
a = numeric(length = 5)
a
```

```
a = logical(length = 5)
a
```

```
a = character(length = 5)
a
```

```
ertekek = c(12, 87, 54, 23)
ertekek
```

Az indexrendszer nagyon rugalmas és hatékony eszköz az egyes adattároló objektumok elemeinek kiolvasására, bármely adattípus esetén. Az indexeket az objektum után írt szögletes zárójellel adjuk meg. Fontos megjegyezni, hogy (több programnyelvvvel ellentétben) az indexelés nem 0-ról, hanem 1-ről indul. Ha az ertekek-vektor harmadik elemét szeretnénk kiolvasni, így tehetjük meg:

```
ertekek[3]
```

1.3 Sanger szekvenálás

<https://www.youtube.com/watch?v=ONGdehkB8jU>

<https://www.youtube.com/watch?v=e2G5zx-OJIw>

```
setwd('/home/sn')
```

```
setwd('data')
```

```
getwd()
```

```
list.files()
```

```
# R
library(sangerseqR)

abif.F = read.abif('sangerF.ab1')
abif.R = read.abif('sangerR.ab1')

abif.F
```

```
Warning message in read.abif("sangerF.ab1"):
"unimplemented legacy type found in file"
Warning message in read.abif("sangerR.ab1"):
"unimplemented legacy type found in file"
```

An object of class "abif"

```

Slot "header":
An object of class "abifHeader"
Slot "abif":
[1] "ABIF"

Slot "version":
[1] 101

Slot "name":
[1] 74 64 69 72

Slot "number":
[1] 1

Slot "elementtype":
[1] 1023

Slot "elementsiz":
[1] 28

Slot "numelements":
[1] 166

Slot "dataoffset":
[1] 331824

Slot "datahandle":
[1] 0

Slot "directory":
An object of class "abifDirectory"
Slot "name":
  [1] "AAct" "ABED" "ABID" "ABlt" "ABRn" "ABTp" "AEpt" "AEpt" "APFN" "APXV"
 [11] "APrN" "APrV" "APrX" "ARTN" "ASPF" "ASPt" "ASPt" "AVld" "AmbT" "AsyC"
 [21] "AsyN" "AsyV" "B1Pt" "B1Pt" "BCTS" "BcRn" "BcRs" "BcRs" "CAED" "CAID"
 [31] "CALt" "CARn" "CASN" "CBED" "CBID" "CBLt" "CBRn" "CBTp" "CRLn" "CRLn"
 [41] "CTNM" "CTow" "CTTL" "CkSm" "CpEP" "DATA" "DATA" "DATA" "DATA" "DATA"
 [51] "DATA" "DATA" "DATA" "DATA" "DATA" "DATA" "DATA" "DSam" "DySN" "Dye#"
 [61] "DyeN" "DyeN" "DyeN" "DyeN" "DyeW" "DyeW" "DyeW" "DyeW" "EPVt" "EVNT"
 [71] "EVNT" "EVNT" "EVNT" "FWO_" "GTyp" "HCFG" "HCFG" "HCFG" "HCFG" "InSc"
 [81] "InVt" "InjN" "LANE" "LAST" "LIMS" "LNTD" "LsrP" "MCHN" "MODF" "MODL"
 [91] "NAVG" "NLNE" "NOIS" "P1AM" "P1RL" "P1WD" "P2AM" "P2BA" "P2RL" "PBAS"
[101] "PBAS" "PCON" "PCON" "PDMF" "PDMF" "PLOC" "PLOC" "PSZE" "PTYP" "PXLB"
[111] "PuSc" "PuSc" "QV20" "QV20" "QcPa" "QcRn" "QcRs" "QcRs" "RGNm" "RMXV"
[121] "RMdN" "RMdV" "RMdX" "RNmF" "RPrN" "RPrV" "RUND" "RUND" "RUND" "RUND"
[131] "RUNT" "RUNT" "RUNT" "RUNT" "Rate" "RevC" "RunN" "S/N%" "SCAN" "SMED"
[141] "SMID" "SMLt" "SMPL" "SMRn" "SPAC" "SPAC" "SPAC" "SVER" "SVER" "SVER"
[151] "SVER" "ScSt" "Scal" "Scan" "SpeN" "TUBE" "Tmpr" "TrPa" "TrSc" "TrSc"
[161] "phAR" "phCH" "phDY" "phQL" "phTR" "phTR"

Slot "tagnumber":
  [1] 1 1 1 1 1 1 1 2 2 1 1 1 1 1 1 2 1 1 1 1 1 1 2
  ↪ 1

```

```
[26] 1 1 2 1 1 1 1 1 1 1 1 1 1 1 2 1 1 1 1 1 1 2 3 4_
↪ 5
[51] 6 7 8 9 10 11 12 1 1 1 1 2 3 4 1 2 3 4 1 1 2 3 4 1_
↪ 1
[76] 1 2 3 4 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1_
↪ 1
[101] 2 1 2 1 2 1 2 1 1 1 1 2 1 2 1 1 1 2 1 1 1 1 1 1_
↪ 1
[126] 1 1 2 3 4 1 2 3 4 1 1 1 1 1 1 1 1 1 1 2 3 1 2_
↪ 3
[151] 4 1 1 1 1 1 1 1 1 1 2 1 1 1 1 1 2
```

Slot "elementtype":

```
[1] 13 19 19 19 5 19 4 4 18 19 19 19 2 5 _
↪ 4
[16] 4 4 19 4 2 19 19 4 4 18 5 19 19 19 _
↪ 19
[31] 19 5 19 19 19 19 5 19 5 19 19 19 18 19 _
↪ 2
[46] 4 4 4 4 4 4 4 4 4 4 4 4 4 18 _
↪ 4
[61] 18 18 18 18 4 4 4 4 5 18 18 18 18 2 _
↪ 18
[76] 19 19 19 19 5 5 19 4 19 18 4 5 18 18 _
↪ 2
[91] 4 4 7 4 4 4 4 2 4 2 2 1 1 18 _
↪ 18
[106] 4 4 5 19 5 5 19 5 19 19 5 19 19 19 _
↪ 19
[121] 19 19 2 7 19 19 10 10 10 10 11 11 11 11_
↪ 1024
[136] 4 19 4 5 18 19 18 18 5 7 18 7 18 18 _
↪ 18
[151] 18 5 7 4 19 18 5 19 5 19 7 18 18 4 _
↪ 4
[166] 7
```

Slot "elementsiz":

```
[1] 1 1 1 1 4 1 2 2 1 1 1 1 1 4 2 2 2 1 2 1 1 1 2 2 1 4 1 1 1 1 1 4 1 1 1 1_
↪ 4
[38] 1 4 1 1 1 1 1 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 1 2 1 1 1 1 2 2 2 2 4 1 1 1 1_
↪ 1
[75] 1 1 1 1 1 4 4 1 2 1 1 2 4 1 1 1 2 2 4 2 2 2 2 1 2 1 1 1 1 1 1 2 2 4 1 4_
↪ 4
[112] 1 4 1 1 4 1 1 1 1 1 1 1 4 1 1 4 4 4 4 4 4 4 1 2 1 2 4 1 1 1 1 4 4 1 4_
↪ 1
[149] 1 1 1 4 4 2 1 1 4 1 4 1 4 1 1 2 2 4
```

Slot "numelements":

```
[1] 1 22 22 8 1 4 1 1 25 2 20 2
[13] 4677 1 1 1 1 6 1481 14960 9 7 1 1
[25] 27 1 1 1 22 22 3 1 10 22 22 8
[37] 1 4 1 5 11 18 9 25 1 15954 15954 15954
[49] 15954 1481 1481 1481 1481 15156 15156 15156 15156 1 2 1
```

[61]	5	6	6	6	1	1	1	1	1	12	12	19
[73]	19	4	5	3	5	5	63	1	1	34	1	398
[85]	37	1	1	16	17	4	1	1	4	1133	1133	1133
[97]	1133	1133	1133	1133	1133	1133	1133	23	23	1133	1133	1
[109]	8	1	1	5	1	5	255	1	1	1	29	14
[121]	17	14	3344	1	22	7	1	1	1	1	1	1
[133]	1	1	12	1	28	4	1	22	22	8	11	1
[145]	1	7	1	79	11	11	22	1	1	1	28	4
[157]	1	434	1	5	1	5	4	1	2	1		

Slot "datasize":

[1]	1	22	22	8	4	4	2	2	25	2	20	2
[13]	4677	4	2	2	2	6	2962	14960	9	7	2	2
[25]	27	4	1	1	22	22	3	4	10	22	22	8
[37]	4	4	4	5	11	18	9	25	1	31908	31908	31908
[49]	31908	2962	2962	2962	2962	30312	30312	30312	30312	2	2	2
[61]	5	6	6	6	2	2	2	2	4	12	12	19
[73]	19	4	5	3	5	5	63	4	4	34	2	398
[85]	37	2	4	16	17	4	2	2	16	2266	2266	2266
[97]	2266	1133	2266	1133	1133	1133	1133	23	23	2266	2266	4
[109]	8	4	4	5	4	5	255	4	1	1	29	14
[121]	17	14	3344	4	22	7	4	4	4	4	4	4
[133]	4	4	12	2	28	8	4	22	22	8	11	4
[145]	4	7	4	79	11	11	22	4	4	2	28	4
[157]	4	434	4	5	4	5	4	2	4	4		

Slot "dataoffset":

[1]	0	128		150		172		25	1094861568
[7]	872546304	872546304		180	838860800		315390	822083584	
[13]	315410	0	65536	94306304	94306304		205		
[19]	211	3173	18133	18142	92798976		92798976		
[25]	18149	0	0	0	18176		18198		
[31]	909377536	499	18220	18230	18252		18274		
[37]	25	1128416000	907	18282	18287		18298		
[43]	18316	283804	16777216	18325	50233		82141		
[49]	114049	145957	148919	151881	154843		157805		
[55]	188117	218429	248741	131072	22675456		262144		
[61]	279053	279058	279064	279070	35389440		37224448		
[67]	38993920	40304640	8500	279076	279088		279100		
[73]	279119	1195463747	279138	1128595456	279143		279148		
[79]	279153	6	1600	279216	458752		298558		
[85]	279250	3276800	13700	279287	279303		859123760		
[91]	65536	524288	279320	288361	286095		290627		
[97]	295159	297425	292893	325026	327292		326159		
[103]	328425	279336	279359	329558	283829		96		
[109]	279382	3	24	279390	948		279395		
[115]	279400	0	0	0	279655		279684		
[121]	279698	279715	279729	1065353216	283073		283095		
[127]	132186631	132186631	132186631	132186631	286852608		320612608		
[133]	288628480	320610304	283102	0	283114		283142		
[139]	15954	283150	283172	283194	325015		87		
[145]	1094855483	283202	1094855483	283725	283209		283220		
[151]	283231	0	1073741824	1045561344	283253		54997047		
[157]	60	283281	48	283715	-1082130432		283720		

[163] 56781159 6488064 -1 -1082130432

Slot "data":

\$AAct.1

NULL

\$ABED.1

[1] "2017-08-18 14:00:00.0"

\$ABID.1

[1] "2017-02-03 05:30:00.0"

\$ABLt.1

[1] "1611354"

\$ABRn.1

[1] 25

\$ABTp.1

[1] "ABC"

\$AEPt.1

[1] 13314

\$AEPt.2

[1] 13314

\$APFN.2

[1] "BDTv3.1_PA_Protocol-POP7"

\$APXV.1

[1] "2"

\$APrN.1

[1] "3500BDTv3-KB-DeNovo"

\$APrV.1

[1] "1"

\$APrX.1

```
[1] "<?xml version="1.0" encoding="UTF-8" standalone="yes"?>
    <AnalysisProtocolContainer doAnalysis="true" name="3500BDTv3-KB-
    DeNovo" guid="DummyGUID" version="2"><AnalysisProtocol version="1"
    doAnalysis="true" guid="DummyGUID" name="Basecaller"><AnalysisSettings
    version="1" name="BasecallerSettings"><Parameter name="BasecallerName">
    <value>KB.bcp</value></Parameter><Parameter name="Description"><value>This
    is the default analysis protocol for 3500 for BDTv3 and POP7 polymer using
    the KB Basecaller. Phred files will be created after user analyzes and
    saves samples </value></Parameter><Parameter name="MobilityFileName"><value>
    KB_3500_POP7_BDTv3.mob</value></Parameter><Parameter name="MatrixFileName">
    <value></value></Parameter><Parameter name="WriteSeqFile"><value>>false
    </value></Parameter><Parameter name="WriteClearRange"><value>>false</
    value></Parameter><Parameter name="WriteSCFFile"><value>>false</value></
    </AnalysisProtocol></AnalysisProtocolContainer>
```

```

→Parameter><Parameter name="WritePHREDFile"><value>>true</value></Parameter>
→<Parameter name="SeqFileFormat"><value>1</value></Parameter><Parameter_
→name="ProcessedDataProfile"><value>0.0</value></Parameter><Parameter_
→name="NCallQvThreshold"><value>0</value></Parameter></AnalysisSettings>
→<AnalysisSettings version="1" name="MobilitySettings"><Parameter_
→name="MobilityFileName"><value>KB_3500_POP7_BDTv3.mob</value></Parameter>
→<Parameter name="MobilityFileContents"><value>QUJJRgBldGRpcgAAAAED/
→wAcAAAACAAAAdwAAACbAAAAAP//////////////////////////
→//////////////////////////
→8WS0JfMzUwMF9QT1A3X0JEVHYzLmJjYy5iY2NDTU5UAAAAAQASAAEAAAABAAAAQAAAAAAAAAAQ05BTQAAAAEAEg
→</value></Parameter></AnalysisSettings><AnalysisSettings version="1"
→name="BasecallerParamsSettings"><Parameter name="StartPoint"><value>
→1439</value></Parameter><Parameter name="BaseOneLocation"><value>
→1416</value></Parameter><Parameter name="AverageSpacing"><value>12.
→135554</value></Parameter><Parameter name="StopPoint"><value>13314</
→value></Parameter></AnalysisSettings><AnalysisSettings version="1"
→name="EndBaseSettings"><Parameter name="StopAtPCRStop"><value>>false
→</value></Parameter><Parameter name="StopAfterNumNs"><value>>false
→</value></Parameter><Parameter name="MaxNumNs"><value>20</value></
→Parameter><Parameter name="StopAfterNumNsInXBases"><value>>false</
→value></Parameter><Parameter name="NumNsPerXBaseInterval"><value>
→5</value></Parameter><Parameter name="XBaseInterval"><value>10</
→value></Parameter><Parameter name="StopAfterNumBases"><value>>false
→</value></Parameter><Parameter name="MaxBasesToCall"><value>800</
→value></Parameter></AnalysisSettings><AnalysisSettings version="1"
→name="MixedBasesSettings"><Parameter name="CallMixedBases"><value>>false
→</value></Parameter><Parameter name="SecondaryPeakThreshold"><value>25.
→0</value></Parameter></AnalysisSettings><AnalysisSettings version="1"
→name="ClearRangeSettings"><Parameter name="UseConfidenceRange"><value>
→false</value></Parameter><Parameter name="UseClearRangeThreshold"><value>
→true</value></Parameter><Parameter name="UseIdentificationOfNcalls">
→<value>>false</value></Parameter><Parameter name="UseReferenceTrimming">
→<value>>false</value></Parameter><Parameter name="FirstBase"><value>
→20</value></Parameter><Parameter name="UseLastBase"><value>true</
→value></Parameter><Parameter name="LastBase"><value>550</value></
→Parameter><Parameter name="BasesToTrimFrom3PrimeEnd"><value>20</
→value></Parameter><Parameter name="MaxLowQualityBases"><value>4</
→value></Parameter><Parameter name="CvThreshold"><value>20</value>
→</Parameter><Parameter name="LowQualityWindow"><value>20</value></
→Parameter><Parameter name="MaxAmbiguities"><value>4</value></Parameter>
→<Parameter name="AmbiguityWindow"><value>20</value></Parameter>
→<Parameter name="UseM13Masking"><value>>false</value></Parameter></
→AnalysisSettings><AnalysisSettings version="1" name="BCPSettings">
→<Parameter name="BCPFileName"><value>KB.bcp</value></Parameter><Parameter_
→name="BCPFileContents"><value># 5Prime Basecaller Paramters</value></
→Parameter></AnalysisSettings></AnalysisProtocol></AnalysisProtocolContainer>
→A"

```

```

$ARTN.1
[1] 0

```

```

$ASPF.1
[1] 1

```

```
$ASPt.1
[1] 1439
```

```
$ASPt.2
[1] 1439
```

```
$AVld.1
[1] "false"
```

```
$AmbT.1
[1] 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26
[25] 26 26 26 26 26 26 26 26 26 26 26 26 26 25 26 26 26 26 26 26 26 25 26
[49] 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26
[73] 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26
[97] 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26
[121] 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26 26
[145] 26 26 26 26 26 26 26 26 26 25 25 25 25 25 26 26 26 26 26 26 26 26 26
[169] 26 26 26 26 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25 26
[193] 26 26 26 26 26 26 26 25 25 26 26 26 26 25 25 25 25 26 26 26 26 25 26
[217] 26 26 26 26 25 25 25 25 25 26 26 26 26 26 25 25 26 26 25 25 25 25 25
[241] 25 25 25 26 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25
[265] 25 26 26 25 25 25 25 25 25 25 25 25 25 25 25 25 26 26 25 25 25 25 25
[289] 25 25 26 26 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25
[313] 25 25 26 26 26 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25
[337] 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25
[361] 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25
[385] 25 25 26 25 25 25 25 25 25 25 25 25 25 25 25 25 26 26 26 25 25 25 25
[409] 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25 25
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\$AsyC.1

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→false</run-parameter><run-parameter name="Array_Heater_Temperature">
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→temperature setting;false</run-parameter><run-parameter name="Poly_Fill_
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→SELF">DC_Cap8_EPS_ARC_SELF;4;uAmps;INTEGER;1;1000;Arc current self test_
→for 8 cap array;false</run-parameter><run-parameter name="Max_Current">
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→Down_Sample">DC_Down_Sample;2;;INTEGER;;;number of frames to average;
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→On."LAS:POW:SETT $DC_Laser_Power_SettingLAS:POW:TOL 1.0LAS On////////Turning_
→Zohana On//SYSTem:MESSagE "Turning Detection Heater On."DETH:TEMP:TOL 3.
→0DETH:TEMP:SETT $Array_Heater_TemperatureDETH On////////Turning Oven On/
→/SYSTem:MESSagE "Setting Oven Temperature."OVEN:TEMP:TOL $Temperature_
→ToleranceOVEN:TEMP:SETT $Oven_TemperatureOVEN On////////Initializing_
→Autosampler//SYSTem:MESSagE "Initializing Autosampler."STR Initialize////
→Filling Polymer Pump//SYSTem:MESSagE "Loading Waste Tray."STR:ROB:MOV:LOAD_
→WasteFLU:POLY:PRES Up 0.34 50FLU:POLY:PRES Down 0.21 200SYSTem:MESSagE_
→"Filling Polymer Pump"FLU:POLY:FILL $Poly_Fill_Vol////Waiting for Oven to_
→Stabilize at Run Temperature//SYSTem:MESSagE "Waiting for Oven to Stabilize_
→at Run Temperature"OVEN:SYNC $Oven_Wait_Time////Waiting for Detection_
→Heater to Stabilize at Run Temperature//SYSTem:MESSagE "Waiting for_
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→Plate_TypeSYST:WAIT 5FLU:CAP:FILL:THRE 1000FLU:CAP:FILL 560SYST:WAIT_
→5STR:ROB:ZOFF 0////Rinsing Capillaries//SYSTem:MESSagE "Rinsing Capillary_
→Tips."STR:ROB:MOV:LOAD WaterSYST:WAIT 5//SYSTem:MESSagE "Loading Buffer_
→Tray."STR:ROB:MOV:LOAD BufferSYST:WAIT 3////Bubble Check//SYSTem:MESSagE_
→"Testing for Normal Current."EPS:Curr:Lim $Max_CurrentEPS:Curr:ARC:THRE
→$Current_StabilityEPS:VOLT:SETT 2.0EPS OnSYST:WAIT 2EPS:Curr:ARC:SELF $DC_
→Cap8_EPS_ARC_SELFEPS Off////Pre Run//SYSTem:MESSagE "PreRun"EPS:VOLT:TOL
→$Voltage_ToleranceEPS:VOLT:SETT $PreRun_VoltageEPS OnEPS:SYNC 10SYST:WAIT
→$DC_PreRun_TimeEPS OffSYST:WAIT 3////////Rinse Capillaries//SYSTem:MESSagE_
→"Rinsing Capillary Tips."STR:ROB:MOV:LOAD WaterSYST:WAIT 3//// Sample_
→injection//SYSTem:MESSagE "Sample Injection."STR:ROB:ZOFF $Injection_Depth_
→OffsetSTR:CSO $DC_RS_CSDataSTR:ROB:MOV:LOAD $DC_RS_Plate_TypeSYST:WAIT_
→15EPS:VOLT:SETT $Injection_VoltageEPS OnSYST:WAIT $DC_Injection_TimeEPS_
→OffEPS:VOLT:SETT 0.0SYST:WAIT 1STR:ROB:ZOFF 0////Rinsing Capillaries//
→SYSTem:MESSagE "Rinsing Capillary Tips."STR:ROB:MOV:LOAD WaterSYST:WAIT_
→3////Starting Electrophoresis//SYSTem:MESSagE "Loading Buffer Tray.
→"STR:ROB:MOV:LOAD BufferSYST:WAIT 3SYSTem:MESSagE "Ramping Cycle.
→"EPS:VOLT:TOL $Voltage_ToleranceEPS:VOLT:SQRR $Run_Voltage $Voltage_Number_
→Of_Steps $Voltage_Step_IntervalePS OnSYST:WAIT $Voltage_Ramp_WaitEPS:SYNC_
→10////Data Delay//SYSTem:MESSagE "Waiting for Data."SYST:WAIT $DC_Data_
→Delay////CCD beginning data read//CAM:SHUT AutoDAT:SRAT $First_ReadOut_
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→Collection."DAT:COLL StartSYST:WAIT $DC_Run_TimeSYSTem:MESSagE "Stop Data_
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[1] 1416

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→154																			
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→4																			
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→6																			
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→9																			
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→220																			
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→44																			
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[10387] -37 -35 -40 -34 -35 -25 -20 -14 0 8 27 38 59 56 63 70 63
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[10405] 47 33 12 8 -5 -19 -22 -23 -32 -36 -37 -41 -45 -42 -44 -45 -42 -
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↪98																		
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↪101																		
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↪7																		
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↪37																		
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↪31																		
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↪7																		
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↪37																		
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↪41																		
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↪26																		
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↪37																		
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↪0																		
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↪31																		
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↪4																		
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[11413] 71 48 22 1 -14 -20 -29 -36 -37 -38 -43 -41 -44 -45 -40 -40 -40 -
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[11431] -35 -41 -38 -39 -37 -40 -34 -32 -34 -27 -30 -24 -23 -14 -4 1 10 _
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[11449] 32 47 53 63 76 95 117 120 133 146 165 179 192 201 214 223 225 _
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[11467] 209 194 185 169 168 150 129 106 88 64 44 32 16 6 -7 -7 -18 -
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[11485] -18 -18 -12 -7 2 9 29 42 68 84 113 154 183 214 232 243 247 _
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[11503] 250 236 221 197 185 185 165 156 150 122 104 74 59 43 18 2 -8 -
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[11593] 80 74 67 55 43 37 36 40 45 62 82 104 126 154 175 189 215 _
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[11611] 216 210 181 150 114 84 63 41 13 -3 -16 -25 -29 -39 -41 -41 -41 -
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[11701] 70 64 59 38 23 14 20 14 12 12 35 46 64 78 92 112 119 _
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[11719] 123 120 117 113 114 111 106 100 94 82 63 54 40 20 -1 -4 -17 -
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→169
[11953] 164 153 145 130 108 87 58 42 27 23 19 14 26 20 26 35 37 _
→38
[11971] 32 34 30 22 13 11 2 -9 -17 -24 -18 -29 -33 -42 -37 -36 -32 -
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→104
[12007] 113 126 144 153 155 152 160 157 141 127 106 98 80 66 50 37 31 _
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[12061] -34 -32 -21 -12 -10 -8 3 24 39 49 53 65 69 67 73 67 59 _
→48
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[12097] 67 68 71 88 104 133 152 171 194 209 218 225 220 214 184 154 125 _
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[12115] 63 39 16 1 -12 -25 -33 -34 -38 -39 -46 -46 -44 -47 -45 -41 -41 -
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[12295] 53 60 59 60 47 41 29 25 8 -6 -9 -21 -26 -35 -39 -42 -41 -

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[12709] 15 12 13 8 -1 -7 -15 -19 -27 -32 -31 -32 -34 -36 -38 -37 -31 -
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↪63
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[1387] -12 -13 -18 -4 -9 -14 -24 -15 -17 -11 -11 -12 -15 -14 -5 -10 -12 -
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[1423] -17 -14 -8 -8 -3 -14 -6 0 0 -1 7 0 3 5 19 4 13 _
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↪17
[1459] 21 28 29 31 32 46 51 42 54 61 57 58 65 72 57 52 46 _
↪29
[1477] 24 20 13 14 17 10 30 29 33 22 36 29 25 19 13 6 1 _
↪6
[1495] -3 -7 0 -2 -8 4 3 3 3 11 16 30 42 39 50 33 30 _
↪28
[1513] 16 9 4 6 -4 -11 -11 -1 -18 -12 -13 -20 -6 -14 -12 -10 -11 -
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↪41
[1549] 31 15 13 -1 -11 -14 -11 -19 -17 -15 -20 -8 -17 -4 -11 -12 -1 -
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[1567] -10 -17 -9 -9 -11 -16 -11 -9 -7 -4 -5 11 8 19 28 46 58 _
↪65
[1585] 67 67 58 35 29 29 19 14 17 24 34 47 53 68 69 73 62 _
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[1639]	-13	-13	-22	-14	-17	-18	-15	-12	-16	-17	-20	-16	-18	-15	-18	-16	-10	↪19
[1657]	-15	-13	-8	-8	-2	-11	2	-5	-10	-8	2	0	-1	-4	-2	3	6	↪19
[1675]	26	48	59	84	103	105	114	100	92	80	79	76	85	92	99	109	122	↪122
[1693]	103	86	63	38	32	10	0	3	-6	-12	-11	-1	-8	-3	-11	-13	-9	↪16
[1711]	-14	-8	-5	-1	-6	0	-1	0	3	0	16	22	34	59	70	90	100	↪105
[1729]	95	86	71	50	28	12	0	0	-3	-7	-7	-7	-1	-6	-17	-18	-9	↪20
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[1801]	25	44	71	97	133	141	150	148	142	117	104	92	86	97	117	144	153	↪148
[1819]	132	112	83	65	31	16	12	10	-5	-4	2	7	3	13	14	36	52	↪73
[1837]	87	99	101	92	81	70	65	60	73	93	111	127	128	117	106	88	54	↪38
[1855]	15	4	7	0	-5	-1	-10	-3	-10	-16	-13	-19	-17	-16	-18	-12	-15	↪27
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[1927]	-19	-17	-14	-21	-13	-21	-15	-20	-16	-13	-6	-7	-4	-10	-9	-10	-3	↪9
[1945]	-6	-9	-8	-5	-13	-20	-18	-21	-16	-19	-18	-18	-15	-8	-5	-10	-12	↪4
[1963]	0	-2	-7	-11	-11	-5	-11	-10	0	-7	-7	0	-7	13	12	30	53	↪68
[1981]	77	101	84	77	67	39	23	2	0	-13	-2	-11	-9	-2	-10	-15	-9	↪14
[1999]	-14	-7	-18	-11	-9	-17	-12	-14	-4	-19	-8	-7	-11	-15	-13	-14	-12	↪6
[2017]	-9	-11	-18	-19	-16	-26	-20	-21	-17	-15	-13	-14	-18	-18	-14	-21	-28	↪30
[2035]	-20	-18	-20	-23	-14	-16	-13	-15	-8	-11	-13	-10	-14	-7	-10	-6	-20	↪9
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↪35
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↪15
[2287] -17 -15 -23 -16 -8 -3 0 -5 8 2 2 -2 1 9 22 43 72_
↪111
[2305] 153 185 205 180 163 107 72 34 13 -4 -1 -8 1 7 5 9 15 _
↪16
[2323] 11 20 41 75 106 147 170 190 191 173 146 116 80 66 92 128 172_
↪222
[2341] 251 266 249 203 141 94 40 32 7 0 -8 0 1 0 1 8 18 _
↪38
[2359] 44 68 116 141 166 158 137 112 74 44 23 12 -15 0 -11 -7 -11 -
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[2377] 0 -4 -15 -11 -4 -4 -13 -5 -3 -19 -13 -9 -11 -14 -16 -16 -11 -
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[2395] -6 -5 1 0 4 -1 25 37 63 101 140 188 212 219 202 150 114 _
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[2413] 36 2 2 -12 -8 -6 -10 -11 -18 -20 -21 -19 -18 -17 -17 -10 -5 -
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[2467] -15 -18 -6 -26 -11 -24 -7 -15 -13 -15 -12 -14 -16 -12 -22 -12 -12 -
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[2503] 10 -7 3 4 -2 0 14 28 52 77 125 172 201 193 203 165 136 _
↪81
[2521] 68 55 74 104 158 193 216 233 226 187 129 85 49 26 3 4 6 _
↪2
[2539] -3 -6 0 -1 -13 -7 -8 -2 -1 0 -16 -11 -22 -12 -17 -10 -14 -
↪16
[2557] -7 4 2 -5 -1 11 11 19 34 61 92 153 192 216 230 205 174_
↪115

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[2665]	165	152	137	94	61	34	12	4	5	-4	0	-2	2	2	-8	-4	1	↪1
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[2737]	93	139	174	222	236	219	187	147	105	71	62	79	100	144	189	214	226	↪212
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[2845]	152	106	75	70	91	142	186	235	272	278	232	193	141	92	67	68	84	↪125
[2863]	163	204	235	232	199	173	121	66	35	15	8	-5	-8	2	0	0	9	↪0
[2881]	3	3	-14	-3	-15	-7	-7	-5	-10	-2	-10	-12	-14	-22	-15	-13	-7	↪11
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[3043]	62	34	4	5	6	11	12	11	16	18	17	20	44	80	134	193	219	↪259

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[3079]	97	147	204	252	300	304	285	243	171	99	64	27	5	-7	-7	8	11	↪9
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[3115]	146	209	251	273	275	240	172	109	63	29	3	0	-3	-2	-3	0	2	↪2
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[3205]	-2	3	4	5	6	3	10	14	22	38	70	104	165	217	247	250	232	↪195
[3223]	139	73	40	9	-6	-13	5	-4	-3	1	7	10	4	11	11	29	54	↪104
[3241]	128	169	216	239	219	191	142	92	44	30	3	-2	-13	3	0	4	-2	↪2
[3259]	-7	5	9	3	6	10	0	10	0	0	6	0	22	39	68	103	150	↪185
[3277]	221	219	191	155	101	57	22	0	-13	-14	-7	-6	-2	7	13	4	10	↪7
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[3457]	33	6	3	3	-6	17	15	16	16	11	22	30	41	68	106	158	203	↪247
[3475]	261	246	198	170	119	87	67	90	142	208	273	334	377	355	284	230	160	↪104
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↪12																		
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↪6																		
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↪7																		
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↪32																		
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↪3																		
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↪10																		
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↪23																		
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↪13																		
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↪11																		
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↪23																		
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↪3																		
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↪8																		
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↪275																		
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↪12																		
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↪53																		
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↪247																		

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↪6																		
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↪218																		
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↪90																		
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↪220																		
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↪3																		
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↪163																		
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↪301																		
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↪24																		
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↪2																		
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↪113																		
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↪17																		
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[10333] -6 4 1 5 13 6 18 29 36 49 63 78 106 117 120 123 138 _
↪114

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↪183
[10369] 185 163 150 127  92  73  48  28  14  11   4  -4  -6  -2   1 -10  -5  -
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↪17

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↳238
[11089] 246 248 251 256 247 242 229 212 182 147 130 100 68 48 28 13 7↳
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[11251] 112 147 160 192 214 224 242 237 239 252 253 267 266 271 279 276 267↳
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[11269] 257 251 248 232 237 221 200 196 176 156 124 83 66 43 30 14 -9↳
↳3
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↳49
[11305] 62 76 100 115 145 152 170 170 172 163 138 120 94 81 52 37 22↳
↳9

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↳138
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↳97
[11575] 110 121 143 152 161 163 158 142 137 106  97  64  42  27  22  12   1  -
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↳115

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↪54
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↪68
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↪152
[12187] 143 150 150 152 139 115 103 81 57 46 31 29 20 25 25 30 39 _
↪47
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↪27
[12223] 25 13 11 18 19 37 47 46 75 79 98 103 123 134 148 128 133_
↪127
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↪60
[12277] 41 33 14 8 6 -7 -9 2 -12 -14 -12 -18 -19 -13 -14 -3 -8 -
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↳325
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↳286
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[793]	63	95	54	4	86	48	11	18	7	83	66	52	77	83	17	31	64	83
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[847]	89	15	8	0	92	85	20	80	12	98	19	67	10	46	96	15	50	85
[865]	82	68	15	50	85	0	79	0	21	41	50	49	60	74	26	43	18	82
[883]	32	92	88	79	21	85	67	18	82	10	94	30	66	79	21	23	77	15
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[919]	18	9	89	87	13	84	50	16	20	7	0	50	55	50	50	75	80	82
[937]	50	18	40	76	86	36	0	31	69	48	80	7	43	26	33	39	62	45
[955]	34	70	71	22	9	31	50	69	43	50	60	75	45	86	22	31	62	70
[973]	33	50	93	2	3	46	61	48	96	14	51	89	53	3	0	82	33	69
[991]	38	50	62	46	49	51	54	41	77	10	46	77	0	23	90	50	29	58
[1009]	77	92	50	8	65	23	52	53	89	25	23	54	9	45	85	93	77	15
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[1081]	54	82	81	42	52	54	55	6	42	14	100	72	26	20	45	76	49	0
[1099]	55	58	56	53	49	5	42	62	0	38	95	54	34	70	17	20	92	29
[1117]	58	50	42	21	57	45	82	55	58	53	61	47	42	68	100	74	5	

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[43]	337	280	331	363	311	284	322	332	321	305	304	340	338	332
[57]	253	275	315	223	302	230	300	231	357	320	313	296	284	292
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[113]	251	251	260	249	255	256	266	253	264	242	240	260	237	271
[127]	249	247	262	256	256	241	231	250	242	243	253	249	249	242
[141]	255	240	223	244	249	235	240	251	237	252	244	249	248	251
[155]	259	255	240	244	241	246	234	225	240	249	234	233	252	229
[169]	244	236	241	229	246	254	248	243	243	247	238	243	247	246
[183]	231	241	219	230	247	256	246	229	228	233	240	247	250	248
[197]	230	216	229	240	226	222	234	234	236	233	223	246	237	244
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[491]	270	284	266	291	295	297	296	289	267	273	285	265	260	276
[505]	286	291	284	301	294	289	285	287	278	293	286	301	286	297
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[533]	287	302	299	315	285	294	288	297	298	318	300	267	295	286
[547]	291	304	287	300	294	319	296	291	305	278	281	305	301	313
[561]	317	292	303	298	303	310	317	318	313	309	319	299	305	312
[575]	315	323	308	313	301	306	305	299	302	319	318	311	322	313
[589]	303	325	328	313	313	297	302	312	328	320	308	306	309	320
[603]	305	314	319	333	315	335	317	307	350	323	334	325	336	327
[617]	322	344	333	330	326	397	335	344	332	361	363	354	347	345
[631]	333	352	324	361	333	403	358	331	339	339	355	368	347	347
[645]	341	373	349	344	345	356	349	363	354	347	368	346	359	354
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[673]	373	378	365	385	394	388	372	382	361	425	470	420	368	373
[687]	396	394	403	397	405	441	377	373	415	391	428	424	381	418
[701]	438	418	373	425	429	390	386	443	382	396	451	427	397	379
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[729]	414	415	419	438	607	857	530	460	436	399	441	418	427	389
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[911]	582	582	644	618	582	582	644	582	582	605	581	587	587	691
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[1023]	985	985	977	985	977	977	985	977	977	977	977	1161	1161	1171
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[71]	61	35	95	55	29	28	46	20	48	30	48	34	33	49
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[99]	25	27	28	37	25	20	39	23	25	20	32	31	92	37
[113]	31	39	21	26	35	70	22	47	13	18	31	15	32	31
[127]	24	28	33	50	25	24	12	33	14	26	33	34	24	23
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[743]	88	176	134	78	113	43	96	100	120	136	101	66	98	244
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[771]	109	77	143	127	112	153	99	87	177	191	267	162	107	70
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[855]	140	78	222	55	103	361	194	166	52	207	198	164	59	73
[869]	78	203	91	19	67	239	266	473	293	211	72	214	44	181
[883]	237	304	215	322	348	209	291	332	255	263	9	13	109	177
[897]	447	341	126	292	211	9	158	318	250	183	198	270	384	204
[911]	443	309	540	191	151	156	301	333	171	216	158	233	74	282
[925]	228	338	278	386	406	239	216	229	514	312	327	717	8	212
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[967]	244	100	235	137	82	194	158	350	384	284	327	283	338	463
[981]	308	153	50	318	293	219	257	402	96	133	168	56	117	53
[995]	11	10	178	322	83	15	172	290	37	219	223	645	371	372
[1009]	179	29	31	29	20	186	290	232	31	244	310	296	382	36
[1023]	12	436	441	395	263	195	141	174	6	8	13	22	210	373
[1037]	236	23	44	764	406	684	495	439	198	96	17	68	174	215
[1051]	250	168	71	16	87	322	427	409	69	531	549	564	224	458
[1065]	1131	644	359	1738	849	1083	658	432	91	357	602	499	582	228
[1079]	382	152	736	292	551	24	1547	966	82	387	460	1431	732	116
[1093]	300	162	322	985	195	830	557	290	39	28	186	360	300	637
[1107]	829	1305	772	195	132	384	556	1346	531	1684	17	29	440	158
[1121]	42	76	71	154	83	363	709	271	21	135	524	774	1096	

\$P2BA.1

[1] "TAGTTTAAGACTCAACGTGGTATAGTCCGTACAATTTAACATTTTCGTACACAACGTGGTTTGTGGCAACCACTATGCGGGTAAC"

\$P2RL.1

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[43]	-482	-310	-93	-300	304	400	-173	83	400	-400	-300	500	-300	-216
[57]	-300	433	-303	-502	491	194	-51	219	400	-423	400	-300	400	-300
[71]	-401	400	-400	-535	-300	-82	73	27	218	400	-436	-347	-395	-237
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[113]	173	-254	209	-164	-29	10	98	198	445	-235	-168	-355	-259	-300
[127]	400	-304	400	-450	78	201	-89	-107	0	-130	223	-166	-419	-135
[141]	25	206	71	-197	401	156	400	-46	45	60	100	-248	540	-300
[155]	32	73	-33	25	-115	126	80	-29	0	-114	216	-182	98	164
[169]	400	8	192	91	400	-148	-55	194	-24	400	-345	240	28	-238
[183]	3	-300	-275	-143	100	306	-100	193	78	3	-229	165	28	182
[197]	13	-199	-67	-73	267	300	101	-41	-45	293	-10	326	-300	-117
[211]	400	-14	-102	400	-167	-150	125	16	-200	32	-300	-102	400	-183
[225]	430	400	-123	400	-242	42	-342	-56	96	58	-336	400	-439	102
[239]	167	-163	-300	671	500	-198	-148	-25	43	400	-264	-400	55	11
[253]	-300	-59	395	108	37	15	-6	112	55	-19	-127	-376	289	-314
[267]	-50	36	8	211	-200	263	-94	66	400	-435	-109	-93	232	-328
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[295]	-433	-86	-75	-66	64	25	5	-66	-126	-172	51	-114	400	-484
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\$PLOC.2

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[13]	141	148	154	164	176	192	206	210	225	239	251	260
[25]	275	285	299	315	328	340	350	358	370	385	396	410
[37]	423	431	445	453	464	478	492	504	518	530	542	556
[49]	568	580	593	602	611	623	633	646	658	673	686	698
[61]	710	722	736	748	762	772	782	792	803	813	824	836
[73]	847	858	870	882	895	908	920	933	946	958	971	983
[85]	993	1003	1015	1027	1041	1049	1062	1074	1085	1097	1110	1123
[97]	1135	1147	1158	1171	1183	1194	1206	1218	1229	1241	1255	1267
[109]	1279	1290	1301	1311	1323	1336	1347	1358	1369	1381	1394	1406
[121]	1419	1433	1445	1458	1470	1481	1493	1503	1514	1526	1537	1549
[133]	1562	1575	1586	1597	1608	1622	1634	1645	1656	1668	1680	1693
[145]	1704	1716	1727	1737	1750	1763	1775	1787	1797	1810	1822	1835
[157]	1849	1860	1873	1884	1896	1908	1920	1932	1944	1956	1967	1977
[169]	1990	2002	2014	2026	2038	2048	2060	2072	2085	2097	2108	2119
[181]	2131	2144	2155	2166	2178	2189	2200	2212	2225	2237	2249	2260
[193]	2271	2282	2295	2307	2320	2332	2344	2356	2367	2378	2390	2402
[205]	2415	2426	2437	2448	2459	2470	2481	2494	2506	2517	2528	2539
[217]	2552	2564	2577	2589	2602	2613	2625	2638	2650	2661	2673	2684
[229]	2695	2707	2720	2732	2743	2755	2766	2778	2790	2802	2813	2826
[241]	2838	2850	2862	2873	2885	2897	2909	2922	2933	2944	2955	2967
[253]	2978	2990	3001	3013	3025	3037	3050	3062	3075	3087	3098	3111
[265]	3122	3134	3145	3156	3169	3181	3192	3204	3216	3228	3241	3254
[277]	3266	3277	3288	3300	3312	3324	3336	3348	3359	3369	3381	3393
[289]	3405	3418	3429	3443	3455	3468	3480	3492	3502	3514	3526	3538
[301]	3550	3563	3576	3588	3599	3611	3623	3634	3646	3659	3671	3683
[313]	3694	3706	3719	3731	3743	3756	3768	3780	3792	3804	3816	3828
[325]	3840	3852	3864	3875	3887	3899	3912	3923	3935	3947	3959	3972
[337]	3985	3995	4008	4019	4030	4042	4055	4066	4077	4089	4102	4113
[349]	4125	4138	4149	4161	4174	4187	4199	4210	4222	4234	4245	4257
[361]	4268	4280	4293	4305	4316	4328	4340	4352	4364	4377	4389	4401
[373]	4413	4424	4435	4447	4459	4471	4483	4495	4506	4517	4529	4541

[385]	4553	4566	4578	4589	4601	4613	4625	4636	4648	4660	4672	4686
[397]	4698	4709	4720	4732	4744	4755	4767	4778	4790	4802	4814	4826
[409]	4838	4850	4861	4875	4887	4899	4911	4922	4934	4944	4957	4970
[421]	4983	4994	5005	5017	5028	5040	5053	5065	5078	5090	5102	5115
[433]	5127	5138	5149	5161	5174	5186	5198	5210	5221	5233	5245	5257
[445]	5268	5280	5291	5303	5314	5326	5338	5351	5363	5375	5388	5400
[457]	5412	5423	5435	5446	5458	5470	5482	5494	5507	5518	5530	5542
[469]	5553	5565	5578	5589	5600	5612	5623	5634	5646	5658	5670	5682
[481]	5693	5706	5717	5729	5741	5752	5765	5778	5790	5802	5815	5825
[493]	5837	5849	5859	5872	5885	5897	5910	5922	5934	5946	5958	5970
[505]	5982	5995	6006	6018	6031	6042	6053	6064	6077	6088	6101	6112
[517]	6124	6136	6147	6160	6172	6184	6196	6209	6220	6232	6245	6257
[529]	6270	6282	6294	6307	6319	6331	6344	6355	6366	6378	6390	6402
[541]	6414	6427	6438	6449	6462	6474	6486	6499	6510	6522	6534	6546
[553]	6559	6571	6584	6595	6607	6619	6631	6644	6657	6669	6681	6693
[565]	6705	6717	6729	6740	6752	6763	6776	6788	6800	6813	6827	6839
[577]	6850	6862	6875	6888	6900	6914	6926	6939	6951	6962	6974	6985
[589]	6997	7010	7023	7034	7047	7058	7071	7083	7096	7108	7120	7132
[601]	7144	7157	7169	7182	7194	7206	7218	7231	7243	7255	7268	7280
[613]	7293	7305	7317	7329	7341	7353	7365	7378	7390	7402	7414	7427
[625]	7439	7451	7463	7475	7488	7500	7512	7524	7537	7549	7563	7575
[637]	7587	7599	7611	7624	7635	7648	7661	7673	7686	7699	7710	7723
[649]	7736	7748	7760	7772	7785	7797	7808	7821	7834	7847	7859	7872
[661]	7884	7897	7908	7920	7933	7946	7959	7970	7982	7994	8006	8018
[673]	8030	8043	8057	8069	8082	8095	8107	8122	8132	8145	8158	8170
[685]	8181	8193	8206	8220	8232	8245	8257	8270	8282	8295	8307	8321
[697]	8332	8344	8356	8368	8381	8395	8407	8417	8430	8442	8455	8468
[709]	8481	8493	8505	8518	8530	8542	8555	8567	8579	8591	8604	8618
[721]	8630	8642	8655	8668	8680	8692	8705	8718	8730	8742	8755	8768
[733]	8782	8794	8807	8819	8834	8846	8858	8873	8884	8897	8910	8922
[745]	8933	8946	8959	8972	8985	8998	9012	9024	9037	9050	9062	9074
[757]	9087	9099	9111	9123	9136	9148	9160	9174	9189	9201	9213	9226
[769]	9239	9263	9276	9290	9303	9316	9328	9341	9353	9367	9380	9393
[781]	9404	9418	9430	9442	9456	9469	9482	9494	9506	9519	9531	9544
[793]	9558	9571	9585	9599	9611	9625	9638	9650	9663	9676	9689	9701
[805]	9713	9725	9738	9751	9764	9777	9791	9805	9819	9831	9845	9857
[817]	9872	9882	9895	9909	9920	9933	9946	9959	9972	9984	9998	10011
[829]	10024	10037	10050	10064	10077	10089	10106	10116	10132	10143	10157	10169
[841]	10183	10196	10209	10223	10236	10246	10261	10275	10288	10301	10313	10326
[853]	10339	10352	10367	10378	10393	10407	10419	10432	10445	10459	10472	10485
[865]	10498	10512	10526	10539	10552	10566	10579	10593	10606	10620	10634	10646
[877]	10660	10673	10687	10699	10713	10728	10741	10754	10767	10779	10795	10808
[889]	10823	10835	10850	10863	10885	10895	10904	10917	10933	10945	10961	10975
[901]	10987	11000	11013	11028	11042	11056	11070	11084	11099	11113	11126	11142
[913]	11153	11167	11180	11196	11209	11221	11236	11251	11266	11279	11294	11310
[925]	11324	11338	11351	11365	11378	11392	11407	11423	11436	11450	11466	11477
[937]	11492	11507	11521	11530	11549	11565	11580	11593	11605	11621	11635	11658
[949]	11667	11681	11695	11710	11724	11737	11760	11769	11783	11798	11815	11829
[961]	11843	11857	11871	11886	11901	11917	11932	11951	11961	11979	12001	12019
[973]	12035	12050	12064	12080	12095	12109	12124	12138	12155	12173	12194	12215
[985]	12230	12246	12263	12278	12298	12307	12327	12338	12349	12373	12387	12401
[997]	12415	12433	12442	12468	12477	12496	12510	12523	12540	12558	12574	12594
[1009]	12610	12629	12639	12649	12658	12668	12686	12703	12712	12722	12737	12753
[1021]	12769	12778	12808	12819	12836	12853	12870	12882	12902	12921	12934	12946

```
[1033] 12959 12971 12984 13003 13021 13033 13046 13072 13088 13104 13113 13125
[1045] 13144 13165 13176 13186 13213 13235 13254 13264 13289 13304 13319 13345
[1057] 13364 13380 13407 13437 13451 13472 13493 13511 13520 13531 13558 13571
[1069] 13581 13599 13608 13627 13661 13690 13711 13729 13749 13773 13798 13825
[1081] 13851 13879 13903 13928 13935 13973 13994 14023 14032 14054 14068 14087
[1093] 14150 14184 14219 14233 14258 14292 14310 14339 14353 14381 14409 14437
[1105] 14465 14497 14519 14550 14563 14601 14646 14655 14689 14699 14717 14759
[1117] 14768 14789 14810 14839 14848 14882 14895 14922 14957 14985 14995 15013
[1129] 15041 15080 15098 15136 15148
```

\$PSZE.1

[1] 96

\$PTYP.1

[1] "96-Well"

\$PXLB.1

[1] 3

\$PuSc.1

[1] 24

\$PuSc.2

[1] "pass"

\$QV20.1

[1] 948

\$QV20.2

[1] "pass"

\$QcPa.1

[1] "UseSampleScore=true,SampleScoreLowCutoff=15,SampleScoreMedCutoff=30,
 ↳UseCRL=true,CRLLowCutoff=100,CRLMedCutoff=300,CRLWindow=20,
 ↳CRLQvThreshold=20,UseQV20+=true,QV20+LowCutoff= 100,QV20+MedCutoff=300,
 ↳UsePupScore=true,PupScoreLowCutoff=4,PupScoreMedCutoff=8"

\$QcRn.1

[1] 0

\$QcRs.1

[1] ""

\$QcRs.2

[1] ""

\$RGNm.1

[1] "SZBK_ResultsGroup-20170227_1"

\$RMXV.1

[1] "TestVersion 1"

\$RMdN.1

[1] "BDxStdSeq50_POP7"

\$RMdV.1

[1] "TestVersion 1"

\$RMdX.1

```
[1] "////SYSTEM:MESSaGe "$DC_RunModule_Comment"////////Turning Laser_
↳On//SYSTEM:MESSaGe "Turning Laser On."LAS:POW:SETT $DC_Laser_Power_
↳SettingLAS:POW:TOL 1.0LAS On////////Turning Zohana On//SYSTEM:MESSaGe_
↳"Turning Detection Heater On."DETH:TEMP:TOL 3.0DETH:TEMP:SETT $Array_
↳Heater_TemperatureDETH On////////Turning Oven On//SYSTEM:MESSaGe "Setting_
↳Oven Temperature."OVEN:TEMP:TOL $Temperature_ToleranceOVEN:TEMP:SETT
↳$Oven_TemperatureOVEN On////////Initializing Autosampler//SYSTEM:MESSaGe_
↳"Initializing Autosampler."STR Initialize///Filling Polymer Pump//
↳SYSTEM:MESSaGe "Loading Waste Tray."STR:ROB:MOV:LOAD WasteFLU:POLY:PRES_
↳Up 0.34 50FLU:POLY:PRES Down 0.21 200SYSTEM:MESSaGe "Filling Polymer_
↳Pump"FLU:POLY:FILL $Poly_Fill_Vol///Waiting for Oven to Stabilize at_
↳Run Temperature//SYSTEM:MESSaGe "Waiting for Oven to Stabilize at Run_
↳Temperature"OVEN:SYNC $Oven_Wait_Time///Waiting for Detection Heater_
↳to Stabilize at Run Temperature//SYSTEM:MESSaGe "Waiting for Detection_
↳Heater to Stabilize at Run Temperature"DETH:SYNC 1800///Waiting for_
↳Laser to Stabilize at Power Setting//SYSTEM:MESSaGe "Waiting for Laser to_
↳Stabilize at Power Setting"LAS:SYNC 600///Filling Array//SYSTEM:MESSaGe_
↳"Filling Array with Polymer."FLU:CAP:FILL:THRE 1000FLU:CAP:FILL $Cap_
↳Fill_Vol///POP Injection//SYSTEM:MESSaGe "POP Injection."STR:ROB:ZOFF
↳$Injection_Depth_OffsetSTR:CSO $DC_RS_CSDaSTR:ROB:MOV:LOAD $DC_RS_
↳Plate_TypeSYST:WAIT 5FLU:CAP:FILL:THRE 1000FLU:CAP:FILL 560SYST:WAIT_
↳5STR:ROB:ZOFF 0///Rinsing Capillaries//SYSTEM:MESSaGe "Rinsing Capillary_
↳Tips."STR:ROB:MOV:LOAD WaterSYST:WAIT 5//SYSTEM:MESSaGe "Loading Buffer_
↳Tray."STR:ROB:MOV:LOAD BufferSYST:WAIT 3///Bubble Check//SYSTEM:MESSaGe_
↳"Testing for Normal Current."EPS:Curr:Lim $Max_CurrentEPS:Curr:ARC:THRE
↳$Current_StabilityEPS:VOLT:SETT 2.0EPS OnSYST:WAIT 2EPS:Curr:ARC:SELF $DC_
↳Cap8_EPS_ARC_SELFEPS Off///Pre Run//SYSTEM:MESSaGe "PreRun"EPS:VOLT:TOL
↳$Voltage_ToleranceEPS:VOLT:SETT $PreRun_VoltageEPS OnEPS:SYNC 10SYST:WAIT
↳$DC_PreRun_TimeEPS OffSYST:WAIT 3////////Rinse Capillaries//SYSTEM:MESSaGe_
↳"Rinsing Capillary Tips."STR:ROB:MOV:LOAD WaterSYST:WAIT 3/// Sample_
↳injection//SYSTEM:MESSaGe "Sample Injection."STR:ROB:ZOFF $Injection_Depth_
↳OffsetSTR:CSO $DC_RS_CSDaSTR:ROB:MOV:LOAD $DC_RS_Plate_TypeSYST:WAIT_
↳15EPS:VOLT:SETT $Injection_VoltageEPS OnSYST:WAIT $DC_Injection_TimeEPS_
↳OffEPS:VOLT:SETT 0.0SYST:WAIT 1STR:ROB:ZOFF 0///Rinsing Capillaries//
↳SYSTEM:MESSaGe "Rinsing Capillary Tips."STR:ROB:MOV:LOAD WaterSYST:WAIT_
↳3///Starting Electrophoresis//SYSTEM:MESSaGe "Loading Buffer Tray.
↳"STR:ROB:MOV:LOAD BufferSYST:WAIT 3SYSTEM:MESSaGe "Ramping Cycle.
↳"EPS:VOLT:TOL $Voltage_ToleranceEPS:VOLT:SQR $Run_Voltage $Voltage_Number_
↳Of_Steps $Voltage_Step_IntervaleEPS OnSYST:WAIT $Voltage_Ramp_WaitEPS:SYNC_
↳10///Data Delay//SYSTEM:MESSaGe "Waiting for Data."SYST:WAIT $DC_Data_
↳Delay///CCD beginning data read//CAM:SHUT AutoDAT:SRAT $First_ReadOut_
↳Time $Second_ReadOut_TimeDAT:MODE BinningSYSTEM:MESSaGe "Starting Data_
↳Collection."DAT:COLL StartSYST:WAIT $DC_Run_TimeSYSTEM:MESSaGe "Stop Data_
↳Collection."DAT:COLL StopEPS OffEPS:VOLT:SETT 0.0SYST:WAIT 1//LAS Off//DETH_
↳Off//OVEN Off////////End Of ModuleB"
```

\$RNmF.1

[1] 4.600603e-41

```
$RPrN.1  
[1] "BDxStdSeq50_POP7_6sec"
```

```
$RPrV.1  
[1] "v1.0.0"
```

```
$RUND.1  
$RUND.1$year  
[1] 2017
```

```
$RUND.1$month  
[1] 2
```

```
$RUND.1$day  
[1] 7
```

```
$RUND.2  
$RUND.2$year  
[1] 2017
```

```
$RUND.2$month  
[1] 2
```

```
$RUND.2$day  
[1] 7
```

```
$RUND.3  
$RUND.3$year  
[1] 2017
```

```
$RUND.3$month  
[1] 2
```

```
$RUND.3$day  
[1] 7
```

```
$RUND.4  
$RUND.4$year  
[1] 2017
```

```
$RUND.4$month  
[1] 2
```

```
$RUND.4$day  
[1] 7
```

```
$RUNT.1  
$RUNT.1$hour  
[1] 17
```

\$RUNT.1\$minute
[1] 25

\$RUNT.1\$second
[1] 6

\$RUNT.1\$hsecond
[1] 0

\$RUNT.2
\$RUNT.2\$hour
[1] 19

\$RUNT.2\$minute
[1] 28

\$RUNT.2\$second
[1] 41

\$RUNT.2\$hsecond
[1] 0

\$RUNT.3
\$RUNT.3\$hour
[1] 17

\$RUNT.3\$minute
[1] 52

\$RUNT.3\$second
[1] 31

\$RUNT.3\$hsecond
[1] 0

\$RUNT.4
\$RUNT.4\$hour
[1] 19

\$RUNT.4\$minute
[1] 28

\$RUNT.4\$second
[1] 32

\$RUNT.4\$hsecond
[1] 0

\$Rate.1
[1] 00 00 00 00 00 00 00 01 68 00 00 00 01 52

\$RevC.1

[1] 0

\$RunN.1

[1] "Run 2017-02-07-04-42-30-726"

\$`S/N%.1`

[1] 108 138 169 140

\$SCAN.1

[1] 15954

\$SMED.1

[1] "2017-05-16 14:00:00.0"

\$SMID.1

[1] "2017-01-31 02:33:00.0"

\$SMLt.1

[1] "1609087"

\$SMPL.1

[1] "10 KE CR-F"

\$SMRn.1

[1] 87

\$SPAC.1

[1] 0.002613202

\$SPAC.2

[1] "KB.bcp"

\$SPAC.3

[1] 0.002613202

\$SVER.1

[1] "3500 Data Collection Version 1.0 - 3500 FC_090519180136-0700 Build_↵
↵Number: 837"

\$SVER.2

[1] "KB 1.4.1.8"

\$SVER.3

[1] "6228001-05"

\$SVER.4

[1] "Sequencing Format 2.0"

\$ScSt.1

[1] 0

\$Scal.1

```
[1] 8.96831e-44
```

```
$Scan.1
```

```
[1] 15954
```

```
$SpeN.1
```

```
[1] "Run 2016-09-02-08-58-30-610"
```

```
$TUBE.1
```

```
[1] "G07"
```

```
$Tmpr.1
```

```
[1] 60
```

```
$TrPa.1
```

```
[1] "UseClearRangeThreshold=true,UseLastBase=true,MaxLowQualityBases3PrimeEnd=4,  
→UseFirstBase=true,UseClearRangeThreshold5PrimeEnd=true,LastBase=550,  
→UseClearRangeThreshold3PrimeEnd=true,LowQualityWindow5PrimeEnd=20,  
→UseAmpliconPrimerTrimming=false,LowQualityWindow3PrimeEnd=20,  
→FirstBase=20,CvThreshold5PrimeEnd=20,CvThreshold3PrimeEnd=20,  
→UseM13Masking=false,UseConfidenceRange=false,MaxLowQualityBases5PrimeEnd=4,  
→BasesToTrimFrom3PrimeEnd=30"
```

```
$TrSc.1
```

```
[1] 48
```

```
$TrSc.2
```

```
[1] "pass"
```

```
$phAR.1
```

```
[1] 4.61854e-41
```

```
$phCH.1
```

```
[1] "term"
```

```
$phDY.1
```

```
[1] "big"
```

```
$phQL.1
```

```
[1] 99
```

```
$phTR.1
```

```
[1] -1 -1
```

```
$phTR.2
```

```
[1] 4.61854e-41
```

A nevek attribútumok, amelyeknek több fajtája is lehet (nevek, oszlopnevek, sornevek, dimenziónevek). Többek között arra is alkalmasak, hogy objektumok elemeire hivatkozhassunk. Ahhoz, hogy nevek segítségével hivatkozzunk elemekre, tudnunk kell, hogy milyen nevek vannak az objektumban. Az objektumban előforduló neveket többféleképpen is kiolvashatjuk, például a `slotNames()`-függvénnyel.

```
slotNames(abif.F)
```

```
abif.F@header
```

```
An object of class "abifHeader"
Slot "abif":
[1] "ABIF"

Slot "version":
[1] 101

Slot "name":
[1] 74 64 69 72

Slot "number":
[1] 1

Slot "elementtype":
[1] 1023

Slot "elementsiz":
[1] 28

Slot "numelements":
[1] 166

Slot "dataoffset":
[1] 331824

Slot "datahandle":
[1] 0
```

```
slotNames(abif.F@header)
```

```
abif.F@header@abif
```

```
slotNames(abif.F@directory)
```

```
names(abif.F@data)
```

```
# a FWO_.1 objektum tartalmazza, hogy a DATA.9 - DATA.11 objektumban tárolt
# intenzitásértékeket mely bázisoknak felelnek meg
abif.F@data$FWO_.1
```

```
# így a G bázisra vonatkozó intenzitások a DATA.9 vektorban vannak
abif.F@data$DATA.9
```

```
typeof(abif.F@data$DATA.9)
```

```
range(abif.F@data$DATA.9)
```

```
length(abif.F@data$DATA.9)
```

1.3.1 data.frame

A `data.frame` vagy más néven adattábla olyan táblázat, aminek oszlopai vektorként foghatók fel. A adattáblákat alkotják numerikus, szöveges, komplex vagy logikai adattípusok. Ugyanazon `data.frame`-en belül többféle típus használható. Fájlból beolvasott táblázatok általában ilyen objektumként jelennek meg az R-ben, de létrehozhatjuk a `data.frame()`-függvénnyel is.

```
bazis1 = abif.F@data$DATA.9
bazis2 = abif.F@data$DATA.10
bazis3 = abif.F@data$DATA.11
bazis4 = abif.F@data$DATA.12

tablazat = data.frame(bazis1, bazis2, bazis3, bazis4)
```

Az adattábla létrehozásakor ügyeljünk arra, hogy az alkotó vektorok egyforma hosszúságúak legyenek. Ha az egyik vektor rövidebb a másikonál, és a hosszabb vektor hossza osztható a rövidebb vektor hosszával, akkor a függvény a rövidebb vektor ismétlésével kipótolja a különbséget.

```
dim(tablazat)
```

```
head(tablazat)
```

Ha `data.frame`-ből szeretnénk kiolvasni értékeket, azt két index alkalmazásával tehetjük meg. A `tablazat` `data.frame`-ből egy elemet a `tablazat[i, j]` utasítással olvashatunk ki, ahol *i* a `data.frame` sorát, *j* pedig az oszlopát meghatározó index.

Egy egész sor olvasásához a `tablazat[i, ]`, egy egész oszlophoz pedig az `x[, j]` parancsot használhatjuk.

Az indexek segítségével nem csak kiolvashatunk értékeket az adattároló objektumokból, hanem lekérdezéseket is végezhetünk azokból, illetve elemeiket módosíthatjuk. Továbbá indexek segítségével az objektumból eltávolíthatunk elemeket, sorokat, oszlopokat.

```
tablazat[2, 4]
```

```
tablazat[2, ]
```

```
tablazat[, 1]
```

Az objektumok elemeire nem csak indexekkel hivatkozhatunk, de nevekkel is.

```
names(tablazat)
```

```
colnames(tablazat)
```

```
rownames(tablazat)
```

```
tablazat$bazis1
```

```
tablazat[, 'bazis1']
```

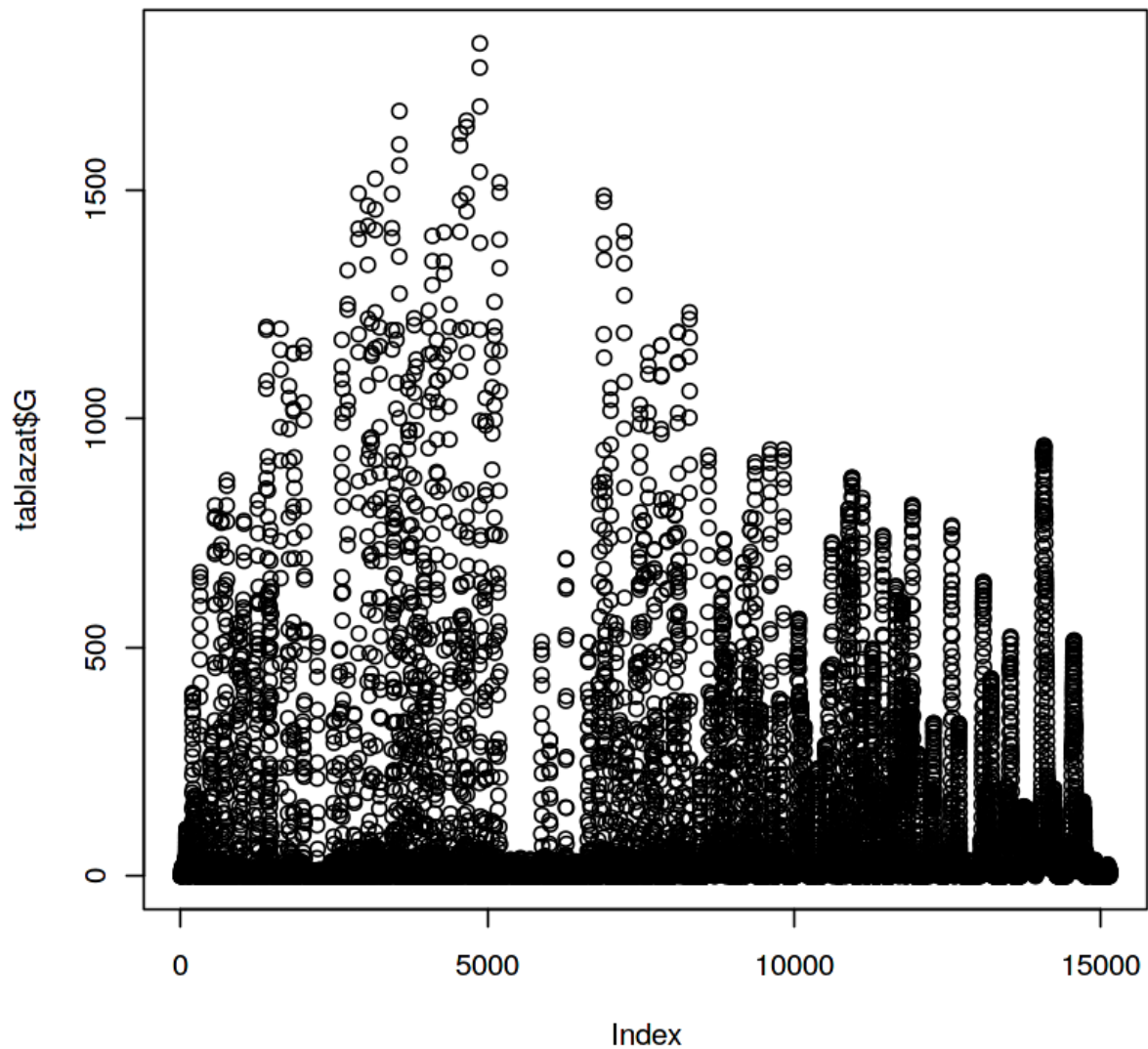
```
colnames(tablazat) = c('G', 'A', 'T', 'C')
head(tablazat)
```

```
tablazat$G
```

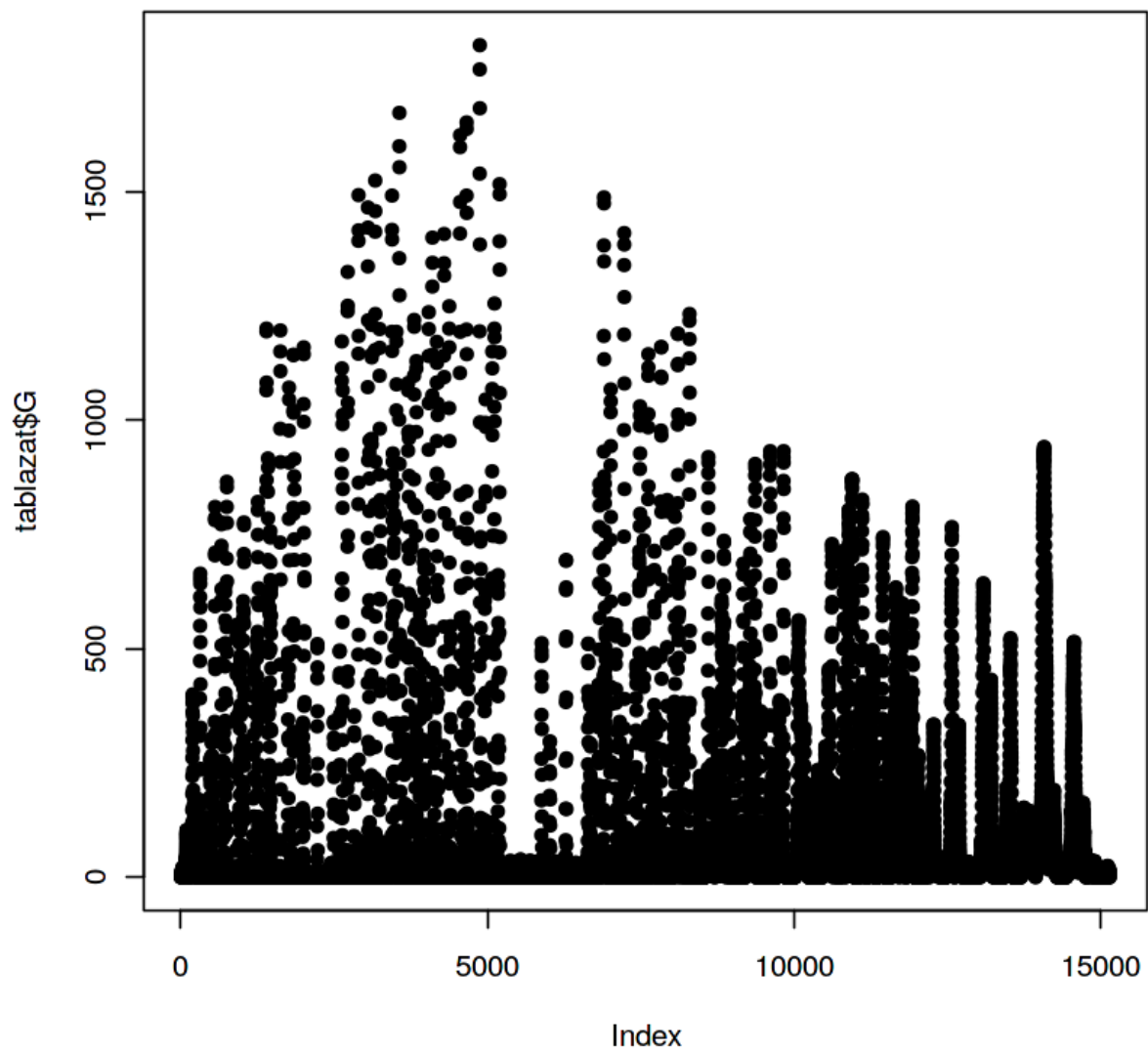
```
tablazat = tablazat[,c('A', 'C', 'G', 'T')]  
head(tablazat)
```

```
tablazat$G
```

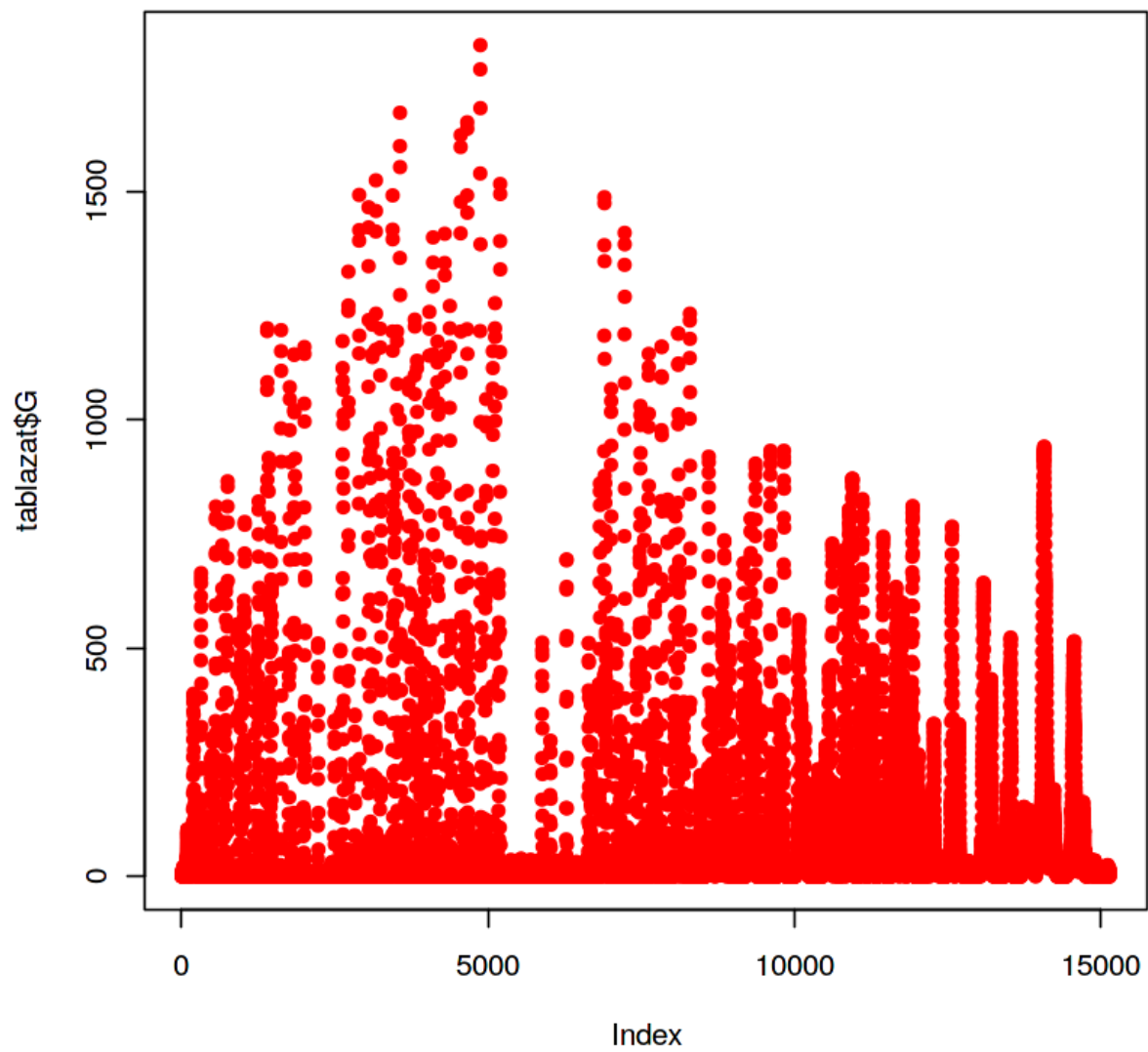
```
plot(tablazat$G)
```



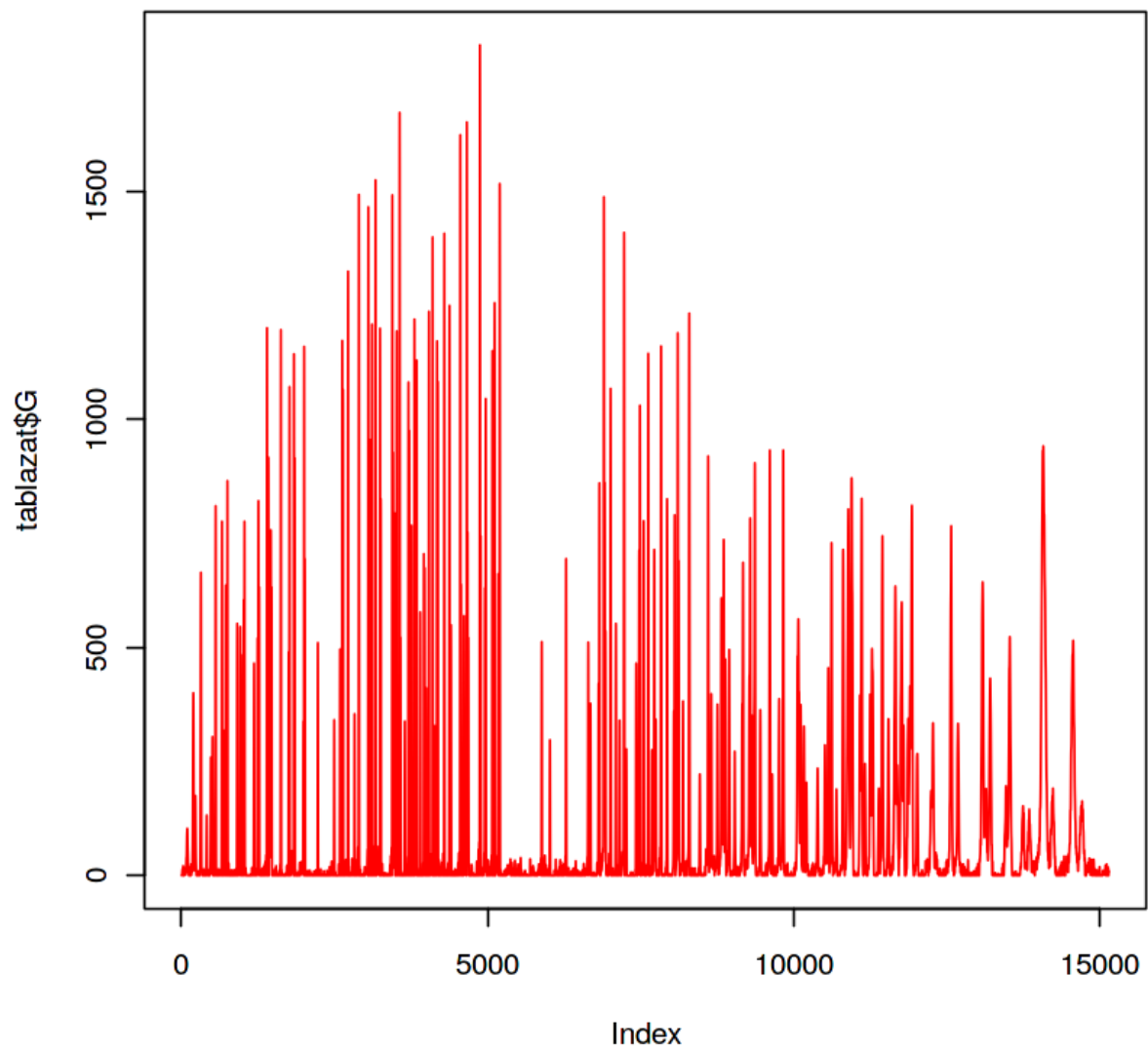
```
plot(tablazat$G, pch=16)
```



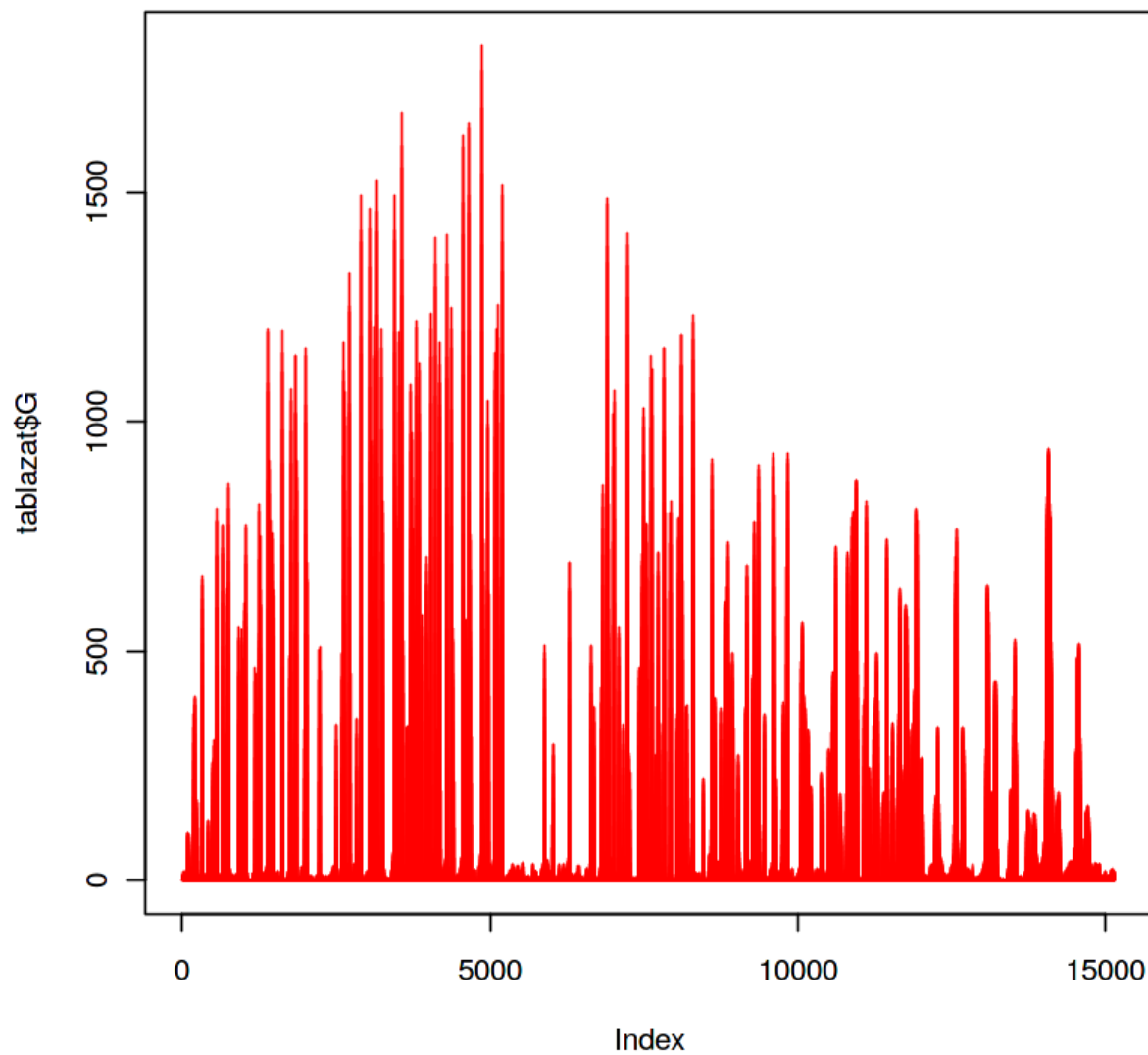
```
plot(tablazat$G, pch=16, col='red')
```



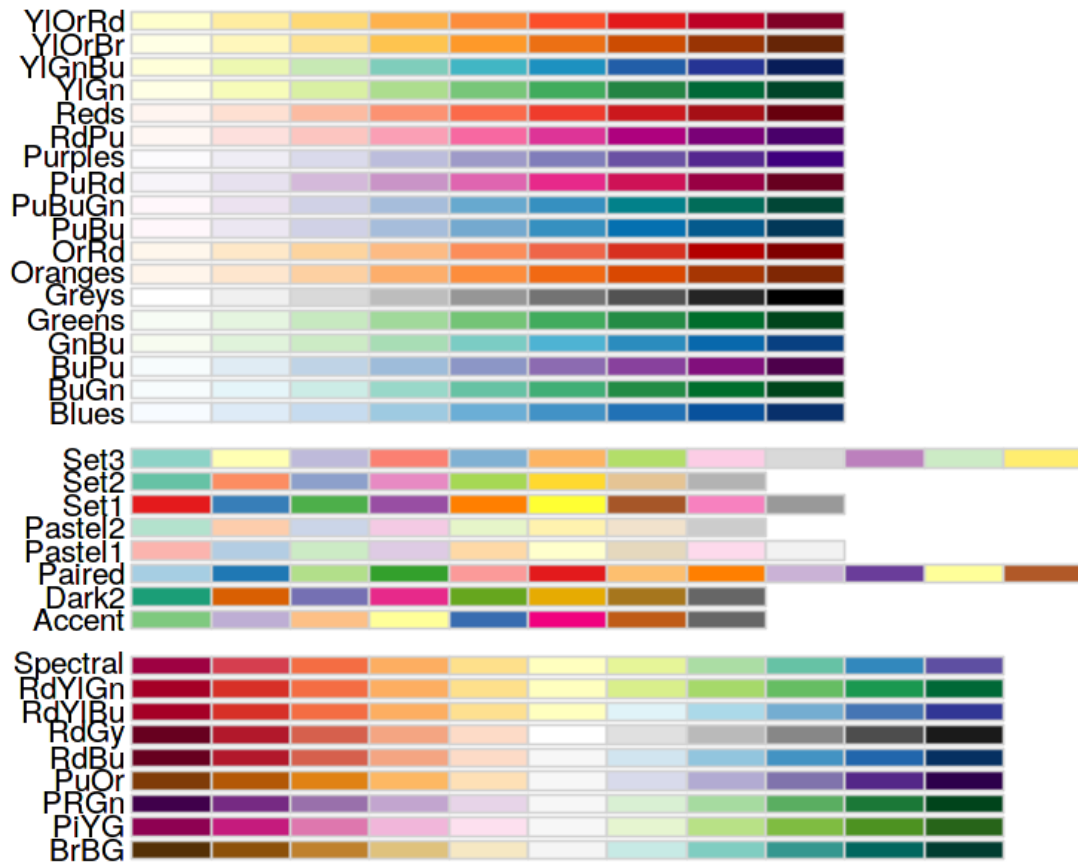
```
plot(tablazat$G, col='red', type='l')
```



```
plot(tablazat$G, col='red', type='h')
```



```
library(RColorBrewer)
display.brewer.all()
```



```
# az elsődleges szekvencia, amelyet módosíthattak
abif.F@data$PBAS.1
```

```
nchar(abif.F@data$PBAS.1)
```

```
# mindig az eredeti elsődleges szekvencia
abif.F@data$PBAS.2
```

```
# a másodlagos szekvencia
abif.F@data$P2BA.1
```

```
# az intenzitásvektorokon belül a peak-eket meghatározó indexek
abif.F@data$PLOC.1
```

```
length(abif.F@data$PLOC.1)
```

```
length(abif.F@data$PLOC.2)
```

```
abif.F@data$PLOC.2 == abif.F@data$PLOC.1
```

```
sum(abif.F@data$PLOC.2 == abif.F@data$PLOC.1)
```

```
sum(abif.F@data$PLOC.2 != abif.F@data$PLOC.1)
```

```
# az elsődleges basecalling peakjeinek amplitúdója
abif.F@data$P1AM.1
```

```
# a másodlagos basecalling peakjeinek amplitúdója, opcionális
abif.F@data$P2AM.1
```

```
sseq = sangerseq(abif.F)
str(sseq)
```

```
Formal class 'sangerseq' [package "sangerseqR"] with 7 slots
 ..@ primarySeqID : chr "From ab1 file"
 ..@ primarySeq   :Formal class 'DNAString' [package "Biostrings"] with 5 slots
 .. .. ..@ shared :Formal class 'SharedRaw' [package "XVector"] with 2 slots
 .. .. .. ..@ xp   :<externalptr>
 .. .. .. ..@ .link_to_cached_object:<environment: 0x726e118>
 .. .. ..@ offset  : int 0
 .. .. ..@ length  : int 1133
 .. .. ..@ elementMetadata: NULL
 .. .. ..@ metadata : list()
 ..@ secondarySeqID: chr "From ab1 file"
 ..@ secondarySeq  :Formal class 'DNAString' [package "Biostrings"] with 5 slots
 .. .. ..@ shared  :Formal class 'SharedRaw' [package "XVector"] with 2 slots
 .. .. .. ..@ xp   :<externalptr>
 .. .. .. ..@ .link_to_cached_object:<environment: 0x726e118>
 .. .. ..@ offset  : int 0
 .. .. ..@ length  : int 1133
 .. .. ..@ elementMetadata: NULL
 .. .. ..@ metadata : list()
 ..@ traceMatrix   : int [1:15156, 1:4] 54 49 43 35 26 20 21 27 37 44 ...
 ..@ peakPosMatrix : num [1:1133, 1:4] 3 15 25 37 46 55 68 76 85 94 ...
 ..@ peakAmpMatrix : int [1:1133, 1:4] 146 350 540 311 524 524 382 379 461 403 ...
```

```
slotNames(sseq)
```

```
# traceMatrix A,C,G,T oszlopsorrendben tartalmazza az összes intenzitásértéket
head(traceMatrix(sseq))
```

```
# mindegyik bázisra vonatkozóan tartalmazza azt az indexet, ahol a Basecall window-al
↳ a maximális peaket találta
# vegyük észre, hogy ezek az indexértékek 1-el nagyobbak, mint a nyers adatokból
↳ lekérdezett volt
head(peakPosMatrix(sseq))
```

```
sum(peakPosMatrix(sseq)[,1] != peakPosMatrix(sseq)[,2])
```

```
is.na(peakPosMatrix(sseq)[,3])
```

```
sum(is.na(peakPosMatrix(sseq)[,3]))
```

```
sum(is.na(peakPosMatrix(sseq)[,4]))
```

```
# az adott ablakban mért legnagyobb amplitúdó, a függvény leírása szerint mindegyik_
↪bázisra,
# azonban itt csak az első és második oszlop tartalmaz adatot, és ezek pedig_
↪meggyeznek a P1AM.1 és P2AM.1 értékekkel
head(peakAmpMatrix(sseq))
```

```
summary(peakAmpMatrix(sseq))
```

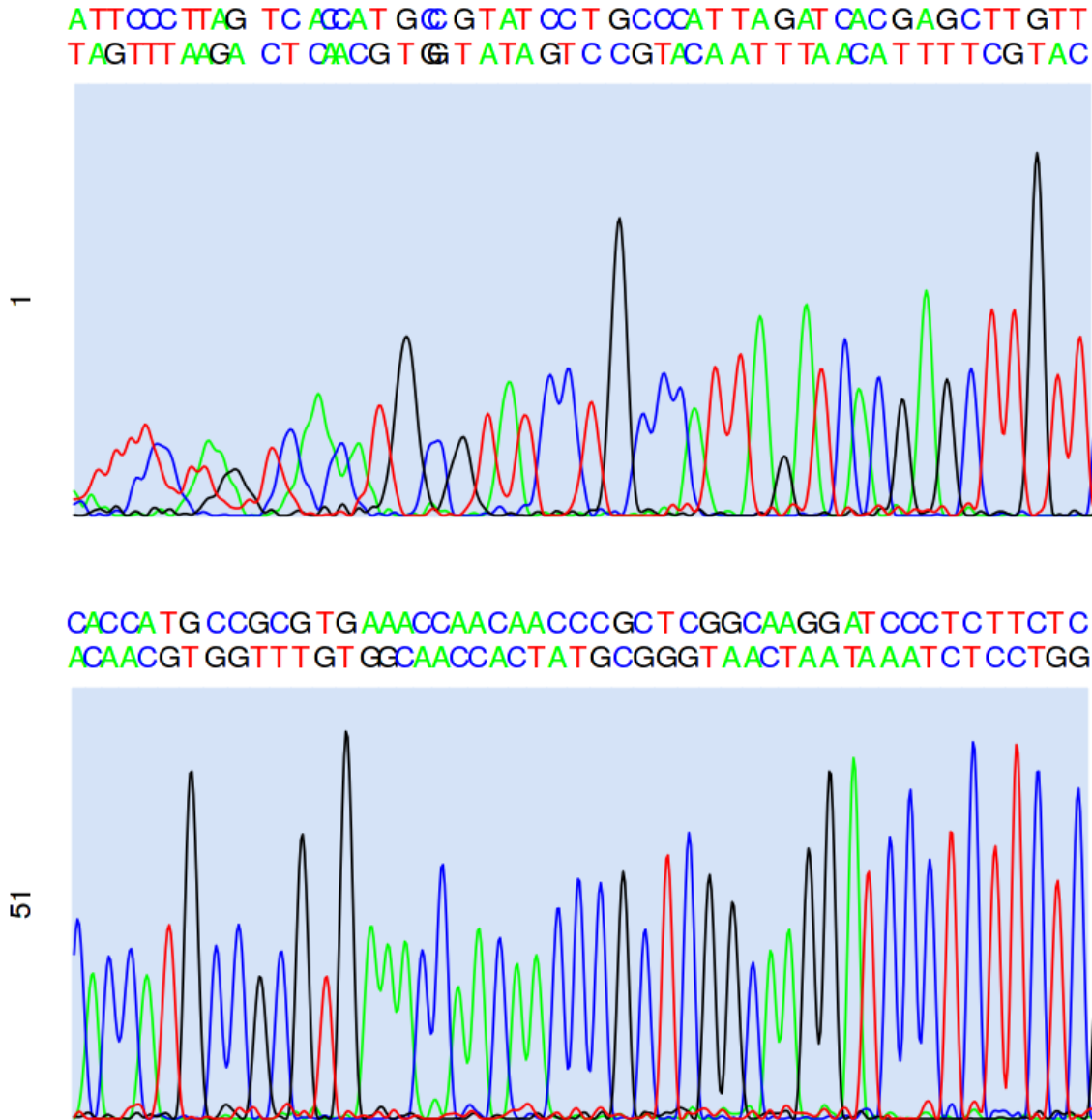
V1	V2	V3	V4
Min. : 11.0	Min. : 5.0	Min. : NA	Min. : NA
1st Qu.: 786.0	1st Qu.: 28.0	1st Qu.: NA	1st Qu.: NA
Median : 976.0	Median : 48.0	Median : NA	Median : NA
Mean : 993.2	Mean : 123.2	Mean : NaN	Mean : NaN
3rd Qu.: 1163.0	3rd Qu.: 140.0	3rd Qu.: NA	3rd Qu.: NA
Max. : 2395.0	Max. : 1738.0	Max. : NA	Max. : NA
		NA's : 1133	NA's : 1133

```
primarySeq(sseq)
```

```
1133-letter "DNAStrng" instance
seq: ATTCCCTTAGTCACCATGCCGTATCCTGCCCATTAG...TACCCCACTATGCTTAGGTCCCTTAAACCACCAGAT
```

```
primarySeq(sseq, string=T)
```

```
chromatogram(sseq, trim5=0, trim3=1033, width=50, showcalls='both')
```



```
peakAmpMatrix(sseq) [1:28,1]
```

Az ábrán a kék háttér azt jelzi, ahol az elsodleges és a másodlagos szekvencia eltér egymástól, azonban ha megvizsgáljuk az ábrát, akkor azt is láthatjuk, hogy a másodlagos szekvencia bázisai az elsodlegeshez viszonyítva nagyon alacsony intenzitásból lehetnek meghatározva.

Homozigóta szekvencia esetén ez nem jelent problémát, mivel akkor használhatjuk csak az elsodleges szekvenciát. Homozigóta szekvenciáknál azonban nem mindegy, hogy a másodlagos szekvencia milyen megalapozottságú.

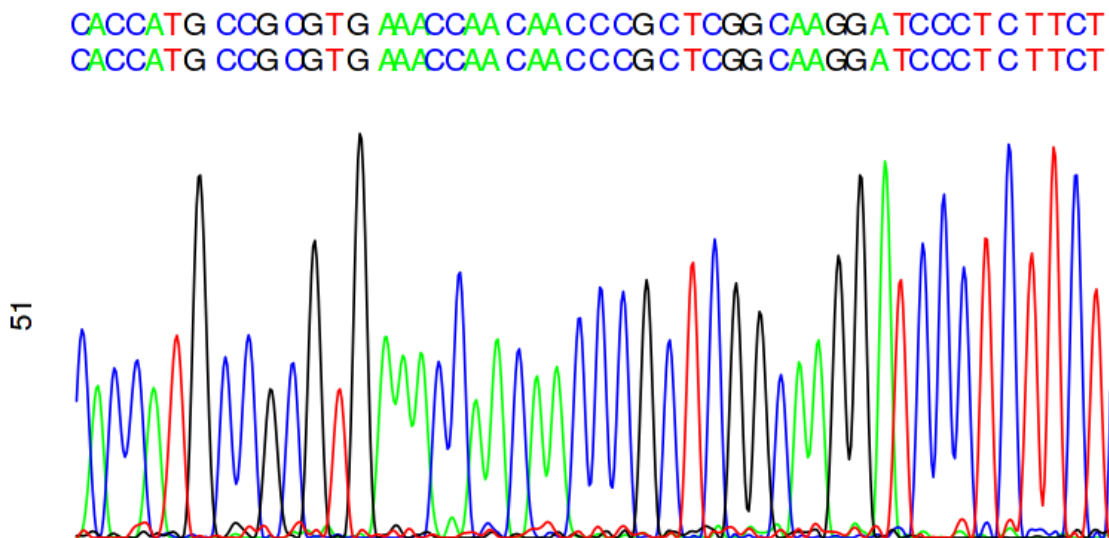
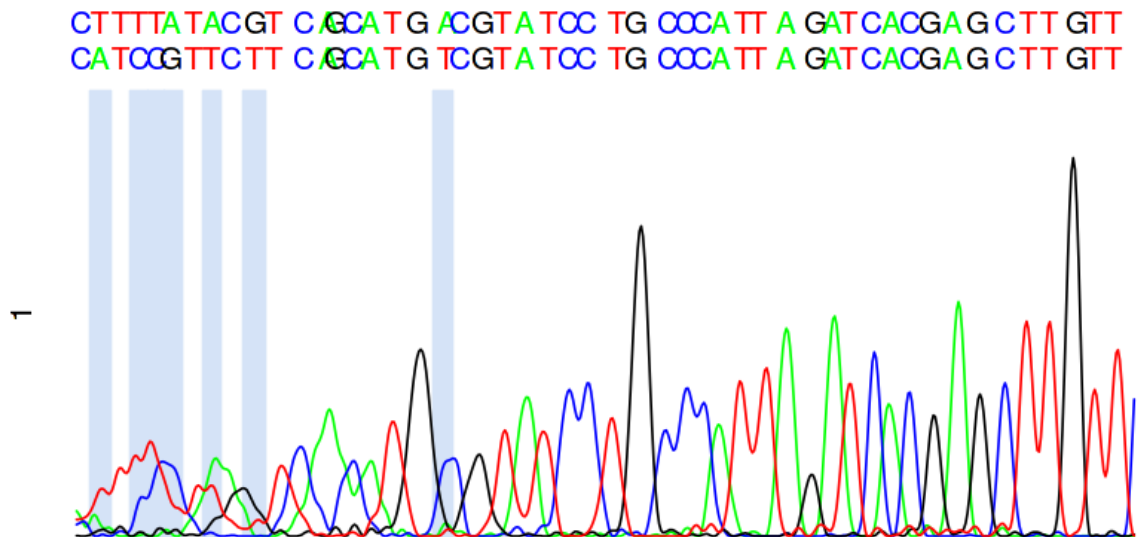
A makeBaseCalls()-függvénnyel saját beállításokkal azonosíthatunk másodlagos szekvenciát. A függvény ratio-argumentumával azt állíthatjuk be, hogy az elsodleges szekvencia intenzitásértékéhez viszonyítva mekkora intenzitás értéktől tekintjük azt jelnek és nem zajnak.

```
seq.new = makeBaseCalls(sseq, ratio = 0.33)
```

(continues on next page)

(folytatás az előző oldalról)

```
chromatogram(seq.new, trim5=0, trim3=1033, width=50, showcalls='both')
```



```
illesztes = pairwiseAlignment(primarySeq(seq.new), primarySeq(sseq), type='global-  

    ↪local')  

writePairwiseAlignments(illesztes)
```

```
#####  

# Program: Biostrings (version 2.46.0), a Bioconductor package  

# Rundate: Tue Apr 3 10:54:25 2018  

#####  

#=====
```

(continues on next page)

(folytatás az előző oldalról)

```
#
# Aligned_sequences: 2
# 1: P1
# 2: S1
# Matrix: NA
# Gap_penalty: 14.0
# Extend_penalty: 4.0
#
# Length: 1133
# Identity: 1071/1133 (94.5%)
# Similarity: NA/1133 (NA%)
# Gaps: 6/1133 (0.5%)
# Score: 1738.12
#
#
#=====
P1          1 CTTTATACGTACGATGACGTATCCTGCCCATTAGATCACGAGCTTGTT      50
|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
S1          4 CCCTTA--GTCACCATGCCGTATCCTGCCCATTAGATCACGAGCTTGTT      50

P1         51 CACCATGCCGCGTGAAACCAACAACCCGCTCGGCAAGGATCCCTCTTCTC     100
|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
S1         51 CACCATGCCGCGTGAAACCAACAACCCGCTCGGCAAGGATCCCTCTTCTC     100

P1        101 GCTCCGGGGCCCATTAAGTGTGGGGGTAAGTATTTAATGAAGTTTAACAGG     150
|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
S1        101 GCTCCGGGGCCCATTAAGTGTGGGGGTAAGTATTTAATGAAGTTTAACAGG     150

P1        151 CATCTGGTTCTTTCTTCAGGGCCATCTCATCTAAAATCGCCCACTCTTTC     200
|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
S1        151 CATCTGGTTCTTTCTTCAGGGCCATCTCATCTAAAATCGCCCACTCTTTC     200

P1        201 CCCTTAAATAAGACATCTCGATGGACTAATGACTAATCAGCCCATGCCCA     250
|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
S1        201 CCCTTAAATAAGACATCTCGATGGACTAATGACTAATCAGCCCATGCCCA     250

P1        251 ACATAACTGTGATGTCATGCATTGGTATTTTTTAATTTTTGGGGATGCT     300
|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
S1        251 ACATAACTGTGATGTCATGCATTGGTATTTTTTAATTTTTGGGGATGCT     300

P1        301 TGGACTCAGCTATGGCCGTCTGAGGCCCGACCCGGAGCATGAATTGTAG     350
|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
S1        301 TGGACTCAGCTATGGCCGTCTGAGGCCCGACCCGGAGCATGAATTGTAG     350

P1        351 CTGGACTTAACTGCATCTTGAGCATCCTCATAATGGTAAGCATGGGCATA     400
|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
S1        351 CTGGACTTAACTGCATCTTGAGCATCCTCATAATGGTAAGCATGGGCATA     400

P1        401 ATATAATTAATGGTCACAGGACATATCTGCTGTATCGTGCATTATATAT     450
|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
S1        401 ATATAATTAATGGTCACAGGACATATCTGCTGTATCGTGCATTATATAT     450

P1        451 TCTTTTCCCCCTTCCCCTTAAATATTTATCACCATTTTAAACACGCTT     500
|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
S1        451 TCTTTTCCCCCTTCCCCTTAAATATTTATCACCATTTTAAACACGCTT     500
```

(continues on next page)

(folytatás az előző oldalról)

P1	501	CCCCCTAGATATTAATATAAAATTTATCCCGCCCTCAATACTCAAATTCAT	550
S1	501	CCCCCTAGATATTAATATAAAATTTATCCCGCCCTCAATACTCAAATTCAT	550
P1	551	ACTCCAACCGAAGTAAATATATAGGCACCTGGGTCACATGCATAACGCAT	600
S1	551	ACTCCAACCGAAGTAAATATATAGGCACCTGGGTCACATGCATAACGCAT	600
P1	601	AGTTAATGTAGCTTAAACTTAAAGCAAGGCACTGAAAATGCCTAGATGAG	650
S1	601	AGTTAATGTAGCTTAAACTTAAAGCAAGGCACTGAAAATGCCTAGATGAG	650
P1	651	TCTACTGACTCCATGAACATATAGGTTTGGTCCCAGCCTTCCTGTAACT	700
S1	651	TCTACTGACTCCATGAACATATAGGTTTGGTCCCAGCCTTCCTGTAACT	700
P1	701	TTCAATAGACTTATACATGCAAGCATCCACGCCCCGGTGAGTAACGCCCT	750
S1	701	TTCAATAGACTTATACATGCAAGCATCCACGCCCCGGTGAGTAACGCCCT	750
P1	751	TCGAATCACACAGGACTAAACGAGCATGTATCAAGCACACACTCTTGTAG	800
S1	751	TCGAATCACACAGGACTAAAGGAGCAGGTATCAAGCACACACTCTTGTAG	800
P1	801	CTCACAACGCCGTGCTTAGCCACACCCCCACGGGAGACAGCAGTAACAAA	850
S1	801	CTCACAACGCCGTGCTTAACCACACCCCCACGGGAGACAGCAGTAACAAA	850
P1	851	AATTAAGTCATAAACGAAAGTTAGACTACGCCATATCGACCACGCCTGAT	900
S1	851	AATTAAGCCATAAACGAAAGTTGACTAAGCCATATTGACCAGGGTTGGT	900
P1	901	AAATCTCGTGACAGTCACGGCGCTCATAACGAATGATCTATGCTGACAAGA	950
S1	901	AAATCTCGTGCCAGCCACCGCGGTCATACGATTGACCCAAGCTAACAGGA	950
P1	951	GTACTGCGTAAAGCGTGCTAAGCATCATACTAATAAAGCTGAACTTCAAT	1000
S1	951	GTACGGCGTAAAGCGTGTTAAGCATCATACTAATAGAGTTAAATTTAAT	1000
P1	1001	AAAGCTGTAAAAACGACCATGATTA--AACAAAAAGTAAATGAACGAATGT	1049
S1	1001	TAAACTGTAAAAAAGCCATAATTATAACAAAAATAAATGAACGAAAGT	1050
P1	1050	AACCTTACAAAGCATGATAACACATAGCTAAAGCGAACCTGGATAAAAAA	1099
S1	1051	AACCTTACATAGCTTGATTACACATAGCTAGACCGGACCTGGATAGATAC	1100
P1	1100	CCCCCTATGCTTAGGGCCAGTAAAACCCAAAAG	1132
S1	1101	CCCACTATGCTTAGGTCCCTTAAA--CCACCAG	1131
#-----			
#-----			

Ehhez hasonlóan illeszthetjük az elsodleges szekvenciát a referencia genomra. Ennek első lépése, hogy letöltjük a

referencia-szekvenciát FASTA formátumban és DNASTring-é alakítjuk.

```
library(rentrez)

fasta.mt = entrez_fetch(db='nucore', id='NC_001941.1', rettype='fasta')
fasta.mt
```

```
library(seqinr)

seq.mt = read.fasta(textConnection(fasta.mt), as.string=T)
ref = DNASTring(seq.mt[[1]][1])
ref
```

```
Attaching package: 'seqinr'

The following object is masked from 'package:sangerseqR':

  read.abif

The following object is masked from 'package:Biostrings':

  translate
```

```
16616-letter "DNASTring" instance
seq: GTTAATGTAGCTTAACTTAAAGCAAGGCACTGAAA...AATATATAGGCACCTGGGTCACATACATAACGCATA
```

```
illesztes = pairwiseAlignment(primarySeq(seq.new), ref, type='global-local')
writePairwiseAlignments(illesztes)
```

```
#####
# Program: Biostrings (version 2.46.0), a Bioconductor package
# Rundate: Tue Apr  3 10:54:29 2018
#####
#=====
#
# Aligned_sequences: 2
# 1: P1
# 2: S1
# Matrix: NA
# Gap_penalty: 14.0
# Extend_penalty: 4.0
#
# Length: 1133
# Identity:      587/1133 (51.8%)
# Similarity:    NA/1133 (NA%)
# Gaps:          535/1133 (47.2%)
# Score: -1091.574
#
#
#=====

P1              1 CTTTATACGTCAGCATGACGTATCCTGCCCATTAGATCACGAGCTTGTT      50
                  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
S1             16018 CCATTCTA-GTCAACATG-CGTATCCTGTCCATTAGATCACGAGCTTGTT 16065

P1              51 CACCATGCCGCGTGAAACCAACAACCCGCTCGGCAAGGATCCCTCTTCTC      100
```

(continues on next page)

(folytatás az előző oldalról)

S1	16066	 CACCATGCCGCGTGAAACCAACAACCCGCTCAGCAAGGATCCCTCTTCTC	16115
P1	101	GCTCCGGGGCCATTAACTGTGGGGGTAACATTTAATGAACCTTAACAGG	150
S1	16116	 GCTCCGGGGCCACTAACTGTGGGGGTAACATTTAATGAACCTTAACAGG	16165
P1	151	CATCTGGTTCTTTCTTCAGGGCCATCTCATCTAAAATCGCCCACTCTTTC	200
S1	16166	 CATCTGGTTCTTTCTTCAGGGCCATCTCATCTAAAATCGCCCACTCTTTC	16215
P1	201	CCCTTAAATAAGACATCTCGATGGACTAATGACTAATCAGCCCATGCCCA	250
S1	16216	 CCCTTAAATAAGACATCTCGATGGACTAATGACTAATCAGCCCATGCCCA	16265
P1	251	ACATAACTGTGATGTCATGCATTGGTATTTTTTAATTTTTGGGGATGCT	300
S1	16266	 ACATAACTGTGGTGTGTCATGCATTGGTATTTTTTAATTTTTGGGGATGCT	16315
P1	301	TGGACTCAGCTATGGCCGTCTGAGGCCCGACCCGGAGCATGAATTGTAG	350
S1	16316	 TGGACTCAGCTATGGCCGTCTGAGGCCT-GACCCGGAGCATGAATTGTAG	16364
P1	351	CTGGACTTAACTGCATCTTGAGCATCCTCATAATGGTAAGCATGGGCATA	400
S1	16365	 CTGGACTTAACTGCATCTTGAGCATCCTCATAATGGTAAGCATGGGCATA	16414
P1	401	ATATAATTAATGGTCACAGGACATATCTGCTGTATCGTGCATTTATATAT	450
S1	16415	 ATATAATTAATGGTCACAGGACATATCTGCTGTATCGTGCATTTATATAT	16464
P1	451	TCTTTTT-CCCCCTTCCCCTTAAATATTTATCACCATTTTAAACACGCT	499
S1	16465	 TCTTTTTTCCCCCTTCCCCTTAAATATTTATCACCATTTTAAACACGCT	16514
P1	500	TCCCCCTAGATATTAATATAAATTTATCCCGCCCTCAATACTCAAATTCA	549
S1	16515	 TCCCCCTAGATATTAATATAAATTTATCCCGCCCTCAATACTCAAATTCA	16564
P1	550	TACTCCAACCGAAGTAAATATATAGGCACCTGGGTACATGCATAACGCA	599
S1	16565	 TACTCCAACCGAAGTAAATATATAGGCACCTGGGTACATGCATAACGCA	16614
P1	600	TAGTTAATGTAGCTTAACTTAAAGCAAGGCACTGAAAATGCCTAGATGA	649
S1	16615	 TA-----	16616
P1	650	GTCTACTGACTCCATGAACATATAGGTTTGGTCCCAGCCTTCCTGTAAAC	699
S1	16617	-----	16616
P1	700	TTTCAATAGACTTATACATGCAAGCATCCACGCCCCGGTGAGTAACGCCC	749
S1	16617	-----	16616
P1	750	TTCGAATCACACAGGACTAAACGAGCATGTATCAAGCACACACTCTTGTA	799

(continues on next page)

(folytatás az előző oldalról)

S1	16617	-----	16616
P1	800	GCTCACAACGCCGTGCTTAGCCACACCCCCACGGGAGACAGCAGTAACAA	849
S1	16617	-----	16616
P1	850	AAATTAAGTCATAAACGAAAGTTAGACTACGCCATATCGACCACGCCTGA	899
S1	16617	-----	16616
P1	900	TAAATCTCGTGACAGTCACGGCGCTCATACGAATGATCTATGCTGACAAG	949
S1	16617	-----	16616
P1	950	AGTACTGCGTAAAGCGTGCTAAGCATCATACTAATAAAGCTGAACCTTCAA	999
S1	16617	-----	16616
P1	1000	TAAAGCTGTAAAAACGACCATGATTAAACAAAAAGTAAATGAACGAATGT	1049
S1	16617	-----	16616
P1	1050	AACCTTACAAAGCATGATAACACATAGCTAAAGCGAACCTGGATAAAAAA	1099
S1	16617	-----	16616
P1	1100	CCCCCTATGCTTAGGGCCAGTAAAACCCAAAAG	1132
S1	16617	-----	16616
#-----			
#-----			

A forward és reverse szekvenciákból a sangeranalyseR-csomaggal létrehozhatunk konszenzus szekvenciát.

```
fwd = readsangerseq('sangerF.ab1')
fwd = primarySeq(fwd, string=T)

rev = readsangerseq('sangerR.ab1')
rev = primarySeq(rev)
rev = reverseComplement(rev)

reads = DNASTringSet(c(fwd, as.character(rev)))
names(reads) = c('+', '-')
reads
```

```
Warning message in read.abif(filename):
"unimplemented legacy type found in file"Warning message in read.abif(filename):
"unimplemented legacy type found in file"
```

```
A DNASTringSet instance of length 2
width seq names
[1] 1133 ATTCCCTTAGTCACCATGCCGTA...TTAGGTCCCTTAAACCACCAGAT +
[2] 1132 ATCATTGACCGCGTGAAACAATC...GTACTACCGGCAACAGCCCCGAT -
```

```
library(sangeranalyseR)

merged.reads = merge.reads(reads)
merged.reads
```

```
Loading required package: ape

Attaching package: 'ape'

The following objects are masked from 'package:seqinr':

    as.alignment, consensus

The following object is masked from 'package:Biostrings':

    complement

Loading required package: DECIPHER
Loading required package: RSQLite
Loading required package: reshape2
Loading required package: phangorn
Loading required package: stringi
Loading required package: stringr
```

```
[1] "Aligning reads"
[1] "Calling consensus sequence"
[1] "Calculating differences between reads and consensus"
```

```
$consensus
 1190-letter "DNAString" instance
seq: ATTCCCTTAGTCACCATGCCGTATCCTGCCCATAG...ATTATTCGCCAGAGTACTACCGGCAACAGCCCCGAT

$alignment
  A DNAStringSet instance of length 3
    width seq
[1] 1190 ATTCCCTTAGTCACCATGCCGT...-----+
[2] 1190 -----...GTACTACCGGCAACAGCCCCGAT -
[3] 1190 ATTCCCTTAGTCACCATGCCGT...GTACTACCGGCAACAGCCCCGAT consensus

$differences
  name pairwise.diffs.to.consensus unused.chars
1    +                      43          0
2    -                      43          0

$distance.matrix
      +      -
+ 0.00000000 0.04110618
- 0.04110618 0.00000000
attr(,"correction")
[1] "Jukes-Cantor"

$dendrogram
'dendrogram' with 2 branches and 2 members total, at height 0.02055309

$indels
NULL
```

(continues on next page)

(folytatás az előző oldalról)

```
$stop.codons
NULL

$secondary.peak.columns
NULL

attr(,"class")
[1] "merged.read"
```

```
res = DNASTringSet(merged.reads$consensus)
names(res) = 'első konszenzus'
writeXStringSet(res, filepath='konszenzus.fa')
```

```
res
```

```
A DNASTringSet instance of length 1
width seq                                     names
[1] 1190 ATCCCTTAGTCACCATGCCGTA...GTACTACGGCAACAGCCCCGAT első konszenzus
```

Eddig nem foglalkoztunk azzal, hogy a meghatározott bázisok különböző minőségűek. A kiindulási ABIF-állomány tartalmazza a bázisok minőségére vonatkozó információkat is, a PCON.1 és PCON.2 változóként. Az elozot módosítja az adállományt létrehozó, míg az utóbbi az eredeti bázisminőséget tárolja.

Ezt az ún. Phred minőségi pontszámmal (https://en.wikipedia.org/wiki/Phred_quality_score) fejezik ki.

$$Q = -10 \times \log_{10} P$$

Ennek valószínűséggé való átalakítása:

$$P = 10^{\frac{-Q}{10}}$$

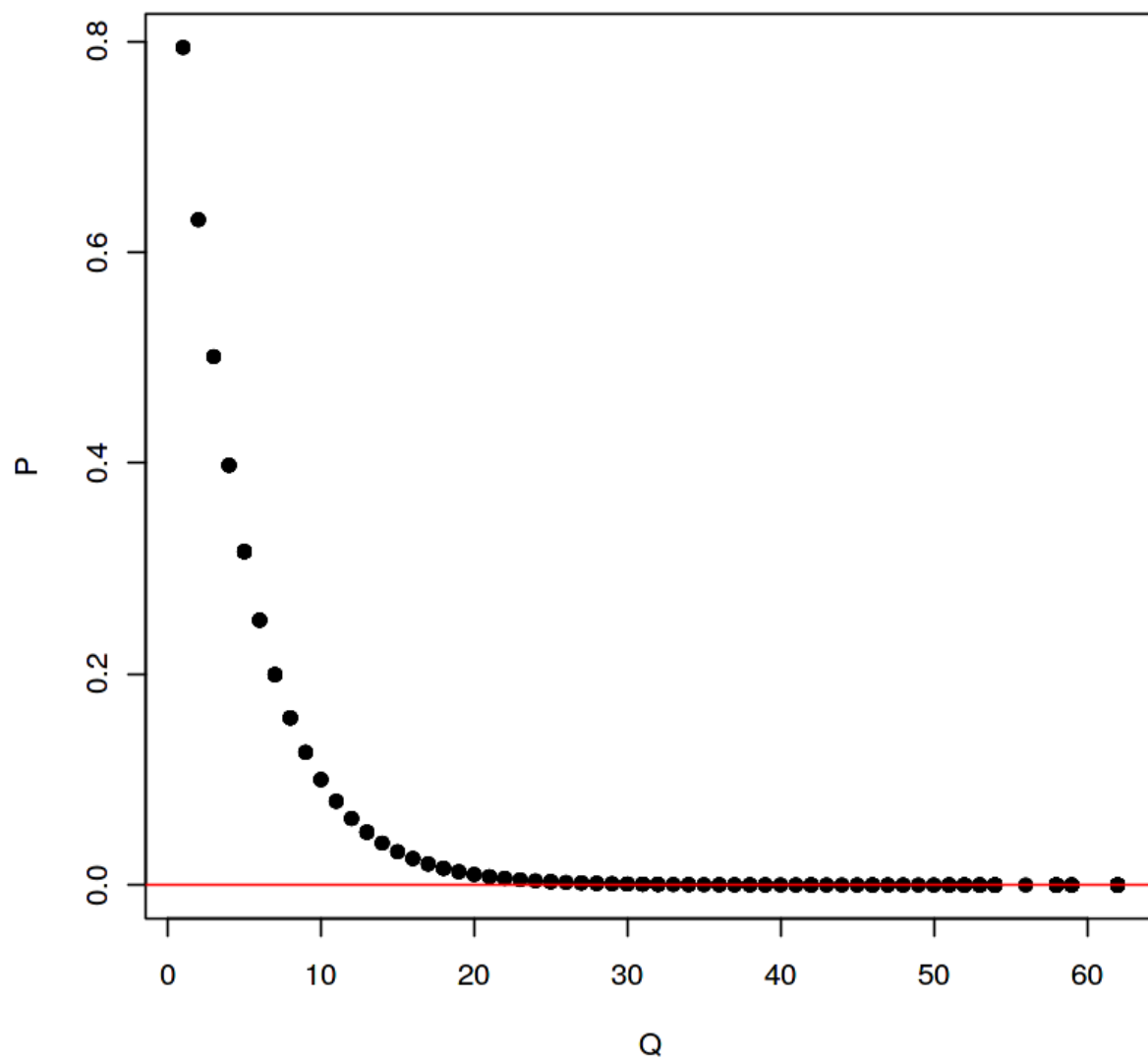
```
Q = abif.F@data$PCON.1
summary(Q)
```

```
Min. 1st Qu. Median Mean 3rd Qu. Max.
1.00 32.00 58.00 46.94 62.00 62.00
```

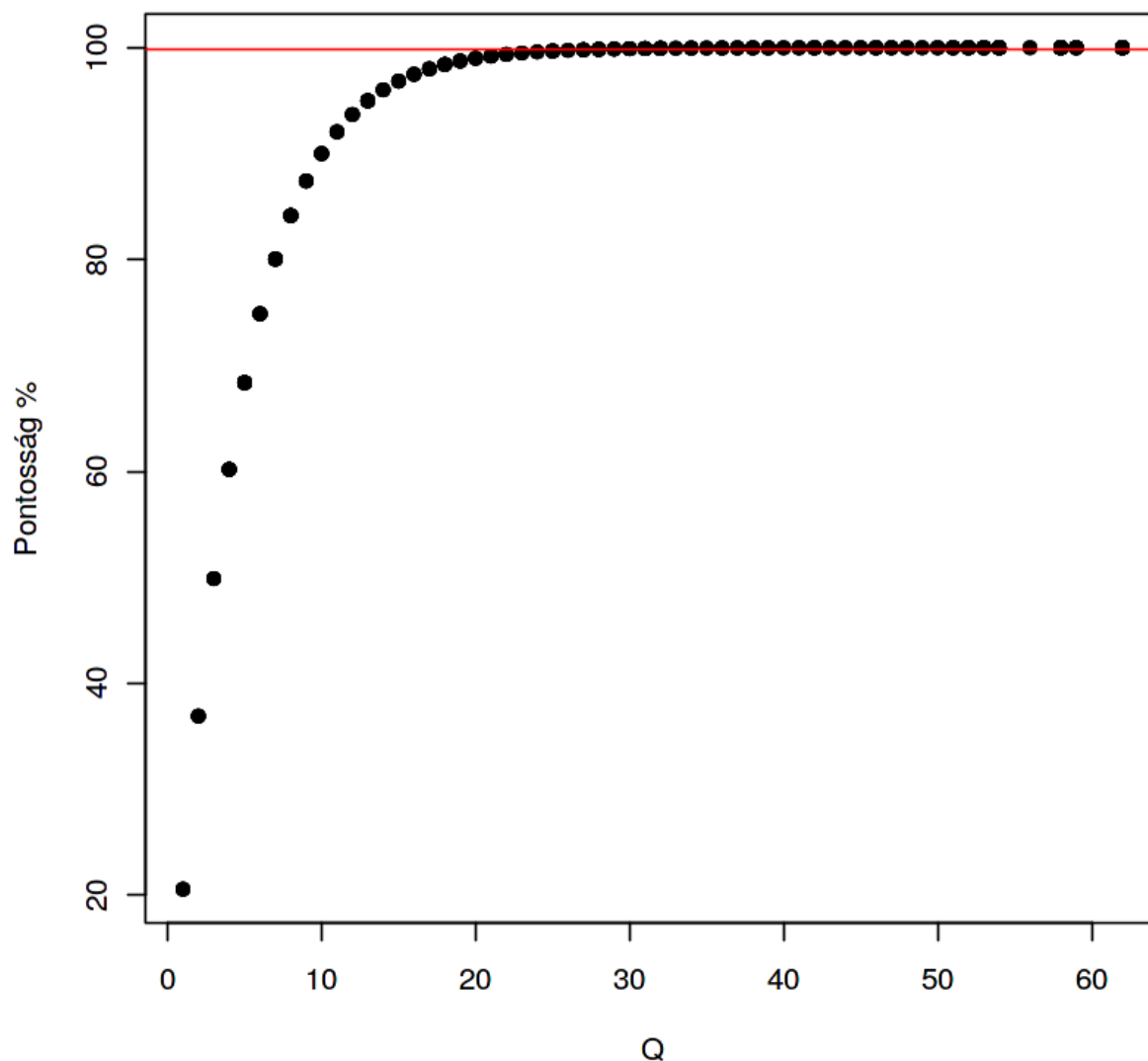
```
P = 10^(-1*Q/10)
summary(P)
```

```
Min. 1st Qu. Median Mean 3rd Qu. Max.
0.0000006 0.0000006 0.0000016 0.0276383 0.0006310 0.7943282
```

```
plot(Q, P, pch=16)
abline(h=0.0001, col='red')
```



```
basecall.pontosság = (1-P)*100
plot(Q, basecall.pontosság, pch=16, ylab='Pontosság %')
abline(h=99.9, col='red')
```



```
(trims = trim.mott(abif.F, cutoff=0.0001))
```

```
(trims = trim.mott(abif.F, cutoff=0.01))
```

A 0.01 határértéssel meghatározott szekvencia-tartományok alapján egy alternatív konszenzus-szekvenciát határoztunk meg

```
seq.f = primarySeq(sangerseq(abif.F), string=T)
seq.f.t = substr(seq.f, trims$start, trims$finish)

trims = trim.mott(abif.R, cutoff=0.01)
seq.r = primarySeq(sangerseq(abif.R))
seq.r = seq.r[trims$start:trims$finish]
seq.r = reverseComplement(seq.r)
```

(continues on next page)

(folytatás az előző oldalról)

```
seq.r.t = as.character(seq.r)

reads = DNASTringSet(c(seq.f.t, seq.r.t))
names(reads) = c('+', '-')

merged.reads = merge.reads(reads)
merged.reads
```

```
[1] "Aligning reads"
[1] "Calling consensus sequence"
[1] "Calculating differences between reads and consensus"
```

```
$consensus
 1151-letter "DNASTring" instance
seq: TGCCGTATCCTGCCCATTAGATCACGAGCTTGTTCA...ATTATAAAAAACAAAATTATTCGCCAGAGTACTACCG

$alignment
  A DNASTringSet instance of length 3
    width seq                                names
[1]  1151 TGCCGTATCCTGCCCATTAGATC...-----+
[2]  1151 -----...AATTATTCGCCAGAGTACTACCG -
[3]  1151 TGCCGTATCCTGCCCATTAGATC...AATTATTCGCCAGAGTACTACCG consensus

$differences
  name pairwise.diffs.to.consensus unused.chars
1   +                                0          0
2   -                                0          0

$distance.matrix
  + -
+ 0 0
- 0 0
attr(,"correction")
[1] "Jukes-Cantor"

$dendrogram
'dendrogram' with 2 branches and 2 members total, at height 0

$indels
NULL

$stop.codons
NULL

$secondary.peak.columns
NULL

attr(,"class")
[1] "merged.read"
```

1.4 Session info

```
sessionInfo()
```

```
R version 3.4.3 (2017-11-30)
Platform: x86_64-pc-linux-gnu (64-bit)
Running under: Ubuntu 16.04.4 LTS

Matrix products: default
BLAS: /usr/local/lib/R/lib/libRblas.so
LAPACK: /usr/local/lib/R/lib/libRlapack.so

locale:
 [1] LC_CTYPE=en_US.UTF-8      LC_NUMERIC=C
 [3] LC_TIME=hu_HU.UTF-8      LC_COLLATE=en_US.UTF-8
 [5] LC_MONETARY=hu_HU.UTF-8  LC_MESSAGES=en_US.UTF-8
 [7] LC_PAPER=hu_HU.UTF-8     LC_NAME=C
 [9] LC_ADDRESS=C             LC_TELEPHONE=C
[11] LC_MEASUREMENT=hu_HU.UTF-8 LC_IDENTIFICATION=C

attached base packages:
[1] stats4      parallel  stats      graphics  grDevices  utils      datasets
[8] methods     base

other attached packages:
 [1] sangeranalyseR_0.1.0 stringr_1.3.0      stringi_1.1.7
 [4] phangorn_2.4.0      reshape2_1.4.3    DECIPHER_2.6.0
 [7] RSQLite_2.1.0       ape_5.0           seqinr_3.4-5
[10] rentrez_1.2.1       RColorBrewer_1.1-2 sangerseqR_1.14.0
[13] Biostrings_2.46.0   XVector_0.18.0    IRanges_2.12.0
[16] S4Vectors_0.16.0    BiocGenerics_0.24.0

loaded via a namespace (and not attached):
 [1] pbdZMQ_0.3-2      repr_0.12.0        lattice_0.20-35
 [4] htmltools_0.3.6   blob_1.1.1         XML_3.98-1.10
 [7] DBI_0.8           bit64_0.9-7        uuid_0.1-2
[10] plyr_1.8.4        zlibbioc_1.24.0    evaluate_0.10.1
[13] memoise_1.1.0     Cairo_1.5-9        httpuv_1.3.6.2
[16] curl_3.2          IRdisplay_0.4.4    Rcpp_0.12.16
[19] xtable_1.8-2      IRkernel_0.8.12.9000 jsonlite_1.5
[22] mime_0.5          bit_1.1-12         fastmatch_1.1-0
[25] digest_0.6.15     shiny_1.0.5        ade4_1.7-10
[28] grid_3.4.3        quadprog_1.5-5     tools_3.4.3
[31] magrittr_1.5      crayon_1.3.4       pkgconfig_2.0.1
[34] MASS_7.3-49       Matrix_1.2-13      httr_1.3.1
[37] R6_2.2.2          igraph_1.2.1       nlme_3.1-131.1
[40] compiler_3.4.3
```


2. fejezet

NGS

<https://www.youtube.com/watch?v=9vNBYYHedLg>

https://www.youtube.com/watch?v=ToKUGz_YhC4

<https://www.youtube.com/watch?v=9YxExTSwgPM>



2.1 Short Reads

2.1.1 SRA

<https://www.ncbi.nlm.nih.gov/sra/docs/>

példa: SRR1660257: <https://www.ncbi.nlm.nih.gov/sra/?term=SRR1660257>

<https://www.ncbi.nlm.nih.gov/geo/>

<https://www.ebi.ac.uk/ena>

```
%bash
export PATH=$PATH:/usr/local/ncbi/sra-tools/bin
mkdir gyak06
cd gyak06
fastq-dump SRR1660257
```

Read 1015290 spots **for** SRR1660257
Written 1015290 spots **for** SRR1660257

```
%%bash
cd gyak06
ls -lh
```

```
total 267M
-rw-rw-r-- 1 sn sn 267M ápr  3 11:18 SRR1660257.fastq
```

```
%%bash
cd gyak06
head SRR1660257.fastq
```

[illegible]

2.1.2 FASTQ

http://support.illumina.com/content/dam/illumina-support/help/BaseSpaceHelp_v2/Content/Vault/Informatics/Sequencing_Analysis/BS/swSEQ_mBS_FASTQFiles.htm

https://en.wikipedia.org/wiki/FASTQ_format

Minden readhez négy sor tartozik: - az első sorban van a read azonosítója, illetve lehet még hozzá tartozó leírás - a második sorban van a read nyers nukleotidsora - a harmadik + jellel kezdődő sor a jel után lehet üres vagy a read azonosítóját is tartalmazhatja - a negyedik sorban az egyes nukleotidok leolvasási minőségére vonatkozó kódolás (részletek 74. lapon: Csaba Ortutay, Zsuzsanna Ortutay: *Molecular Data Analysis Using R*. 2017, Wiley-Blackwell, ISBN: 978-1-119-16502-6. <https://www.wiley.com/en-hu/Molecular+Data+Analysis+Using+R-p-9781119165026>)

BAM-ban tárolva: <https://gatkforums.broadinstitute.org/gatk/discussion/5990/what-is-ubam-and-why-is-it-better-than-fastq-for-storing>

2.1.3 CSFASTA

ABi SOLiD sequencer: <http://cutadapt.readthedocs.io/en/stable/colospace.html>

CSFASTA-file

```
# Title: s0205_20110422_FRAG_BC_miRNA_MeDIP
>1_5_224_F3
T.222200232103..132..030.020..000.00
>1_5_656_F3
T.31311231.331..233..122.122..122.10
>1_5_1005_F3"
T.122221311011..212..312.033..303.32
```

	A	C	G	T
A	0	1	2	3
C	1	0	3	2
G	2	3	0	1
T	3	2	1	0

QUAL-file

```
# Title: s0205_20110422_FRAG_BC_miRNA_MeDIP
>1_5_224_F3"
-1 27 28 30 32 21 31 30 31 27 4 28 31 -1 -1 17 28 31 -1 -1 24 30 25 -1 22 25 21 -1 -1
↪ 30 28 21 -1 27 31
>1_5_656_F3"
-1 33 4 31 29 16 33 4 32 -1 20 33 4 -1 -1 25 31 10 -1 -1 12 25 5 -1 22 4 7 -1 -1 5 4
↪ 7 -1 4 4
>1_5_1005_F3"
-1 31 20 33 32 32 30 32 31 26 26 25 17 -1 -1 30 26 6 -1 -1 27 23 28 -1 25 31 10 -1 -1
↪ 29 14 19 -1 33 33
```

```
%%bash

cd gyak06

fastq-dump -X 10000 --split-files SRR1972739
```

```
Read 10000 spots for SRR1972739
Written 10000 spots for SRR1972739
```

```
library(ShortRead)
library(seqTools)
library(qrqc)
library(BiocParallel)

setwd('gyak06')

fajlom = 'SRR1660257.fastq'

readek = readFastq(fajlom)
```

```
slotNames(readek)
```

```
id(readek)
```

```
A BStringSet instance of length 1015290
      width seq
[1]      25 SRR1660257.1 1 length=100
[2]      25 SRR1660257.2 2 length=100
[3]      25 SRR1660257.3 3 length=100
[4]      25 SRR1660257.4 4 length=100
[5]      25 SRR1660257.5 5 length=100
...      ...
[1015286]    37 SRR1660257.1015286 1015286 length=100
[1015287]    37 SRR1660257.1015287 1015287 length=100
[1015288]    37 SRR1660257.1015288 1015288 length=100
[1015289]    37 SRR1660257.1015289 1015289 length=100
[1015290]    37 SRR1660257.1015290 1015290 length=100
```

```
sread(readek)
```

```
A DNAStringSet instance of length 1015290
width seq
[1] 100 GTAATCTTAGATTCAAGTAATCCATATAATT...GAAGCAATCTGAAGAAAACTAAAAGACAA
[2] 100 CAAGAGCACTGACTTCCTGGACCCCGCCACC...AACAACTCATCACCAAGATACCGGAGAA
[3] 100 GCCCAAGATTGATCGAGGTTGGGTATGTGTT...TGAGCCAATCTCCCTTCCCTCCGAAAGAGG
[4] 100 TAATAATCAGATCTGCGAACCGGTAGAGTTT...CAATAGAAATTTAAACAGTGAGTGAGACA
[5] 100 CTTAGACATCAAAAATTCTTCTGTTTTCTG...ATCCCATTGTTCCATGCTCATTCACTGATG
...
[1015286] 100 CAGATGATGAAGAGCAGGACAGGGACGGAAC...ATACAGAGATCACTCTGAAAAGAAAGAACT
[1015287] 100 CTGGAAGTTTCATAAGAATTTTCTTTCCTGA...TATTGTTGGAGTTGCTTCTCAGCCTCAGTG
[1015288] 100 ATCTTCCAAGATGCTGCTCCACCTGTCATCC...AAAGCTTGCCTCCAGTCCCACCATCGCCCA
[1015289] 100 AGAGCCACAACATGAGCTACGCACCTTTTCAA...GGGGCGGCACATGCCACATTCTGGGACCGG
[1015290] 100 GACGAGGACACTAAGCCGGTGCTAATAGAT...GGCATATAGAGGGCAGACAGACACAATCCG
```

```
kval = quality(readek)
kval
```

```
class: FastqQuality
quality:
  A BStringSet instance of length 1015290
    width seq
      [1]   100 CBCFFFFFFHHHHHJJJJIJJJJJJJJJJ..JJJJJJJJJJJHHGHHHFFFFCEECDDDD
      [2]   100 CCCFFFFFFHHHHHJJJJJJJJJJJJJJ..DDDDDDDDDDDDDDDDDDDDDDDDDDDDDD
      [3]   100 CCCFFFFFFHHHHHJJIJJEGIJJCFGICFFG..HHFFFFFFECEEEDDDDDDDDDDDDDDB
      [4]   100 CCCFFFFFFHHHHHJJJGGJJJJJGHIIJHIJ..EEEEEDCCDDDEDDDDCCDDACDDDDDD
      [5]   100 CCCFFFFFFHHHHHJJJJJJJJJJJJJJJIJ..JJGHHHHFFFFFFFFFECCCEEEEECDDDDD
      ...   ...   ...
[1015286] 100 C@CFFFFFFHHHHHJBHIIIJJIIIGIGGIJ..DDDEEDDDCCDDDDDDDDDDDDDDDDDDDC
[1015287] 100 ?@fBDBBFHHBBGBECFFHIBEHEHDHHEG...CH>DF@@;;(..;AC@C>A@;>9<ACC@:
[1015288] 100 CCCFFFFFFHHHHHJJJJJJJJJJJJJJ..DEEDBCDDDDDBDCCDDDDDDDDCCDDDDDD
[1015289] 100 ;@DDDDDDHHFDCCGBHGI@GHFFCHHGIEC...A92=;59<@><@AACCCBAACDACCB&B&5
[1015290] 100 l=@D4=A#####.....#####
```

PHRED minőségi pontszám:

$$Q_{PHRED} = -10 \times \log_{10} P$$

Ennek valószínűséggé való átalakítása:

$$P = 10^{-Q_{PHRED}/10}$$

Néhány minőségi érték a döntésekhez:

Q_{PHRED}	hiba		megbízhatóság
10	10%	(1/10)	90%
20	1%	(1/100)	99%
30	0.1%	(1/1000)	99.9%
40	0.01%	(1/10000)	99.99%

Solexa:

$$Q_{Solexa} = -10 \times \log_{10} \left(\frac{P}{1-P} \right)$$

Átváltások:

$$Q_{PHRED} = -10 \times \log_{10} (10^{Q_{Solexa}/10} + 1)$$

$$Q_{Solexa} = -10 \times \log_{10} (10^{Q_{PHRED}/10} - 1)$$

ASCII
Minőségi pont

Tartomány
Típus
Tartomány

Sanger standard

fastq-sanger
33 - 126
PHRED
0 - 93

Solexa/régebbi Illumina

fastq-solexa
59 - 126
Solexa
-5 - 62

Illumina 1.3+

```
<td> fastq-illumina</td>
<td>64 - 126</td>
<td>PHRED</td>
<td>0 - 62</td>
```

Cock PJA, Fields CJ, Goto N, Heuer ML, Rice PM The sanger FASTQ file format for sequences with quality scores and the Solexa/Illumina FASTQ variants. Nucleic Acids Research 2010 Vol.38 No.6 1767-1771

```
phredTable()
```

```
m = as(kval, 'matrix')
m[1,]
```

2.2 Readek minőségellenőrzése

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2752612/>

FastQC: <https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>

<https://www.youtube.com/watch?v=bz93ReOv87Y>

```
%%bash

# export PATH=$PATH:/usr/local/ncbi/sra-tools/bin

mkdir gyak07
cd gyak07

fastq-dump SRR1660259

ls -lh
```

```
Read 9130 spots for SRR1660259
Written 9130 spots for SRR1660259
total 2,4M
-rw-rw-r-- 1 sn sn 2,4M ápr  3 11:28 SRR1660259.fastq
```

```
library(fastqcr)

setwd('gyak07')

fastqc()
```

```
minosegi.fajl = 'FASTQC/SRR1660259_fastqc.zip'

qc_report(
  qc.path = minosegi.fajl,
  template = 'minta_jelentes.Rmd',
  result.file = 'osszefoglalo',
  interpret = TRUE,
  experiment = 'minőségellenőrzési példa',
  preview = FALSE
)
```

processing file: minta_jelentes.Rmd

.		2%
inline R code fragments		
..		3%
label: unnamed-chunk-1 (with options)		
List of 1		
\$ echo: logi FALSE		
...		5%
ordinary text without R code		
....		6%
label: unnamed-chunk-2 (with options)		
List of 1		
\$ echo: logi FALSE		
.....		8%
inline R code fragments		
.....		10%
label: unnamed-chunk-3 (with options)		
List of 1		
\$ echo: logi TRUE		

Attaching package: 'dplyr'

The following objects are masked from 'package:stats':

filter, lag

The following objects are masked from 'package:base':

intersect, setdiff, setequal, union

.....		11%
ordinary text without R code		
.....		13%
label: unnamed-chunk-4		

Reading: FASTQC/SRR1660259_fastqc.zip

.....		14%
ordinary text without R code		
.....		16%
label: summary (with options)		
List of 3		
\$ fig.width : num 5		
\$ fig.height: num 4.5		
\$ fig.align : chr "center"		
.....		17%

(continues on next page)

(folytatás az előző oldalról)

ordinary text without R code	
.....	19%
label: basic-statistics (with options)	
List of 3	
\$ fig.width : num 5	
\$ fig.height: num 3	
\$ fig.align : chr "center"	
.....	21%
ordinary text without R code	
.....	22%
label: per-base-sequence-quality (with options)	
List of 3	
\$ fig.width : num 4	
\$ fig.height: num 3.5	
\$ fig.align : chr "center"	
.....	24%
ordinary text without R code	
.....	25%
label: unnamed-chunk-5 (with options)	
List of 2	
\$ type : chr "warning"	
\$ engine: chr "block"	
.....	27%
ordinary text without R code	
.....	29%
label: unnamed-chunk-6 (with options)	
List of 2	
\$ type : chr "block"	
\$ engine: chr "block"	
.....	30%
ordinary text without R code	
.....	32%
label: per-sequence-quality-scores (with options)	
List of 3	
\$ fig.width : num 4	
\$ fig.height: num 3.5	
\$ fig.align : chr "center"	
.....	33%
ordinary text without R code	
.....	35%
label: unnamed-chunk-7 (with options)	
List of 2	
\$ type : chr "warning"	
\$ engine: chr "block"	
.....	37%

(continues on next page)

(folytatás az előző oldalról)

ordinary text without R code	
.....	38%
label: unnamed-chunk-8 (with options)	
List of 2	
\$ type : chr "block"	
\$ engine: chr "block"	
.....	40%
ordinary text without R code	
.....	41%
label: per-base-sequence-content (with options)	
List of 3	
\$ fig.width : num 4	
\$ fig.height: num 3.5	
\$ fig.align : chr "center"	
.....	43%
ordinary text without R code	
.....	44%
label: unnamed-chunk-9 (with options)	
List of 2	
\$ type : chr "notice"	
\$ engine: chr "block"	
.....	46%
ordinary text without R code	
.....	48%
label: unnamed-chunk-10 (with options)	
List of 2	
\$ type : chr "warning"	
\$ engine: chr "block"	
.....	49%
ordinary text without R code	
.....	51%
label: unnamed-chunk-11 (with options)	
List of 2	
\$ type : chr "block"	
\$ engine: chr "block"	
.....	52%
ordinary text without R code	
.....	54%
label: per-sequence-GC-content (with options)	
List of 3	
\$ fig.width : num 4	
\$ fig.height: num 3.5	
\$ fig.align : chr "center"	
.....	56%
ordinary text without R code	

(continues on next page)

(folytatás az előző oldalról)

```

|.....| 57%
label: unnamed-chunk-12 (with options)
List of 2
 $ type : chr "success"
 $ engine: chr "block"

|.....| 59%
ordinary text without R code

|.....| 60%
label: per-base-N-content (with options)
List of 3
 $ fig.width : num 4
 $ fig.height: num 3.5
 $ fig.align : chr "center"

|.....| 62%
ordinary text without R code

|.....| 63%
label: unnamed-chunk-13 (with options)
List of 2
 $ type : chr "warning"
 $ engine: chr "block"

|.....| 65%
ordinary text without R code

|.....| 67%
label: unnamed-chunk-14 (with options)
List of 2
 $ type : chr "block"
 $ engine: chr "block"

|.....| 68%
ordinary text without R code

|.....| 70%
label: sequence-length-distribution (with options)
List of 3
 $ fig.width : num 4
 $ fig.height: num 3.5
 $ fig.align : chr "center"

```

geom_path: Each group consists of only one observation. Do you need to adjust the `group aesthetic`?

```

|.....| 71%
ordinary text without R code

|.....| 73%
label: sequence-duplication-levels (with options)
List of 3
 $ fig.width : num 4
 $ fig.height: num 3.5

```

(continues on next page)

(folytatás az előző oldalról)

\$ fig.align : chr "center"	
.....	75%
ordinary text without R code	
.....	76%
label: unnamed-chunk-15 (with options)	
List of 2	
\$ type : chr "warning"	
\$ engine: chr "block"	
.....	78%
ordinary text without R code	
.....	79%
label: unnamed-chunk-16 (with options)	
List of 2	
\$ type : chr "block"	
\$ engine: chr "block"	
.....	81%
ordinary text without R code	
.....	83%
label: Overrepresented-sequences (with options)	
List of 3	
\$ fig.width : num 4	
\$ fig.height: num 3.5	
\$ fig.align : chr "center"	
.....	84%
ordinary text without R code	
.....	86%
label: unnamed-chunk-17 (with options)	
List of 2	
\$ type : chr "warning"	
\$ engine: chr "block"	
.....	87%
ordinary text without R code	
.....	89%
label: unnamed-chunk-18 (with options)	
List of 2	
\$ type : chr "block"	
\$ engine: chr "block"	
.....	90%
ordinary text without R code	
.....	92%
label: adapter-content (with options)	
List of 3	
\$ fig.width : num 4	
\$ fig.height: num 3.5	
\$ fig.align : chr "center"	

(continues on next page)

(folytatás az előző oldalról)

```
|.....| 94%
ordinary text without R code

|.....| 95%
label: unnamed-chunk-19 (with options)
List of 2
 $ type : chr "warning"
 $ engine: chr "block"

|.....| 97%
ordinary text without R code

|.....| 98%
label: unnamed-chunk-20 (with options)
List of 2
 $ type : chr "block"
 $ engine: chr "block"

|.....| 100%
ordinary text without R code
```

output file: minta_jelentes.knit.md

```
/usr/bin/pandoc +RTS -K512m -RTS minta_jelentes.utf8.md --to html4 --from_
↳markdown+autolink_bare_uris+ascii_identifiers+tex_math_single_backslash --output /
↳home/sn/gyak07/osszefoglalo.html --smart --email-obfuscation none --self-contained -
↳standalone --section-divs --table-of-contents --toc-depth 3 --variable toc_float=1_
↳--variable toc_selectors=h1,h2,h3 --variable toc_smooth_scroll=1 --variable toc_
↳print=1 --template /usr/local/lib/R/library/rmarkdown/rmd/h/default.html --no-
↳highlight --variable highlightjs=1 --variable 'theme:bootstrap' --include-in-header_
↳/tmp/Rtmp25z3BX/rmarkdown-str13c555fdd057.html --mathjax --variable 'mathjax-
↳url:https://mathjax.rstudio.com/latest/MathJax.js?config=TeX-AMS-MML_HTMLorMML'
```

Output created: osszefoglalo.html

Output file: /home/sn/gyak07/osszefoglalo.html

2.3 Gyenge minőségű nukleotidok, readok kiszűrése

trimming, filtering

```
library(seqTools)

fajlom = 'SRR1660259.fastq'

trimFastq(fajlom,
  outfile      = 'marad.fq.gz',
  discard      = 'kimarad.fq.gz',
  qualDiscard  = 10, # All reads which contain one or more phred scores <_
↳qualDiscard will be discarded.
```

(continues on next page)

(folytatás az előző oldalról)

```

fixTrimLeft    = 0, # Prefix of this size will be trimmed.
fixTrimRight   = 0, # Suffix of this size will be trimmed.
qualTrimLeft   = 30, # Prefix where all phred scores are < qualTrimLeft will be
↳trimmed.
qualTrimRight  = 30, # Suffix where all phred scores are < qualTrimRight will be
↳trimmed.
minSeqLen      = 50 # All reads where sequence length after (fixed and quality
↳based) trimming is < minSeqLen will be discarded.
)

```

```

Loading required package: zlibbioc
[trimFastq]      7.843 records written to outfile.
[trimFastq]      1.287 records written to discard.

```

2.4 Readek illesztése referencia-genomra

```

%%bash

cd gyak07

export PATH=$PATH:/home/bioinfo/edirect

efetch -db=nuccore -format=fasta -id=AF086833 > ebola1976.fa

ls -lh

```

```

total 4,6M
-rw-rw-r-- 1 sn sn 19K ápr 3 11:33 ebola1976.fa
drwxrwxr-x 2 sn sn 4,0K ápr 3 11:29 FASTQC
-rw-rw-r-- 1 sn sn 106K ápr 3 11:32 kimarad.fq.gz
-rw-rw-r-- 1 sn sn 453K ápr 3 11:32 marad.fq.gz
-rwxrwxr-x 1 sn sn 13K márc 19 12:04 minta_jelentes.Rmd
-rw-rw-r-- 1 sn sn 1,6M ápr 3 11:31 osszefoglalo.html
-rw-rw-r-- 1 sn sn 2,4M ápr 3 11:28 SRR1660259.fastq

```

```

%%bash

cd gyak07

head ebola1976.fa

```

```

>AF086833.2 Ebola virus - Mayinga, Zaire, 1976, complete genome
CGGACACACAAAAAGAAAGAAGATTTTTAGGATCTTTTGTGTGCGAATAACTATGAGGAAGATTAATAA
TTTTCTCTCATTGAAATTTATATCGGAATTTAAATTGAAATTGTTACTGTAATCACACCTGGTTTGTTT
CAGAGCCACATCACAAAGATAGAGAACAACCTAGGTCTCCGAAGGGAGCAAGGGCATCAGTGTGCTCAGT
TGAAAATCCCTTGTCACACCTAGGTCTTATCACATCACAAGTTCCACCTCAGACTCTGCAGGGTGATCC
AACAACTTAATAGAAACATTATTGTTAAAGGACAGCATTAGTTCACAGTCAAACAAGCAAGATTGAGAA
TTAACCTTGTTTTGAACCTTAGGGGATTGAAGATTCAACAACCTAAAGCTTGGGGTAAAC
ATTGGAAATAGTTAAAGACAAATTGCTCGGAATCACAAAATTCCGAGTATGGATTCTCGTCCTCAGAAA
ATCTGGATGGCGCCGAGTCTCACTGAATCTGACATGGATTACCACAAGATCTTGACAGCAGGTCTGTCCG
TTCAACAGGGGATTGTTTCGGCAAAGAGTCATCCAGTGTATCAAGTAAACAATCTTGAAGAAATTTGCCA

```

```
%bash

cd gyak07

export PATH=$PATH:/home/bioinfo/bwa

bwa index -p Ebola ebola1976.fa

ls -lh
```

```
total 4,6M
-rw-rw-r-- 1 sn sn 19K ápr 3 11:33 ebola1976.fa
-rw-rw-r-- 1 sn sn 10 ápr 3 11:34 Ebola.amb
-rw-rw-r-- 1 sn sn 86 ápr 3 11:34 Ebola.ann
-rw-rw-r-- 1 sn sn 19K ápr 3 11:34 Ebola.bwt
-rw-rw-r-- 1 sn sn 4,7K ápr 3 11:34 Ebola.pac
-rw-rw-r-- 1 sn sn 9,4K ápr 3 11:34 Ebola.sa
drwxrwxr-x 2 sn sn 4,0K ápr 3 11:29 FASTQC
-rw-rw-r-- 1 sn sn 106K ápr 3 11:32 kimarad.fq.gz
-rw-rw-r-- 1 sn sn 453K ápr 3 11:32 marad.fq.gz
-rwxrwxr-x 1 sn sn 13K márc 19 12:04 minta_jelentes.Rmd
-rw-rw-r-- 1 sn sn 1,6M ápr 3 11:31 osszefoglalo.html
-rw-rw-r-- 1 sn sn 2,4M ápr 3 11:28 SRR1660259.fastq
```

```
[bwa_index] Pack FASTA... 0.00 sec
[bwa_index] Construct BWT for the packed sequence...
[bwa_index] 0.00 seconds elapse.
[bwa_index] Update BWT... 0.00 sec
[bwa_index] Pack forward-only FASTA... 0.00 sec
[bwa_index] Construct SA from BWT and Occ... 0.00 sec
[main] Version: 0.7.17-r1188
[main] CMD: bwa index -p Ebola ebola1976.fa
[main] Real time: 0.427 sec; CPU: 0.009 sec
```

```
%bash

cd gyak07

export PATH=$PATH:/home/bioinfo/bwa

bwa mem Ebola SRR1660259.fastq > illeszt01.sam

ls -lh
```

```
total 7,1M
-rw-rw-r-- 1 sn sn 19K ápr 3 11:33 ebola1976.fa
-rw-rw-r-- 1 sn sn 10 ápr 3 11:34 Ebola.amb
-rw-rw-r-- 1 sn sn 86 ápr 3 11:34 Ebola.ann
-rw-rw-r-- 1 sn sn 19K ápr 3 11:34 Ebola.bwt
-rw-rw-r-- 1 sn sn 4,7K ápr 3 11:34 Ebola.pac
-rw-rw-r-- 1 sn sn 9,4K ápr 3 11:34 Ebola.sa
drwxrwxr-x 2 sn sn 4,0K ápr 3 11:29 FASTQC
-rw-rw-r-- 1 sn sn 2,5M ápr 3 11:34 illeszt01.sam
-rw-rw-r-- 1 sn sn 106K ápr 3 11:32 kimarad.fq.gz
-rw-rw-r-- 1 sn sn 453K ápr 3 11:32 marad.fq.gz
-rwxrwxr-x 1 sn sn 13K márc 19 12:04 minta_jelentes.Rmd
```

(continues on next page)

```
-rw-rw-r-- 1 sn sn 1,6M ápr  3 11:31 osszefoglalo.html
-rw-rw-r-- 1 sn sn 2,4M ápr  3 11:28 SRR1660259.fastq
```

Gyakorlati jegyzetek: 269

(folytatás az előző oldalról)

SRR1660259.7	16	AF086833.2	7235	60	100M	*	0	0
   								
   								
   								
SRR1660259.8	16	AF086833.2	3969	60	100M	*	0	0
   								
   								
   								
   								

Fejléc:

```
@SQ SN:AF086833.2 LN:18959
@PG ID:bwa PN:bwa VN:0.7.17-r1188 CL:bwa mem ../genomes/Ebola SRR1660257.fastq
```

Illesztési eredmény 1. sora:

[illegible]

Kötelező elemei:

[illegible]

Opcionális elemek (<http://samtools.github.io/hts-specs/SAMtags.pdf>):

```
NM:i:0          # Edit distance to the reference, including ambiguous bases but
↳excluding clipping
MD:Z:100         # String for mismatching positions, the field ought to match the
↳CIGAR string.
AS:i:100         # Alignment score generated by aligner
XS:i:0          # Reserved for end users
```

A MAPQ azt fejezi ki PHRED-pontszámmal, hogy a read milyen valószínűséggel lett hibásan illesztve.

CIGAR string értelmezéséhez:

```
M alignment match (can be a sequence match or mismatch)
I insertion to the reference
```

(continues on next page)

(folytatás az előző oldalról)

```

D   deletion from the reference
N   skipped region from the reference
S   soft clipping (clipped sequences present in SEQ)
H   hard clipping (clipped sequences NOT present in SEQ)
P   padding (silent deletion from padded reference)
=   sequence match
X   sequence mismatch

```

2.4.2 Binary Alignment Map (BAM) fájl

Bináris, tömörített SAM-fájl. A kisebb mérete mellett nagy előnye, hogy lekérdezhető, kisebb részeket kiolvashatók belőle anélkül, hogy a teljes állományt be kellene tölteni a memóriába.

```

%%bash

cd gyak07

samtools view -Sb illeszt01.sam > illeszt01.bam

ls -lh

```

```

total 7,7M
-rw-rw-r-- 1 sn sn 19K ápr 3 11:33 ebola1976.fa
-rw-rw-r-- 1 sn sn 10 ápr 3 11:34 Ebola.amb
-rw-rw-r-- 1 sn sn 86 ápr 3 11:34 Ebola.ann
-rw-rw-r-- 1 sn sn 19K ápr 3 11:34 Ebola.bwt
-rw-rw-r-- 1 sn sn 4,7K ápr 3 11:34 Ebola.pac
-rw-rw-r-- 1 sn sn 9,4K ápr 3 11:34 Ebola.sa
drwxrwxr-x 2 sn sn 4,0K ápr 3 11:29 FASTQC
-rw-rw-r-- 1 sn sn 639K ápr 3 11:39 illeszt01.bam
-rw-rw-r-- 1 sn sn 2,5M ápr 3 11:34 illeszt01.sam
-rw-rw-r-- 1 sn sn 106K ápr 3 11:32 kimarad.fq.gz
-rw-rw-r-- 1 sn sn 453K ápr 3 11:32 marad.fq.gz
-rwxrwxr-x 1 sn sn 13K márc 19 12:04 minta_jelentes.Rmd
-rw-rw-r-- 1 sn sn 1,6M ápr 3 11:31 osszefoglalo.html
-rw-rw-r-- 1 sn sn 2,4M ápr 3 11:28 SRR1660259.fastq

```

```

%%bash

cd gyak07

samtools sort illeszt01.bam > illeszt01_sorted.bam

samtools index illeszt01_sorted.bam

ls -lh

```

```

total 8,1M
-rw-rw-r-- 1 sn sn 19K ápr 3 11:33 ebola1976.fa
-rw-rw-r-- 1 sn sn 10 ápr 3 11:34 Ebola.amb
-rw-rw-r-- 1 sn sn 86 ápr 3 11:34 Ebola.ann
-rw-rw-r-- 1 sn sn 19K ápr 3 11:34 Ebola.bwt
-rw-rw-r-- 1 sn sn 4,7K ápr 3 11:34 Ebola.pac
-rw-rw-r-- 1 sn sn 9,4K ápr 3 11:34 Ebola.sa

```

(continues on next page)

(folytatás az előző oldalról)

```
drwxrwxr-x 2 sn sn 4,0K ápr 3 11:29 FASTQC
-rw-rw-r-- 1 sn sn 639K ápr 3 11:39 illeszttes01.bam
-rw-rw-r-- 1 sn sn 2,5M ápr 3 11:34 illeszttes01.sam
-rw-rw-r-- 1 sn sn 449K ápr 3 11:39 illeszttes01_sorted.bam
-rw-rw-r-- 1 sn sn 128 ápr 3 11:39 illeszttes01_sorted.bam.bai
-rw-rw-r-- 1 sn sn 106K ápr 3 11:32 kimarad.fq.gz
-rw-rw-r-- 1 sn sn 453K ápr 3 11:32 marad.fq.gz
-rwxrwxr-x 1 sn sn 13K márc 19 12:04 minta_jelentes.Rmd
-rw-rw-r-- 1 sn sn 1,6M ápr 3 11:31 osszefoglalo.html
-rw-rw-r-- 1 sn sn 2,4M ápr 3 11:28 SRR1660259.fastq
```

TERMINÁLBAN !!!

cd gyak07

samtools tview illeszttes01_sorted.bam

samtools tview illeszttes01_sorted.bam ebola1976.fa

- ?: súgó ablakot nyit
- .: forward illesztésű, a referenciával megegyező nukleotid
- ,: reverse illesztésű, a referenciával megegyező nukleotid
- ACGT: forward illesztésű, referenciától eltérő nukleotid
- acgt: reverse illesztésű, referenciától eltérő nukleotid
- *: törölt bázis

%%bash

cd gyak07

samtools view -Sb -F 4 illeszttes01.sam > illeszttes01_van.bam

samtools view -Sb -f 4 illeszttes01.sam > illeszttes01_nincs.bam

ls -lh

```
total 8,8M
-rw-rw-r-- 1 sn sn 19K ápr 3 11:33 ebola1976.fa
-rw-rw-r-- 1 sn sn 10 ápr 3 11:34 Ebola.amb
-rw-rw-r-- 1 sn sn 86 ápr 3 11:34 Ebola.ann
-rw-rw-r-- 1 sn sn 19K ápr 3 11:34 Ebola.bwt
-rw-rw-r-- 1 sn sn 4,7K ápr 3 11:34 Ebola.pac
-rw-rw-r-- 1 sn sn 9,4K ápr 3 11:34 Ebola.sa
drwxrwxr-x 2 sn sn 4,0K ápr 3 11:29 FASTQC
-rw-rw-r-- 1 sn sn 639K ápr 3 11:39 illeszttes01.bam
-rw-rw-r-- 1 sn sn 354 ápr 3 11:41 illeszttes01_nincs.bam
-rw-rw-r-- 1 sn sn 2,5M ápr 3 11:34 illeszttes01.sam
-rw-rw-r-- 1 sn sn 449K ápr 3 11:39 illeszttes01_sorted.bam
-rw-rw-r-- 1 sn sn 128 ápr 3 11:39 illeszttes01_sorted.bam.bai
-rw-rw-r-- 1 sn sn 638K ápr 3 11:41 illeszttes01_van.bam
-rw-rw-r-- 1 sn sn 106K ápr 3 11:32 kimarad.fq.gz
-rw-rw-r-- 1 sn sn 453K ápr 3 11:32 marad.fq.gz
-rwxrwxr-x 1 sn sn 13K márc 19 12:04 minta_jelentes.Rmd
```

(continues on next page)

(folytatás az előző oldalról)

```
-rw-rw-r-- 1 sn sn 1,6M ápr 3 11:31 osszefoglalo.html
-rw-rw-r-- 1 sn sn 2,4M ápr 3 11:28 SRR1660259.fastq
```

```
%%bash
```

```
cd gyak07
```

```
export PATH=$PATH:/home/bioinfo/bwa
```

```
bwa mem Ebola SRR1660259.fastq | samtools view -Sb -F 4 > illeszttes02_van.bam
```

```
ls -lh
```

```
total 9,4M
```

```
-rw-rw-r-- 1 sn sn 19K ápr 3 11:33 ebola1976.fa
-rw-rw-r-- 1 sn sn 10 ápr 3 11:34 Ebola.amb
-rw-rw-r-- 1 sn sn 86 ápr 3 11:34 Ebola.ann
-rw-rw-r-- 1 sn sn 19K ápr 3 11:34 Ebola.bwt
-rw-rw-r-- 1 sn sn 4,7K ápr 3 11:34 Ebola.pac
-rw-rw-r-- 1 sn sn 9,4K ápr 3 11:34 Ebola.sa
drwxrwxr-x 2 sn sn 4,0K ápr 3 11:29 FASTQC
-rw-rw-r-- 1 sn sn 639K ápr 3 11:39 illeszttes01.bam
-rw-rw-r-- 1 sn sn 354 ápr 3 11:41 illeszttes01_nincs.bam
-rw-rw-r-- 1 sn sn 2,5M ápr 3 11:34 illeszttes01.sam
-rw-rw-r-- 1 sn sn 449K ápr 3 11:39 illeszttes01_sorted.bam
-rw-rw-r-- 1 sn sn 128 ápr 3 11:39 illeszttes01_sorted.bam.bai
-rw-rw-r-- 1 sn sn 638K ápr 3 11:41 illeszttes01_van.bam
-rw-rw-r-- 1 sn sn 638K ápr 3 11:41 illeszttes02_van.bam
-rw-rw-r-- 1 sn sn 106K ápr 3 11:32 kimarad.fq.gz
-rw-rw-r-- 1 sn sn 453K ápr 3 11:32 marad.fq.gz
-rwxrwxr-x 1 sn sn 13K márc 19 12:04 minta_jelentes.Rmd
-rw-rw-r-- 1 sn sn 1,6M ápr 3 11:31 osszefoglalo.html
-rw-rw-r-- 1 sn sn 2,4M ápr 3 11:28 SRR1660259.fastq
```

```
[M::bwa_idx_load_from_disk] read 0 ALT contigs
[M::process] read 9130 sequences (911661 bp)...
[M::mem_process_seqs] Processed 9130 reads in 0.325 CPU sec, 0.344 real sec
[main] Version: 0.7.17-r1188
[main] CMD: bwa mem Ebola SRR1660259.fastq
[main] Real time: 0.484 sec; CPU: 0.345 sec
```

```
%%bash
```

```
cd gyak07
```

```
export PATH=$PATH:/home/bioinfo/bwa
```

```
bwa mem Ebola SRR1660259.fastq | samtools view -Sb -F 4 | samtools sort > illeszttes03_
↪van_sorted.bam
```

```
samtools index illeszttes03_van_sorted.bam
```

```
ls -lh
```

```
total 9,8M
-rw-rw-r-- 1 sn sn 19K ápr 3 11:33 ebola1976.fa
-rw-rw-r-- 1 sn sn 10 ápr 3 11:34 Ebola.amb
-rw-rw-r-- 1 sn sn 86 ápr 3 11:34 Ebola.ann
-rw-rw-r-- 1 sn sn 19K ápr 3 11:34 Ebola.bwt
-rw-rw-r-- 1 sn sn 4,7K ápr 3 11:34 Ebola.pac
-rw-rw-r-- 1 sn sn 9,4K ápr 3 11:34 Ebola.sa
drwxrwxr-x 2 sn sn 4,0K ápr 3 11:29 FASTQC
-rw-rw-r-- 1 sn sn 639K ápr 3 11:39 illeszttes01.bam
-rw-rw-r-- 1 sn sn 354 ápr 3 11:41 illeszttes01_nincs.bam
-rw-rw-r-- 1 sn sn 2,5M ápr 3 11:34 illeszttes01.sam
-rw-rw-r-- 1 sn sn 449K ápr 3 11:39 illeszttes01_sorted.bam
-rw-rw-r-- 1 sn sn 128 ápr 3 11:39 illeszttes01_sorted.bam.bai
-rw-rw-r-- 1 sn sn 638K ápr 3 11:41 illeszttes01_van.bam
-rw-rw-r-- 1 sn sn 638K ápr 3 11:41 illeszttes02_van.bam
-rw-rw-r-- 1 sn sn 448K ápr 3 11:42 illeszttes03_van_sorted.bam
-rw-rw-r-- 1 sn sn 128 ápr 3 11:42 illeszttes03_van_sorted.bam.bai
-rw-rw-r-- 1 sn sn 106K ápr 3 11:32 kimarad.fq.gz
-rw-rw-r-- 1 sn sn 453K ápr 3 11:32 marad.fq.gz
-rwxrwxr-x 1 sn sn 13K márc 19 12:04 minta_jelentes.Rmd
-rw-rw-r-- 1 sn sn 1,6M ápr 3 11:31 osszefoglalo.html
-rw-rw-r-- 1 sn sn 2,4M ápr 3 11:28 SRR1660259.fastq
```

```
[M::bwa_idx_load_from_disk] read 0 ALT contigs
[M::process] read 9130 sequences (911661 bp)...
[M::mem_process_seqs] Processed 9130 reads in 0.323 CPU sec, 0.332 real sec
[main] Version: 0.7.17-rl188
[main] CMD: bwa mem Ebola SRR1660259.fastq
[main] Real time: 0.479 sec; CPU: 0.344 sec
```

```
%%bash

cd gyak07

# samtools mpileup illeszttes03_van_sorted.bam > illeszttes03_van_sorted.pileup

samtools mpileup -f ebola1976.fa illeszttes03_van_sorted.bam > illeszttes03_van_sorted.
↪pileup

tail illeszttes03_van_sorted.pileup
```

```
AF086833.2 18924 T 4 ,,,, HHHF
AF086833.2 18925 A 4 ,,,, HFHH
AF086833.2 18926 A 4 ,,,, FDDD
AF086833.2 18927 A 4 ,,,, FDDD
AF086833.2 18928 A 4 ,,,, FDDD
AF086833.2 18929 A 4 ,,,, FDDB
AF086833.2 18930 T 4 ,,,, FDDD
AF086833.2 18931 A 4 ,,,, C@@@
AF086833.2 18932 A 4 ,,,, B@@@
AF086833.2 18933 A 4 ,,$,$,$, $@@@
```

```
[mpileup] 1 samples in 1 input files
<mpileup> Set max per-file depth to 8000
```

Oszlopok jelentése: 1. a referencia szekvencia azonosítója 2. a szekvencia nukleotidjának pozíciója (1-ről induló sorszám) 3. a referencia nukleotid az adott pozíción 4. az adott pozíciót lefedő readok száma 5. az illesztett readok

nukleotidja az adott pozícióban 6. az illesztett readek adott pozícióbeli nukleotidjának minősége

További információ: https://en.wikipedia.org/wiki/Pileup_format

```
library(Rsamtools)
```

```
bam.fajlom = 'illesztes03_van_sorted.bam'
```

```
countBam(bam.fajlom)
```

```
idxstatsBam(bam.fajlom)
```

```
# átlagos lefedettség
```

```
ref.szek.hossza = idxstatsBam(bam.fajlom)$seqlength
```

```
illesztett.nukleotidok.szama = countBam(bam.fajlom)$nucleotides
```

```
illesztett.nukleotidok.szama / ref.szek.hossza
```

```
olvasasi.parameterok = PileupParam(max_depth=50)
```

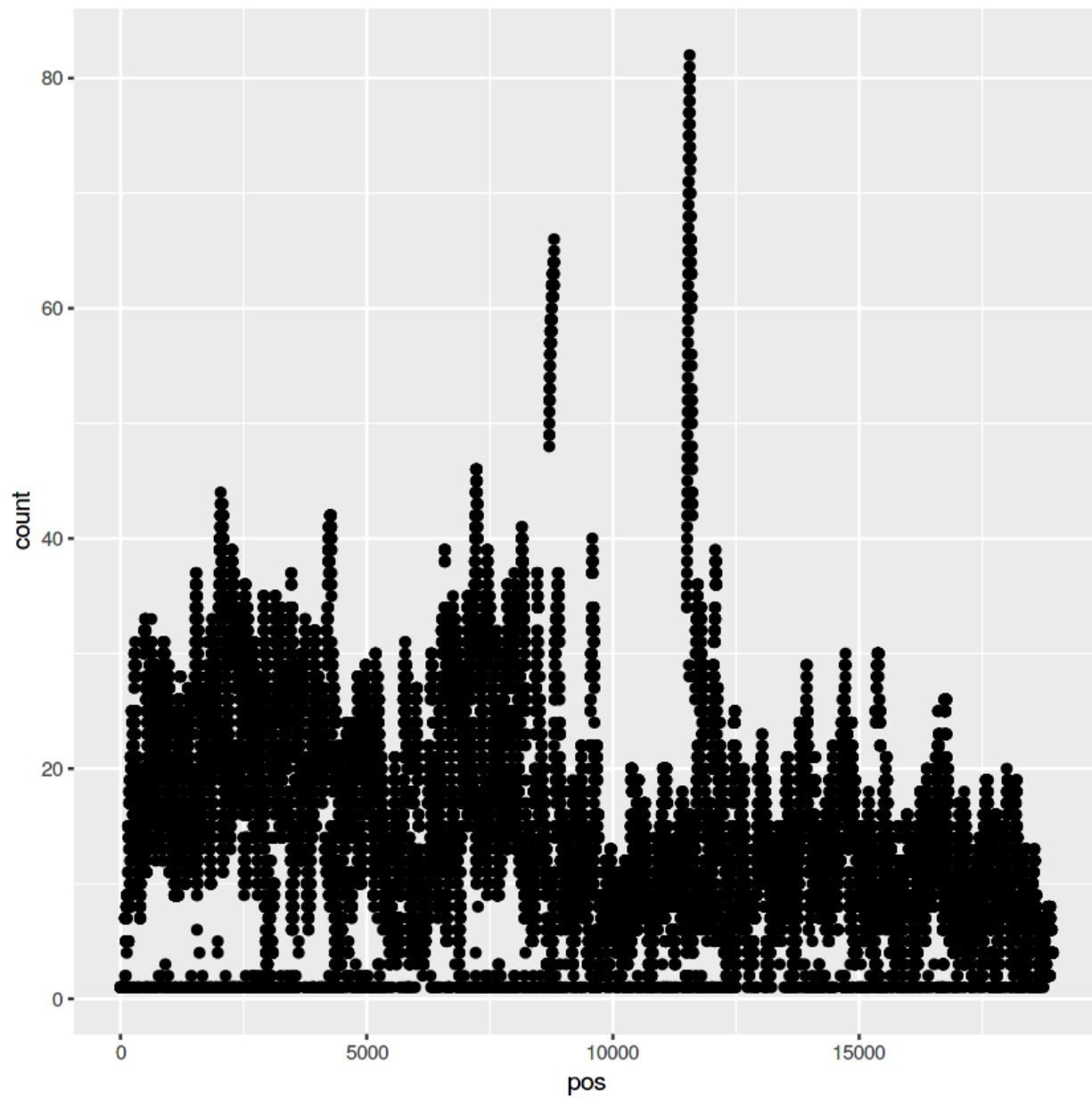
```
my.pileup = pileup(bam.fajlom, pileupParam=olvasasi.parameterok)
```

```
head(my.pileup)
```

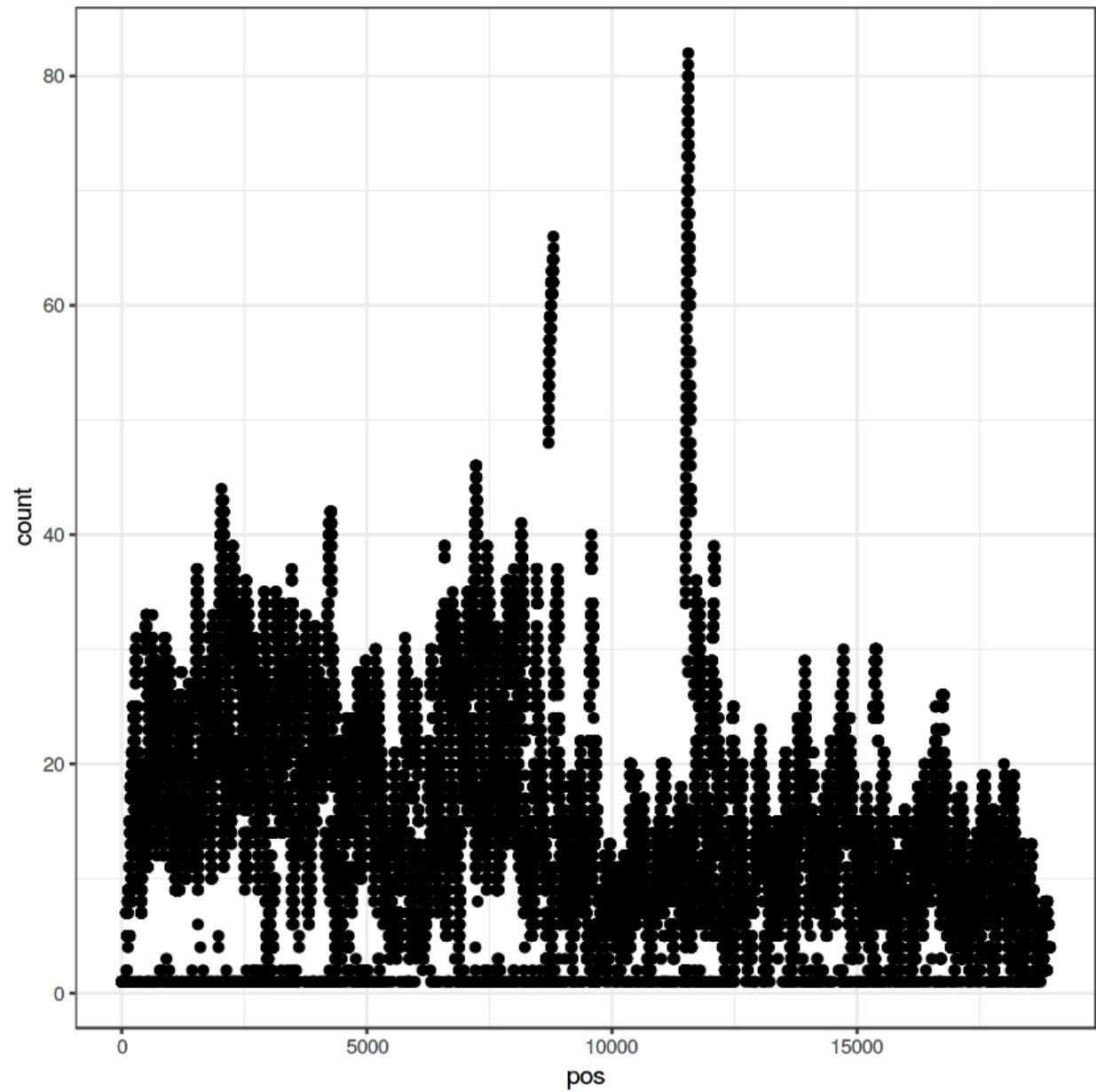
```
# http://ggplot2.org/
```

```
library(ggplot2)
```

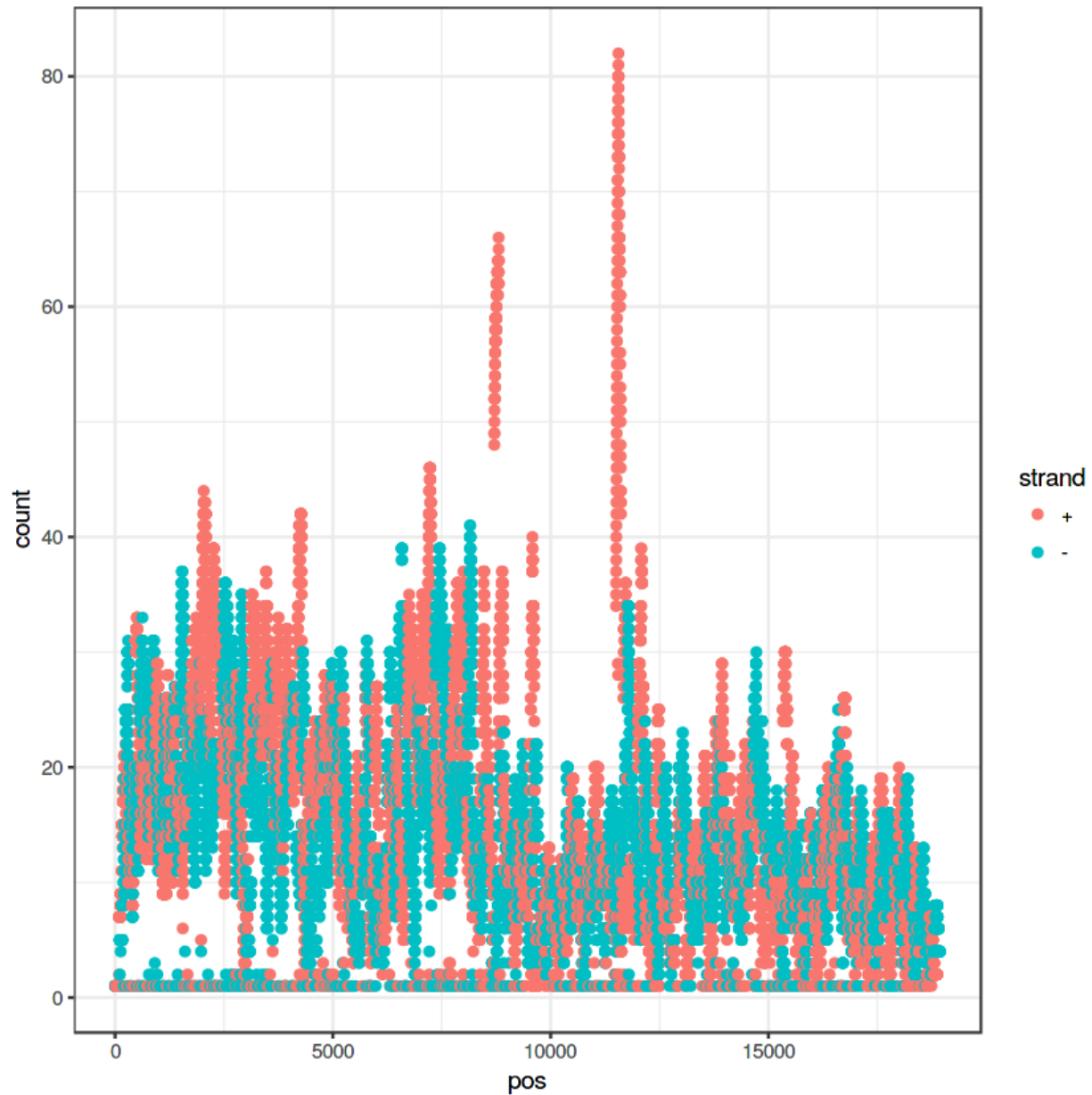
```
ggplot(data=my.pileup, aes(x=pos, y=count)) + geom_point()
```



```
ggplot(data=my.pileup, aes(x=pos, y=count)) + geom_point() + theme_bw()
```

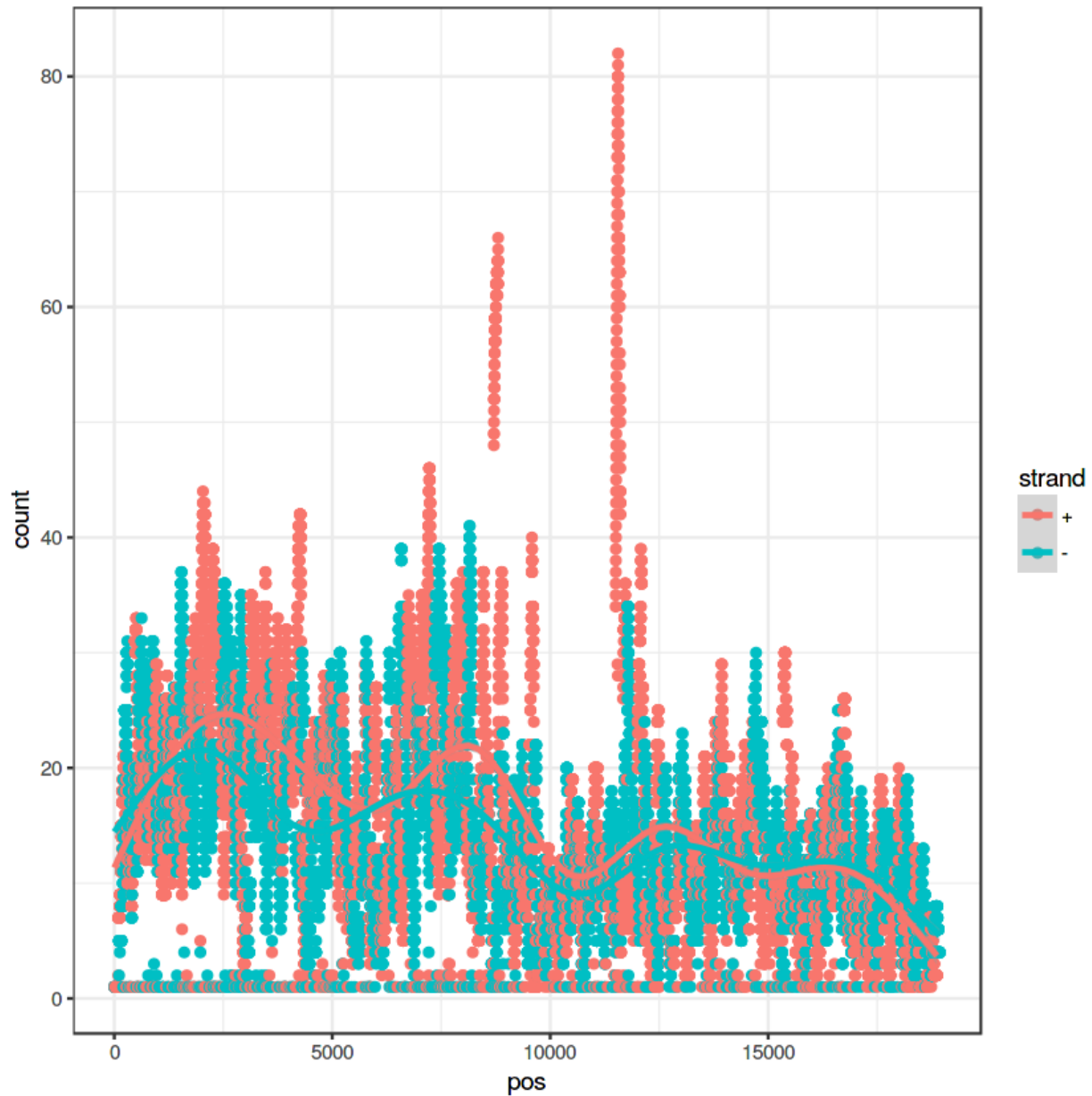


```
ggplot(data=my.pileup, aes(x=pos, y=count, color=strand)) + geom_point() + theme_bw()
```



```
ggplot(data=my.pileup, aes(x=pos, y=count, color=strand)) + geom_point() + theme_bw()
↪ + geom_smooth()
```

`geom_smooth()` using `method = 'gam'`



```
library(Gviz)

# https://www.bioconductor.org/packages/release/bioc/html/Gviz.html
# https://www.bioconductor.org/packages/release/bioc/vignettes/Gviz/inst/doc/Gviz.pdf

options(ucscChromosomeNames=FALSE)
# https://www.ncbi.nlm.nih.gov/nuccore/KM034562.1
# http://hgdownload.cse.ucsc.edu/downloads.html#ebola\_virus

illesztes.track = AlignmentsTrack(bam.fajlom, start=2000, end=3000)

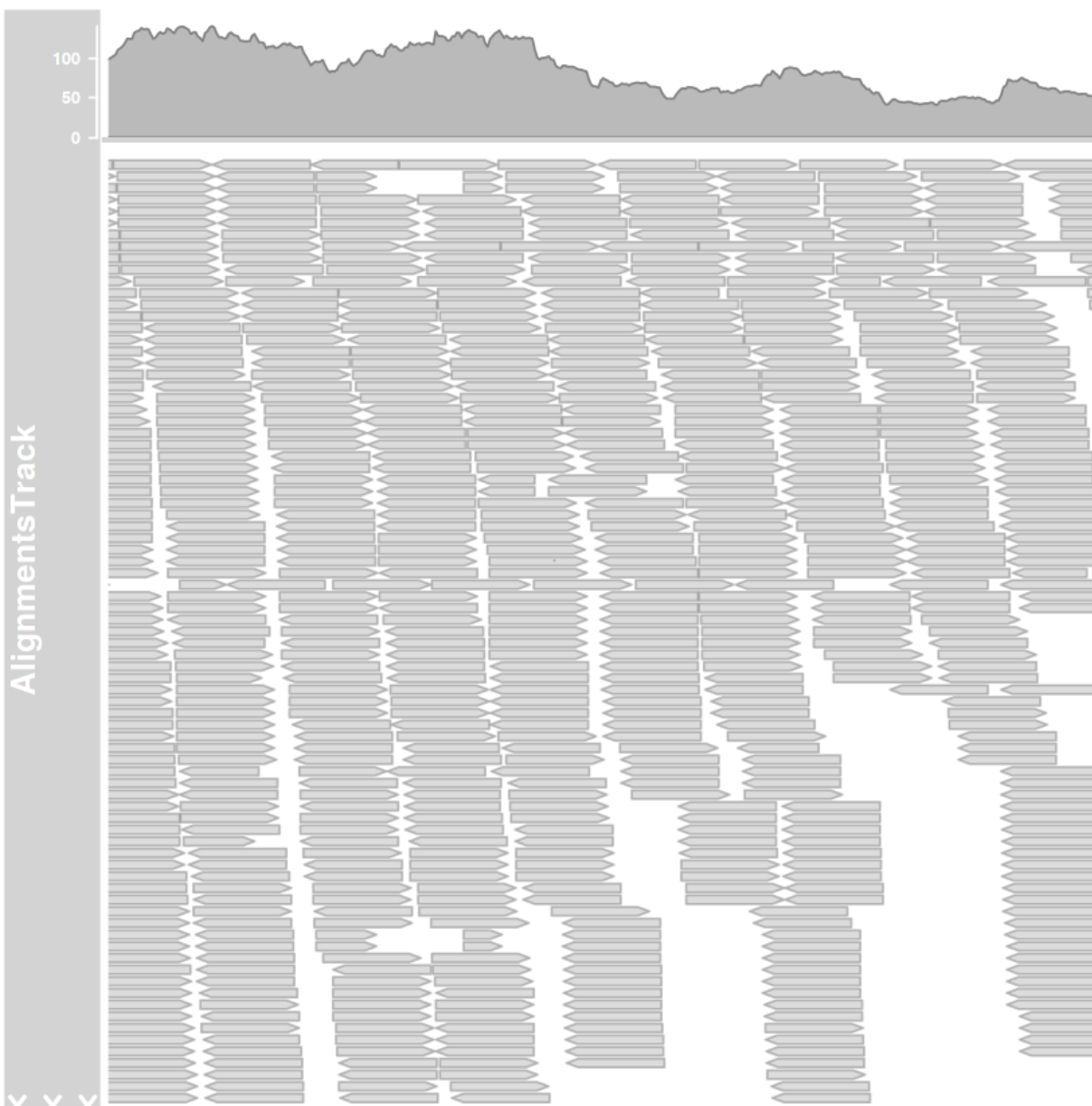
plotTracks(
  list(illesztes.track),
  type=c('coverage', 'pileup'),
```

(continues on next page)

(folytatás az előző oldalról)

```
chromosome='AF086833.2',
from=2000,
to=3000
)
```

Loading required package: grid



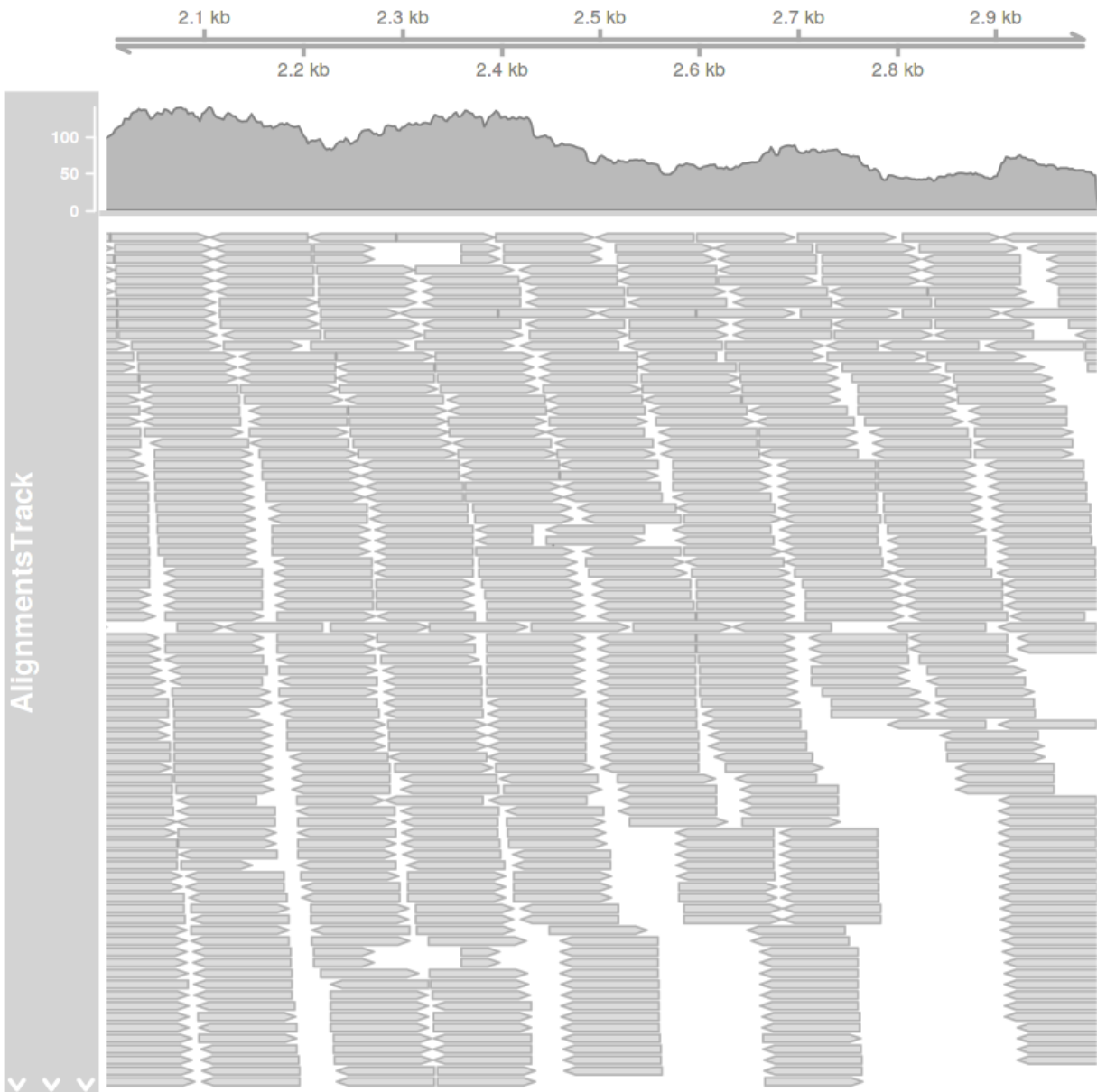
```
tengely.track = GenomeAxisTrack()

plotTracks(
  list(tengely.track, illesztes.track),
  type=c('coverage', 'pileup'),
```

(continues on next page)

(folytatás az előző oldalról)

```
chromosome='AF086833.2',
from=2000,
to=3000
)
```



```
library(rentrez)
library(seqinr)

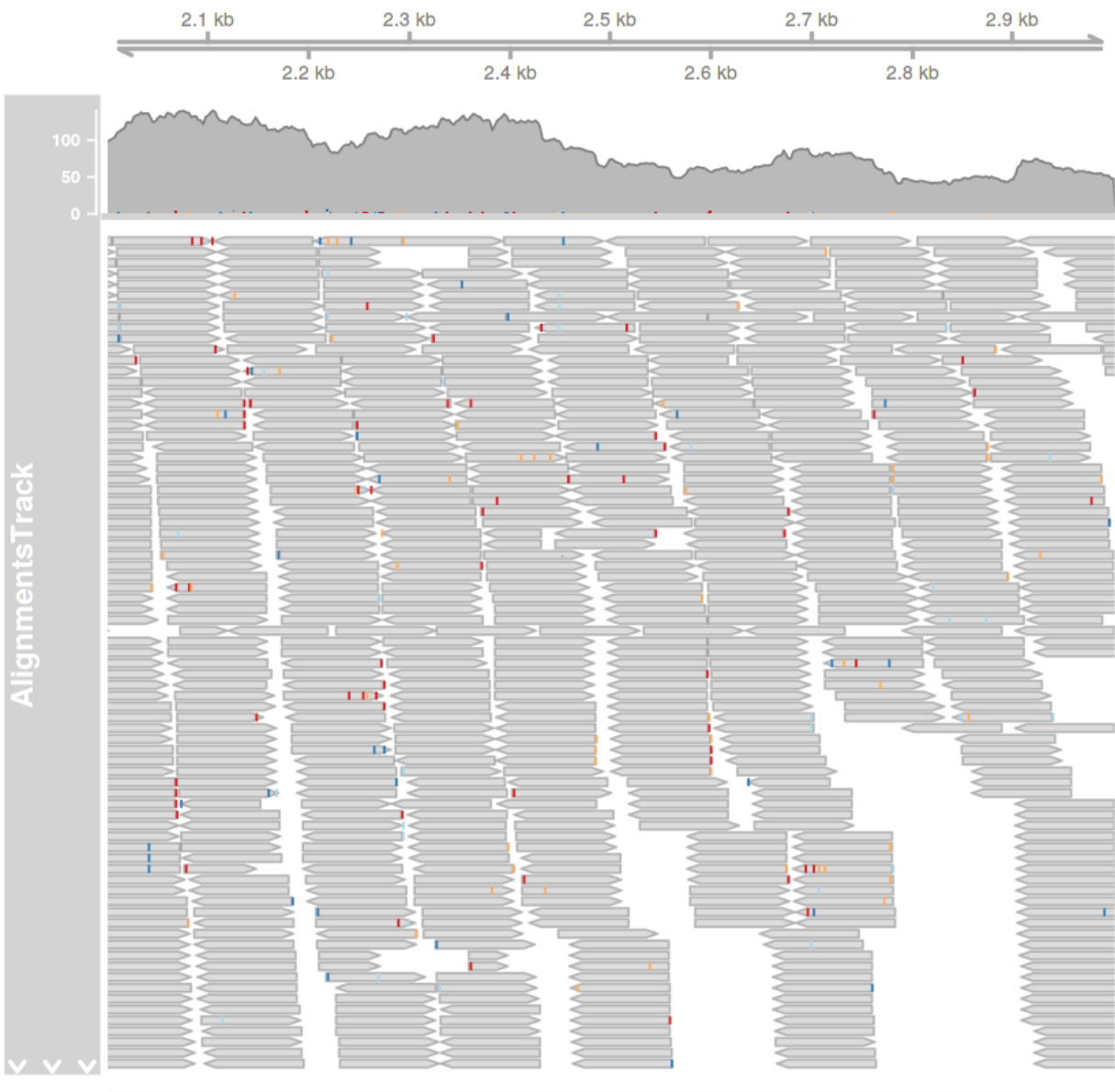
ebola = entrez_fetch(db='nuccore', id='AF086833.2', rettype='fasta')
referencia = read.fasta(textConnection(ebola), as.string=TRUE, seqonly=TRUE)
referencia.szekvencia = referencia[[1]]
referencia.szekvencia = DNASTringSet(referencia.szekvencia)
names(referencia.szekvencia) = 'AF086833.2'
```

(continues on next page)

(folytatás az előző oldalról)

```
szekvencia.track = SequenceTrack(referencia.szekvencia)

plotTracks(
  trackList = list(tengely.track, illesztes.track, szekvencia.track),
  type = c('coverage', 'pileup'),
  chromosome = 'AF086833.2',
  from = 2000,
  to = 3000
)
```



```
kiemeles = HighlightTrack(
  trackList = list(illesztes.track, szekvencia.track),
```

(continues on next page)

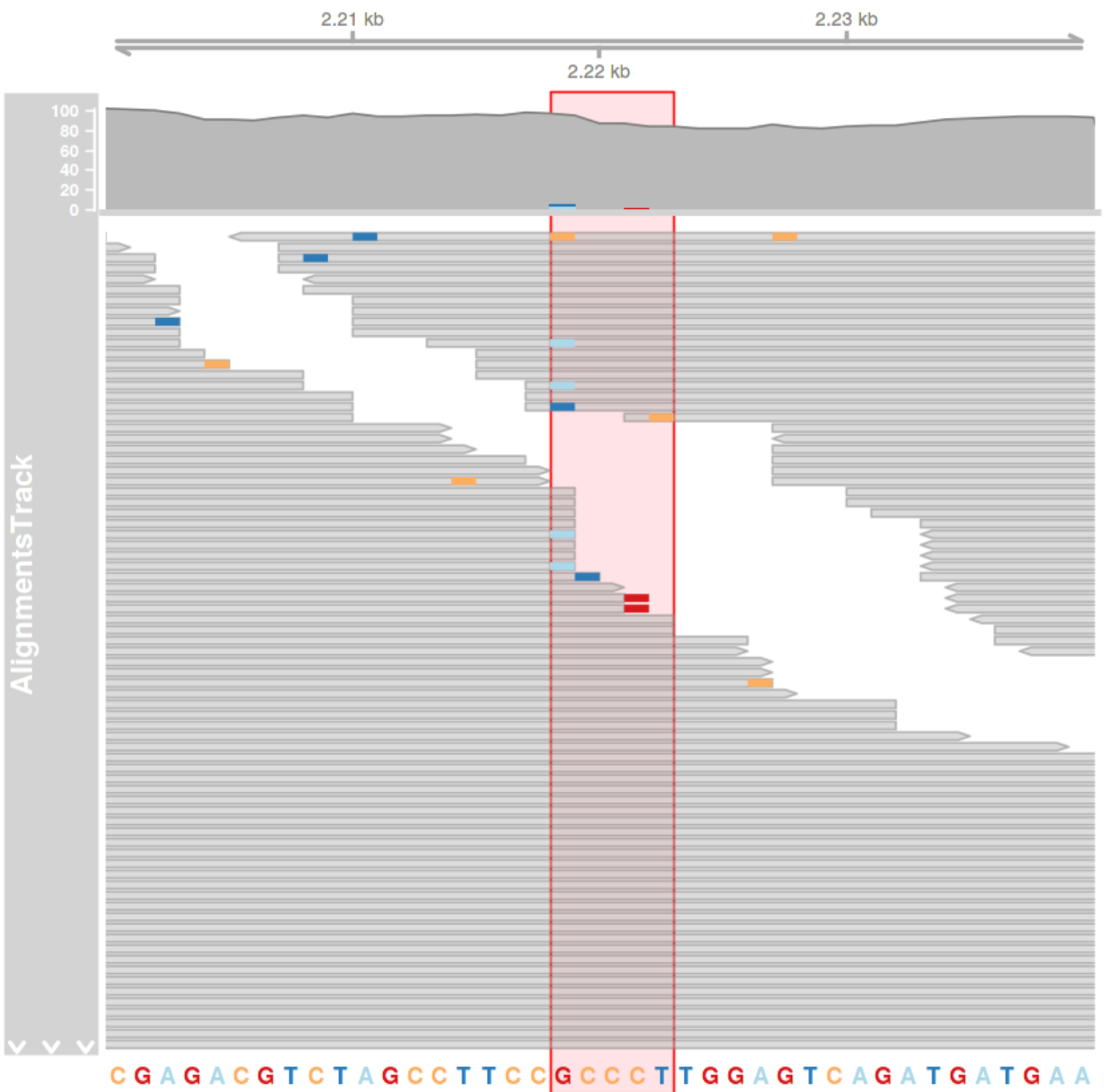
(folytatás az előző oldalról)

```

chromosome = 'AF086833.2',
start = 2218,
end = 2222
)

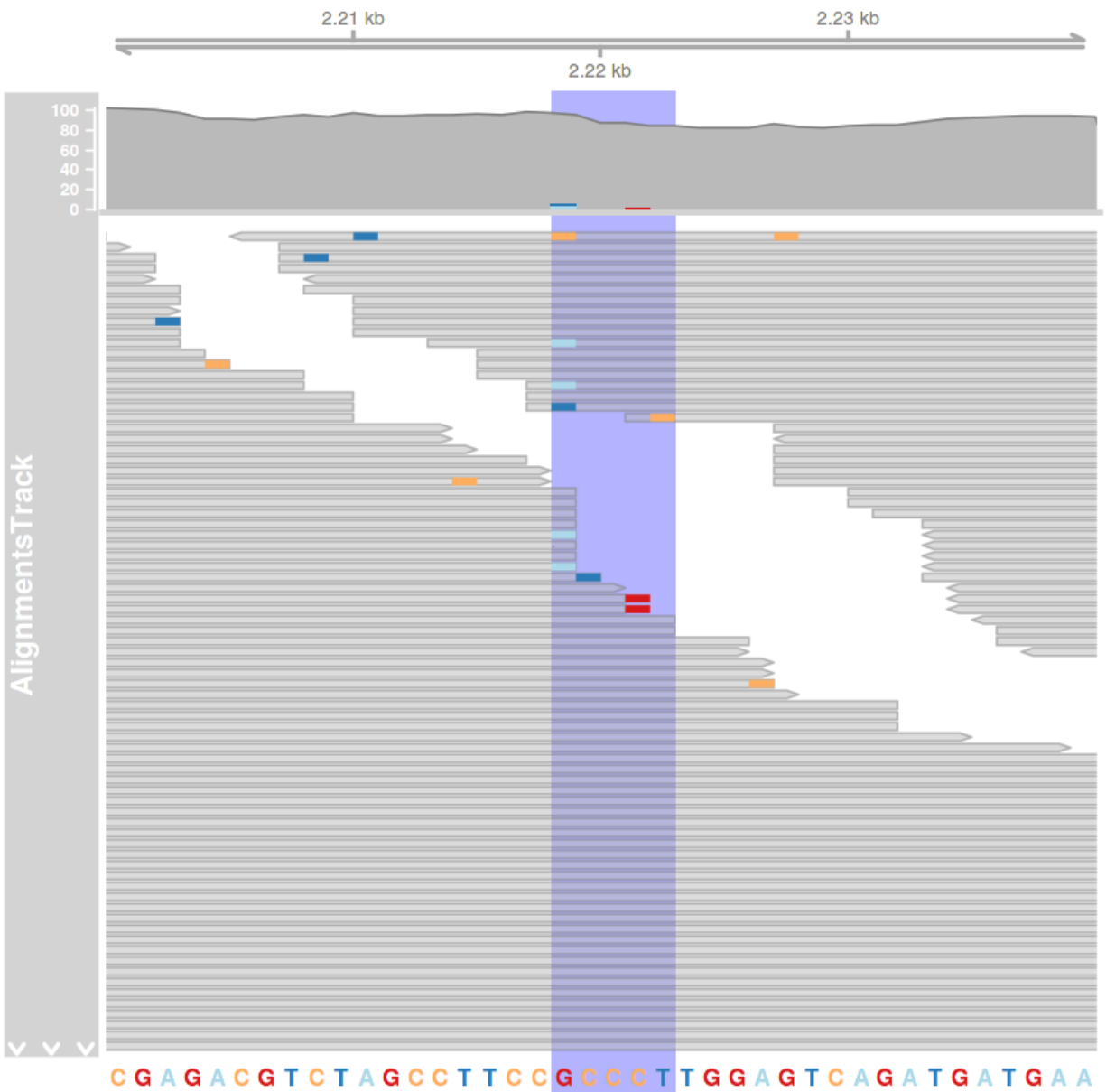
plotTracks(
  list(tengely.track, kiemeles),
  type = c('coverage', 'pileup'),
  chromosome = 'AF086833.2',
  from = 2200,
  to = 2240
)

```



```
displayPars(kiemeles)$fill = 'blue'
displayPars(kiemeles)$col = 'transparent'
displayPars(kiemeles)$alpha = 0.3

plotTracks(
  list(tengely.track, kiemeles),
  type = c('coverage', 'pileup'),
  chromosome = 'AF086833.2',
  from = 2200,
  to = 2240
)
```



3. fejezet

Variant Calling

- variant calling
- variant filtering
- variant annotation

Források: [Genome Analysis in Ebola](#), [viral-ngs](#)

Referencia szekvencia

Az eboVir3.chrom.sizes-fájl alapján a szekvencia 18957 nukleotidból áll.

```
%%bash

mkdir gyak08
cd gyak08

wget http://hgdownload-test.cse.ucsc.edu/goldenPath/currentGenomes/Ebola_virus/
↪bigZips/KM034562v1.fa.gz

gunzip KM034562v1.fa.gz

ls -lh
```

```
total 20K
-rw-rw-r-- 1 sn sn 19K Sep 30 2014 KM034562v1.fa
```

```
--2018-04-10 09:35:30-- http://hgdownload-test.cse.ucsc.edu/goldenPath/
↪currentGenomes/Ebola_virus/bigZips/KM034562v1.fa.gz
Resolving hgdownload-test.cse.ucsc.edu (hgdownload-test.cse.ucsc.edu)... 132.249.245.
↪79
Connecting to hgdownload-test.cse.ucsc.edu (hgdownload-test.cse.ucsc.edu)|132.249.245.
↪79|:80... connected.
HTTP request sent, awaiting response... 200 OK
Length: 6301 (6.2K) [application/x-gzip]
Saving to: 'KM034562v1.fa.gz'

0K ..... 100% 30.6M=0s
```

(continues on next page)

(folytatás az előző oldalról)

```
2018-04-10 09:35:30 (30.6 MB/s) - 'KM034562v1.fa.gz' saved [6301/6301]
```

Az SRA [SRR1553500](#) mintáját használjuk, amiben 101 nukleotid hosszúságú páros read-ek vannak. Ha átlagosan 30-as lefedettséget biztosító readet szeretnénk csak letölteni az SRA-ról, akkor $\frac{18957}{101} \times 30 \approx 5600$, vagyis kb. 3000 read-pár elég lehet.

Sims D, Sudbery I, Illott NE, Heger A, Ponting CP. Sequencing depth and coverage: key considerations in genomic analyses, Nat Rev Genet. 2014 Feb;15(2):121-32. doi: 10.1038/nrg3642 <<https://www.nature.com/articles/nrg3642>>‘__.

```
%%bash

export PATH=$PATH:/usr/local/ncbi/sra-tools/bin

cd gyak08

fastq-dump -X 3000 --split-files SRR1553500

ls -lh
```

```
Read 3000 spots for SRR1553500
Written 3000 spots for SRR1553500
total 1.6M
-rw-rw-r-- 1 sn sn 19K Sep 30 2014 KM034562v1.fa
-rw-rw-r-- 1 sn sn 787K Apr 10 09:47 SRR1553500_1.fastq
-rw-rw-r-- 1 sn sn 787K Apr 10 09:47 SRR1553500_2.fastq
```

```
%%bash

export PATH=$PATH:/home/bioinfo/bwa

cd gyak08

bwa index -p ebolaRef KM034562v1.fa

ls -lh
```

```
total 1.7M
-rw-rw-r-- 1 sn sn 10 Apr 10 09:48 ebolaRef.amb
-rw-rw-r-- 1 sn sn 41 Apr 10 09:48 ebolaRef.ann
-rw-rw-r-- 1 sn sn 19K Apr 10 09:48 ebolaRef.bwt
-rw-rw-r-- 1 sn sn 4.7K Apr 10 09:48 ebolaRef.pac
-rw-rw-r-- 1 sn sn 9.4K Apr 10 09:48 ebolaRef.sa
-rw-rw-r-- 1 sn sn 19K Sep 30 2014 KM034562v1.fa
-rw-rw-r-- 1 sn sn 787K Apr 10 09:47 SRR1553500_1.fastq
-rw-rw-r-- 1 sn sn 787K Apr 10 09:47 SRR1553500_2.fastq
```

```
[bwa_index] Pack FASTA... 0.00 sec
[bwa_index] Construct BWT for the packed sequence...
[bwa_index] 0.00 seconds elapse.
[bwa_index] Update BWT... 0.00 sec
[bwa_index] Pack forward-only FASTA... 0.00 sec
[bwa_index] Construct SA from BWT and Occ... 0.00 sec
```

(continues on next page)

(folytatás az előző oldalról)

```
[main] Version: 0.7.17-r1188
[main] CMD: bwa index -p ebolaRef KM034562v1.fa
[main] Real time: 0.122 sec; CPU: 0.008 sec
```

```
%bash

export PATH=$PATH:/home/bioinfo/bwa

cd gyak08

bwa mem ebolaRef SRR1553500_1.fastq SRR1553500_2.fastq | samtools view -Sb -F 4 |_
↪samtools sort > illesztes01.bam

samtools index illesztes01.bam

ls -lh
```

```
total 2.0M
-rw-rw-r-- 1 sn sn 10 Apr 10 09:48 ebolaRef.amb
-rw-rw-r-- 1 sn sn 41 Apr 10 09:48 ebolaRef.ann
-rw-rw-r-- 1 sn sn 19K Apr 10 09:48 ebolaRef.bwt
-rw-rw-r-- 1 sn sn 4.7K Apr 10 09:48 ebolaRef.pac
-rw-rw-r-- 1 sn sn 9.4K Apr 10 09:48 ebolaRef.sa
-rw-rw-r-- 1 sn sn 389K Apr 10 09:49 illesztes01.bam
-rw-rw-r-- 1 sn sn 152 Apr 10 09:49 illesztes01.bam.bai
-rw-rw-r-- 1 sn sn 19K Sep 30 2014 KM034562v1.fa
-rw-rw-r-- 1 sn sn 787K Apr 10 09:47 SRR1553500_1.fastq
-rw-rw-r-- 1 sn sn 787K Apr 10 09:47 SRR1553500_2.fastq
```

```
[M::bwa_idx_load_from_disk] read 0 ALT contigs
[M::process] read 6000 sequences (606000 bp)...
[M::mem_pestat] # candidate unique pairs for (FF, FR, RF, RR): (234, 2511, 16, 206)
[M::mem_pestat] analyzing insert size distribution for orientation FF...
[M::mem_pestat] (25, 50, 75) percentile: (110, 187, 267)
[M::mem_pestat] low and high boundaries for computing mean and std.dev: (1, 581)
[M::mem_pestat] mean and std.dev: (194.67, 117.50)
[M::mem_pestat] low and high boundaries for proper pairs: (1, 738)
[M::mem_pestat] analyzing insert size distribution for orientation FR...
[M::mem_pestat] (25, 50, 75) percentile: (155, 260, 409)
[M::mem_pestat] low and high boundaries for computing mean and std.dev: (1, 917)
[M::mem_pestat] mean and std.dev: (294.87, 177.15)
[M::mem_pestat] low and high boundaries for proper pairs: (1, 1171)
[M::mem_pestat] analyzing insert size distribution for orientation RF...
[M::mem_pestat] (25, 50, 75) percentile: (65, 107, 189)
[M::mem_pestat] low and high boundaries for computing mean and std.dev: (1, 437)
[M::mem_pestat] mean and std.dev: (114.27, 85.53)
[M::mem_pestat] low and high boundaries for proper pairs: (1, 561)
[M::mem_pestat] analyzing insert size distribution for orientation RR...
[M::mem_pestat] (25, 50, 75) percentile: (103, 190, 307)
[M::mem_pestat] low and high boundaries for computing mean and std.dev: (1, 715)
[M::mem_pestat] mean and std.dev: (213.14, 137.62)
[M::mem_pestat] low and high boundaries for proper pairs: (1, 919)
[M::mem_pestat] skip orientation RF
[M::mem_process_seqs] Processed 6000 reads in 0.374 CPU sec, 0.373 real sec
[main] Version: 0.7.17-r1188
```

(continues on next page)

(folytatás az előző oldalról)

```
[main] CMD: bwa mem ebolaRef SRR1553500_1.fastq SRR1553500_2.fastq
[main] Real time: 0.446 sec; CPU: 0.384 sec
```

```
%%bash

cd gyak08

samtools faidx KM034562v1.fa

freebayes -f KM034562v1.fa illeszt01.bam > sznipek_freebayes.vcf

ls -lh
```

```
total 2.1M
-rw-rw-r-- 1 sn sn 10 Apr 10 09:48 ebolaRef.amb
-rw-rw-r-- 1 sn sn 41 Apr 10 09:48 ebolaRef.ann
-rw-rw-r-- 1 sn sn 19K Apr 10 09:48 ebolaRef.bwt
-rw-rw-r-- 1 sn sn 4.7K Apr 10 09:48 ebolaRef.pac
-rw-rw-r-- 1 sn sn 9.4K Apr 10 09:48 ebolaRef.sa
-rw-rw-r-- 1 sn sn 389K Apr 10 09:49 illeszt01.bam
-rw-rw-r-- 1 sn sn 152 Apr 10 09:49 illeszt01.bam.bai
-rw-rw-r-- 1 sn sn 19K Sep 30 2014 KM034562v1.fa
-rw-rw-r-- 1 sn sn 26 Apr 10 10:02 KM034562v1.fa.fai
-rw-rw-r-- 1 sn sn 787K Apr 10 09:47 SRR1553500_1.fastq
-rw-rw-r-- 1 sn sn 787K Apr 10 09:47 SRR1553500_2.fastq
-rw-rw-r-- 1 sn sn 24K Apr 10 10:02 sznipek_freebayes.vcf
```

```
## TERMINÁL ##

cd gyak08

less sznipek_freebayes.vcf
```

3.1 Variant Call Format (VCF) fájl

What is a VCF and how should I interpret it?

VCFv4.2

3.1.1 Header

```
##fileformat=VCFv4.2
##fileDate=20180405
##source=freeBayes v1.1.0-60-gc15b070
##reference=KM034562v1.fa
##contig=<ID=KM034562v1,length=18957>
##phasing=none
##commandline="freebayes -f KM034562v1.fa illeszt01.bam"
##INFO=<ID=NS,Number=1,Type=Integer,Description="Number of samples with data">
##INFO=<ID=DP,Number=1,Type=Integer,Description="Total read depth at the locus">
##INFO=<ID=DPB,Number=1,Type=Float,Description="Total read depth per bp at the locus;↳
↳bases in reads overlapping / bases in haplotype">
```

(continues on next page)

(folytatás az előző oldalról)

```

##INFO=<ID=AC,Number=A,Type=Integer,Description="Total number of alternate alleles in
↳called genotypes">
##INFO=<ID=AN,Number=1,Type=Integer,Description="Total number of alleles in called
↳genotypes">
##INFO=<ID=AF,Number=A,Type=Float,Description="Estimated allele frequency in the
↳range (0,1]">
##INFO=<ID=RO,Number=1,Type=Integer,Description="Count of full observations of the
↳reference haplotype.">
##INFO=<ID=AO,Number=A,Type=Integer,Description="Count of full observations of this
↳alternate haplotype.">
##INFO=<ID=PRO,Number=1,Type=Float,Description="Reference allele observation count,
↳with partial observations recorded fractionally">
##INFO=<ID=PAO,Number=A,Type=Float,Description="Alternate allele observations, with
↳partial observations recorded fractionally">
##INFO=<ID=QR,Number=1,Type=Integer,Description="Reference allele quality sum in phred
↳">
##INFO=<ID=QA,Number=A,Type=Integer,Description="Alternate allele quality sum in phred
↳">
##INFO=<ID=PQR,Number=1,Type=Float,Description="Reference allele quality sum in phred
↳for partial observations">
##INFO=<ID=PQA,Number=A,Type=Float,Description="Alternate allele quality sum in phred
↳for partial observations">
##INFO=<ID=SRF,Number=1,Type=Integer,Description="Number of reference observations on
↳the forward strand">
##INFO=<ID=SRR,Number=1,Type=Integer,Description="Number of reference observations on
↳the reverse strand">
##INFO=<ID=SAF,Number=A,Type=Integer,Description="Number of alternate observations on
↳the forward strand">
##INFO=<ID=SAR,Number=A,Type=Integer,Description="Number of alternate observations on
↳the reverse strand">
##INFO=<ID=SRP,Number=1,Type=Float,Description="Strand balance probability for the
↳reference allele: Phred-scaled upper-bounds estimate of the probability of
↳observing the deviation between SRF and SRR given  $E(SRF/SRR) \sim 0.5$ , derived using
↳Hoeffding's inequality">
##INFO=<ID=SAP,Number=A,Type=Float,Description="Strand balance probability for the
↳alternate allele: Phred-scaled upper-bounds estimate of the probability of
↳observing the deviation between SAF and SAR given  $E(SAF/SAR) \sim 0.5$ , derived using
↳Hoeffding's inequality">
##INFO=<ID=AB,Number=A,Type=Float,Description="Allele balance at heterozygous sites:
↳a number between 0 and 1 representing the ratio of reads showing the reference
↳allele to all reads, considering only reads from individuals called as heterozygous
↳">
##INFO=<ID=ABP,Number=A,Type=Float,Description="Allele balance probability at
↳heterozygous sites: Phred-scaled upper-bounds estimate of the probability of
↳observing the deviation between ABR and ABA given  $E(ABR/ABA) \sim 0.5$ , derived using
↳Hoeffding's inequality">
##INFO=<ID=RUN,Number=A,Type=Integer,Description="Run length: the number of
↳consecutive repeats of the alternate allele in the reference genome">
##INFO=<ID=RPP,Number=A,Type=Float,Description="Read Placement Probability: Phred-
↳scaled upper-bounds estimate of the probability of observing the deviation between
↳RPL and RPR given  $E(RPL/RPR) \sim 0.5$ , derived using Hoeffding's inequality">
##INFO=<ID=RPPR,Number=1,Type=Float,Description="Read Placement Probability for
↳reference observations: Phred-scaled upper-bounds estimate of the probability of
↳observing the deviation between RPL and RPR given  $E(RPL/RPR) \sim 0.5$ , derived using
↳Hoeffding's inequality">
##INFO=<ID=RPL,Number=A,Type=Float,Description="Reads Placed Left: number of reads
↳supporting the alternate balanced to the left (5') of the alternate allele">

```

(continues on next page)

```
##INFO=<ID=RPR,Number=A,Type=Float,Description="Reads Placed Right: number of reads_
↳supporting the alternate balanced to the right (3') of the alternate allele">
##INFO=<ID=EPP,Number=A,Type=Float,Description="End Placement Probability: Phred-
↳scaled upper-bounds estimate of the probability of observing the deviation between_
↳EL and ER given  $E(EL/ER) \sim 0.5$ , derived using Hoeffding's inequality">
##INFO=<ID=EPPR,Number=1,Type=Float,Description="End Placement Probability for_
↳reference observations: Phred-scaled upper-bounds estimate of the probability of_
↳observing the deviation between EL and ER given  $E(EL/ER) \sim 0.5$ , derived using_
↳Hoeffding's inequality">
##INFO=<ID=DPRA,Number=A,Type=Float,Description="Alternate allele depth ratio. Ratio_
↳between depth in samples with each called alternate allele and those without.">
##INFO=<ID=ODDS,Number=1,Type=Float,Description="The log odds ratio of the best_
↳genotype combination to the second-best.">
##INFO=<ID=GTI,Number=1,Type=Integer,Description="Number of genotyping iterations_
↳required to reach convergence or bailout.">
##INFO=<ID=TYPE,Number=A,Type=String,Description="The type of allele, either snp, mnp,
↳ins, del, or complex.">
##INFO=<ID=CIGAR,Number=A,Type=String,Description="The extended CIGAR representation_
↳of each alternate allele, with the exception that '=' is replaced by 'M' to ease_
↳VCF parsing. Note that INDEL alleles do not have the first matched base (which is_
↳provided by default, per the spec) referred to by the CIGAR.">
##INFO=<ID=NUMALT,Number=1,Type=Integer,Description="Number of unique non-reference_
↳alleles in called genotypes at this position.">
##INFO=<ID=MEANALT,Number=A,Type=Float,Description="Mean number of unique non-
↳reference allele observations per sample with the corresponding alternate alleles.">
##INFO=<ID=LEN,Number=A,Type=Integer,Description="allele length">
##INFO=<ID=MQM,Number=A,Type=Float,Description="Mean mapping quality of observed_
↳alternate alleles">
##INFO=<ID=MQMR,Number=1,Type=Float,Description="Mean mapping quality of observed_
↳reference alleles">
##INFO=<ID=PAIRED,Number=A,Type=Float,Description="Proportion of observed alternate_
↳alleles which are supported by properly paired read fragments">
##INFO=<ID=PAIREDR,Number=1,Type=Float,Description="Proportion of observed reference_
↳alleles which are supported by properly paired read fragments">
##INFO=<ID=MIN_DP,Number=1,Type=Integer,Description="Minimum depth in gVCF output_
↳block.">
##INFO=<ID=END,Number=1,Type=Integer,Description="Last position (inclusive) in gVCF_
↳output record.">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=GQ,Number=1,Type=Float,Description="Genotype Quality, the Phred-scaled_
↳marginal (or unconditional) probability of the called genotype">
##FORMAT=<ID=GL,Number=G,Type=Float,Description="Genotype Likelihood, log10-scaled_
↳likelihoods of the data given the called genotype for each possible genotype_
↳generated from the reference and alternate alleles given the sample ploidy">
##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Read Depth">
##FORMAT=<ID=AD,Number=R,Type=Integer,Description="Number of observation for each_
↳allele">
##FORMAT=<ID=RO,Number=1,Type=Integer,Description="Reference allele observation count_
↳">
##FORMAT=<ID=QR,Number=1,Type=Integer,Description="Sum of quality of the reference_
↳observations">
##FORMAT=<ID=AO,Number=A,Type=Integer,Description="Alternate allele observation count_
↳">
##FORMAT=<ID=QA,Number=A,Type=Integer,Description="Sum of quality of the alternate_
↳observations">
##FORMAT=<ID=MIN_DP,Number=1,Type=Integer,Description="Minimum depth in gVCF output_
↳block.">
```

3.1.2 Records

```
#CHROM POS ID REF ALT QUAL FILTER INFO FORMAT unknown
KM034562v1 800 . C T 874.877 . AB=0;ABP=0;AC=2;AF=1;AN=2;AO=28;CIGAR=1X;
→DP=28;DPB=28;DPRA=0;EPP=3.32051;EPPR=0;GTI=0;LEN=1;MEANALT=1;MQM=60;MQMR=0;NS=1;
→NUMALT=1;ODDS=43.4214;PAIRED=1;PAIREDR=0;PAO=0;PQA=0;PQR=0;PRO=0;QA=1001;QR=0;RO=0;
→RPL=12;RPP=4.25114;RPPR=0;RPR=16;RUN=1;SAF=11;SAP=5.80219;SAR=17;SRF=0;SRP=0;SRR=0;
→TYPE=snp GT:DP:AD:RO:QR:AO:QA:GL 1/1:28:0,28:0:0:28:1001:-90.3858,-8.42884,0
KM034562v1 8928 . A C 909.973 . AB=0;ABP=0;AC=2;AF=1;AN=2;AO=28;CIGAR=1X;
→DP=28;DPB=28;DPRA=0;EPP=7.97367;EPPR=0;GTI=0;LEN=1;MEANALT=1;MQM=60;MQMR=0;NS=1;
→NUMALT=1;ODDS=43.4214;PAIRED=0.964286;PAIREDR=0;PAO=0;PQA=0;PQR=0;PRO=0;QA=1044;
→QR=0;RO=0;RPL=13;RPP=3.32051;RPPR=0;RPR=15;RUN=1;SAF=17;SAP=5.80219;SAR=11;SRF=0;
→SRP=0;SRR=0;TYPE=snp GT:DP:AD:RO:QR:AO:QA:GL 1/1:28:0,28:0:0:28:1044:-94.2605,-8.
→42884,0
KM034562v1 10218 . G A 865.247 . AB=0;ABP=0;AC=2;AF=1;AN=2;AO=28;CIGAR=1X;
→DP=28;DPB=28;DPRA=0;EPP=3.32051;EPPR=0;GTI=0;LEN=1;MEANALT=1;MQM=60;MQMR=0;NS=1;
→NUMALT=1;ODDS=43.4214;PAIRED=1;PAIREDR=0;PAO=0;PQA=0;PQR=0;PRO=0;QA=989;QR=0;RO=0;
→RPL=14;RPP=3.0103;RPPR=0;RPR=14;RUN=1;SAF=17;SAP=5.80219;SAR=11;SRF=0;SRP=0;SRR=0;
→TYPE=snp GT:DP:AD:RO:QR:AO:QA:GL 1/1:28:0,28:0:0:28:989:-89.3026,-8.42884,0
KM034562v1 11811 . T C 919.423 . AB=0;ABP=0;AC=2;AF=1;AN=2;AO=27;CIGAR=1X;
→DP=27;DPB=27;DPRA=0;EPP=3.09072;EPPR=0;GTI=0;LEN=1;MEANALT=1;MQM=60;MQMR=0;NS=1;
→NUMALT=1;ODDS=42.0351;PAIRED=1;PAIREDR=0;PAO=0;PQA=0;PQR=0;PRO=0;QA=1050;QR=0;RO=0;
→RPL=14;RPP=3.09072;RPPR=0;RPR=13;RUN=1;SAF=17;SAP=6.95112;SAR=10;SRF=0;SRP=0;SRR=0;
→TYPE=snp GT:DP:AD:RO:QR:AO:QA:GL 1/1:27:0,27:0:0:27:1050:-94.795,-8.12781,0
KM034562v1 15599 . G A 1672.84 . AB=0;ABP=0;AC=2;AF=1;AN=2;AO=50;CIGAR=1X;
→DP=50;DPB=50;DPRA=0;EPP=3.70517;EPPR=0;GTI=0;LEN=1;MEANALT=1;MQM=60;MQMR=0;NS=1;
→NUMALT=1;ODDS=73.9199;PAIRED=0.98;PAIREDR=0;PAO=0;PQA=0;PQR=0;PRO=0;QA=1891;QR=0;
→RO=0;RPL=28;RPP=4.57376;RPPR=0;RPR=22;RUN=1;SAF=27;SAP=3.70517;SAR=23;SRF=0;SRP=0;
→SRR=0;TYPE=snp GT:DP:AD:RO:QR:AO:QA:GL 1/1:50:0,50:0:0:50:1891:-170.423,-15.0515,0
KM034562v1 15963 . G A 930.435 . AB=0;ABP=0;AC=2;AF=1;AN=2;AO=27;CIGAR=1X;
→DP=27;DPB=27;DPRA=0;EPP=6.95112;EPPR=0;GTI=0;LEN=1;MEANALT=1;MQM=60;MQMR=0;NS=1;
→NUMALT=1;ODDS=42.0351;PAIRED=1;PAIREDR=0;PAO=0;PQA=0;PQR=0;PRO=0;QA=1070;QR=0;RO=0;
→RPL=18;RPP=9.52472;RPPR=0;RPR=9;RUN=1;SAF=15;SAP=3.73412;SAR=12;SRF=0;SRP=0;SRR=0;
→TYPE=snp GT:DP:AD:RO:QR:AO:QA:GL 1/1:27:0,27:0:0:27:1070:-96.5897,-8.12781,0
KM034562v1 17142 . T C 1423.48 . AB=0;ABP=0;AC=2;AF=1;AN=2;AO=42;CIGAR=1X;
→DP=42;DPB=42;DPRA=0;EPP=6.31921;EPPR=0;GTI=0;LEN=1;MEANALT=1;MQM=60;MQMR=0;NS=1;
→NUMALT=1;ODDS=62.8295;PAIRED=1;PAIREDR=0;PAO=0;PQA=0;PQR=0;PRO=0;QA=1614;QR=0;RO=0;
→RPL=22;RPP=3.21711;RPPR=0;RPR=20;RUN=1;SAF=19;SAP=3.83753;SAR=23;SRF=0;SRP=0;SRR=0;
→TYPE=snp GT:DP:AD:RO:QR:AO:QA:GL 1/1:42:0,42:0:0:42:1614:-145.511,-12.6433,0
```

3.1.3 Az első record:

Mező	Érték	Mező
CHROM	KM034562v1	CHROM
POS	800	POS
ID	.	ID
REF	C	REF
ALT	T	ALT
QUAL	874.877	QUAL
FILTER	.	FILTER
INFO	AB=0;ABP=0;AC=2;AF=1;AN=2;AO=28;CIGAR=1X;DP=28;DPB=28;DPRA=0;EPP=3.32051;EPPR=0;GTI=0;LEN=1;MEANALT=1;MQM=60;MQMR=0;NS=1;NUMALT=1;ODDS=43.4214;PAIRED=1;PAIREDR=0;PAO=0;PQA=0;PQR=0;PRO=0;QA=1001;QR=0;RO=0;RPL=12;RPP=4.25114;RPPR=0;RPR=16;RUN=1;SAF=11;SAP=5.80219;SAR=17;SRF=0;SRP=0;SRR=0;TYPE=snp	INFO
FORMAT	GT:DP:AD:RO:QR:AO:QA:GL	FORMAT
unknown	1/1:28:0,28:0:0:28:1001:-90.3858,-8.42884,0	unknown

INFO:

Mező	Érték
AB	0
ABP	0
AC	2
AF	1
AN	2
AO	28
CIGAR	1X
DP	28
DPB	28
DPRA	0
EPP	3.32051
EPPR	0
GTI	0
LEN	1
MEANALT	1
MQM	60
MQMR	0
NS	1
NUMALT	1
ODDS	43.4214
PAIRED	1

A következő oldalon folytatódik

1 – folytatás az előző oldalról

Mező	Érték
PAIREDR	0
PAO	0
PQA	0
PQR	0
PRO	0
QA	1001
QR	0
RO	0
RPL	12
RPP	4.25114
RPPR	0
RPR	16
RUN	1
SAF	11
SAP	5.80219
SAR	17
SRF	0
SRP	0
SRR	0
TYPE	snp

FORMAT:

FORMAT	unknown
GT	1/1
DP	28
AD	0,28
RO	0
QR	0
AO	28
QA	1001
GL	-90.3858,-8.42884,0

3.2 Genomic VCF (gVCF) fájl

<https://software.broadinstitute.org/gatk/documentation/article.php?id=4017>

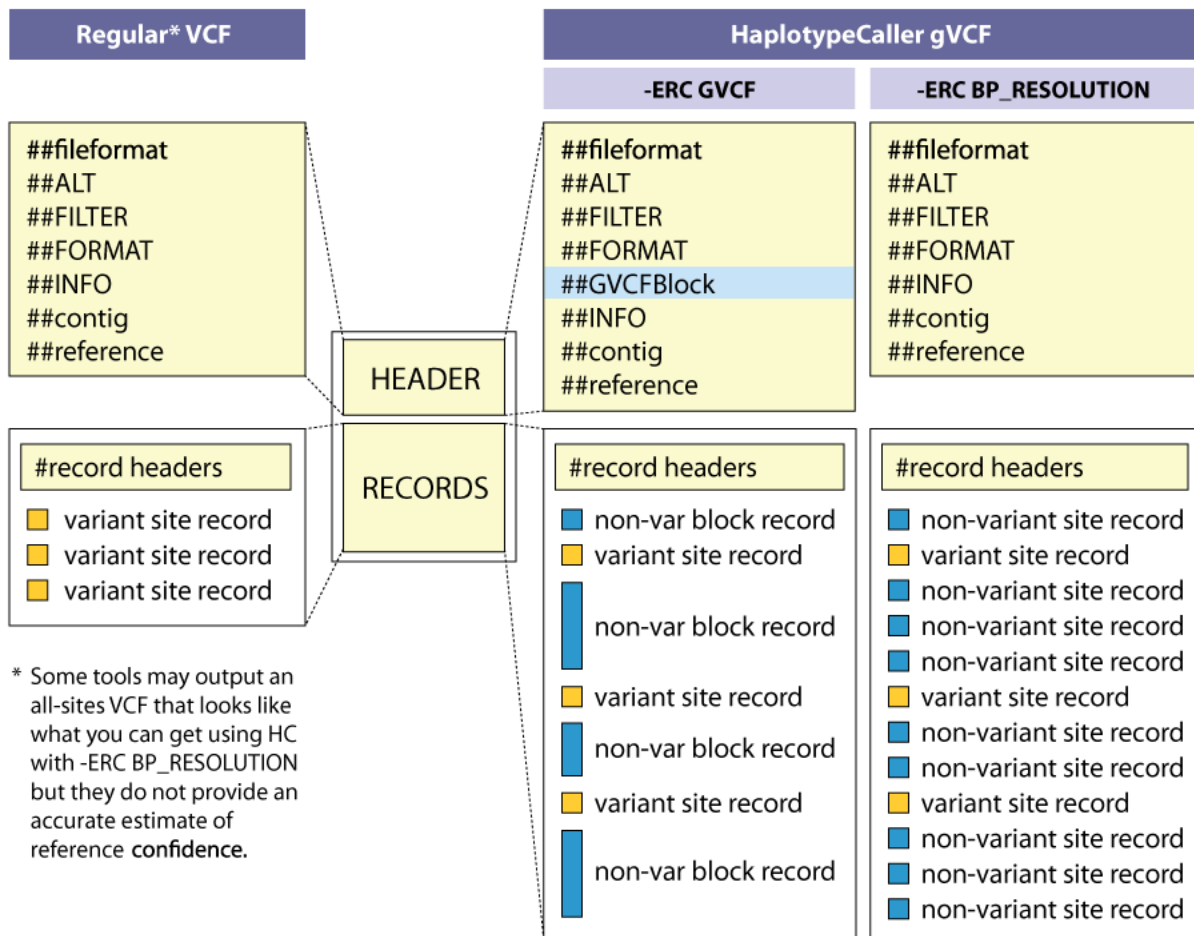
```
# R
library(Gviz)
library(seqinr)
library(Biostrings)

options(ucscChromosomeNames=FALSE)

setwd('gyak08')

# a 15599. pozíció ábrázolása
```

(continues on next page)



1. . title

(folytatás az előző oldalról)

```

poz = 15599
kezdet = poz - 20
veg = poz + 20

bam.fajlom = 'illesztes01.bam'
illesztes.track = AlignmentsTrack(bam.fajlom, start=kezdet, end=veg)

tengely.track = GenomeAxisTrack()

referencia = read.fasta('KM034562v1.fa', as.string=TRUE, seqonly=TRUE)
referencia.szekvencia = referencia[[1]]
referencia.szekvencia = DNASTringSet(referencia.szekvencia)
names(referencia.szekvencia) = 'KM034562v1'

sekvencia.track = SequenceTrack(referencia.szekvencia)

plotTracks(
  list(tengely.track, illesztes.track, sekvencia.track),
  type=c('coverage', 'pileup'),
  chromosome='KM034562v1', #!!!!!!!
  from=kezdet,
  to=veg
)

```

```

Loading required package: S4Vectors
Loading required package: stats4
Loading required package: BiocGenerics
Loading required package: parallel

Attaching package: 'BiocGenerics'

The following objects are masked from 'package:parallel':

  clusterApply, clusterApplyLB, clusterCall, clusterEvalQ,
  clusterExport, clusterMap, parApply, parCapply, parLapply,
  parLapplyLB, parRapply, parSapply, parSapplyLB

The following objects are masked from 'package:stats':

  IQR, mad, sd, var, xtabs

The following objects are masked from 'package:base':

  anyDuplicated, append, as.data.frame, cbind, colMeans, colnames,
  colSums, do.call, duplicated, eval, evalq, Filter, Find, get, grep,
  grepl, intersect, is.unsorted, lapply, lengths, Map, mapply, match,
  mget, order, paste, pmax, pmax.int, pmin, pmin.int, Position, rank,
  rbind, Reduce, rowMeans, rownames, rowSums, sapply, setdiff, sort,
  table, tapply, union, unique, unsplit, which, which.max, which.min

Attaching package: 'S4Vectors'

The following object is masked from 'package:base':

  expand.grid

```

(continues on next page)

(folytatás az előző oldalról)

```
Loading required package: IRanges
Loading required package: GenomicRanges
Loading required package: GenomeInfoDb
Loading required package: grid
Loading required package: XVector

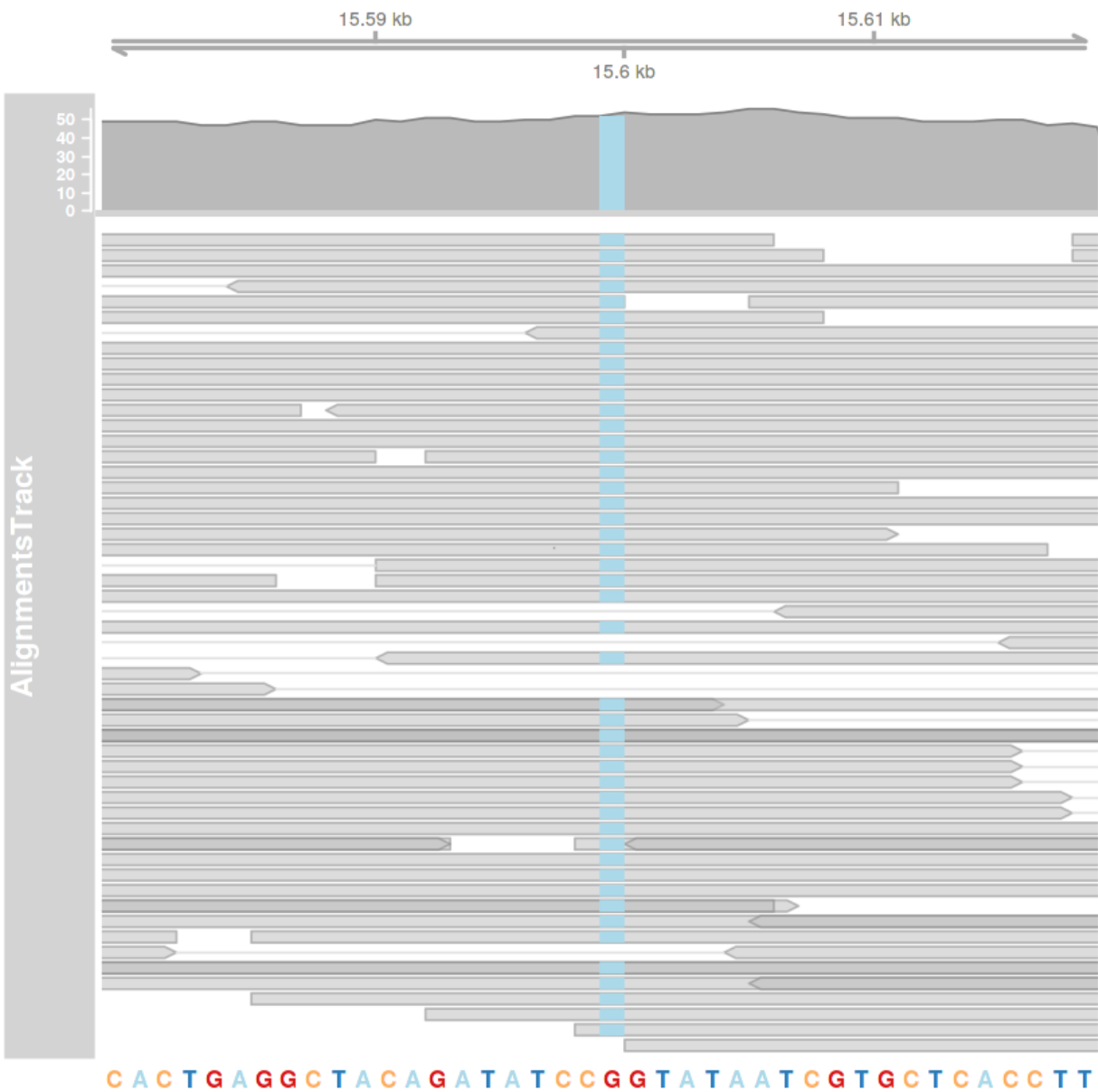
Attaching package: 'Biostrings'

The following object is masked from 'package:seqinr':

    translate

The following object is masked from 'package:base':

    strsplit
```



3.3 GATK Best Practice

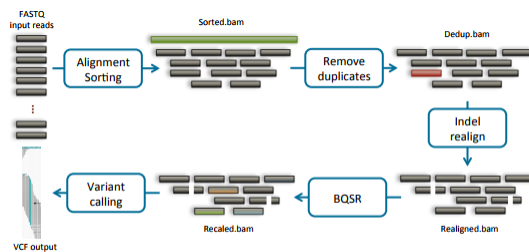
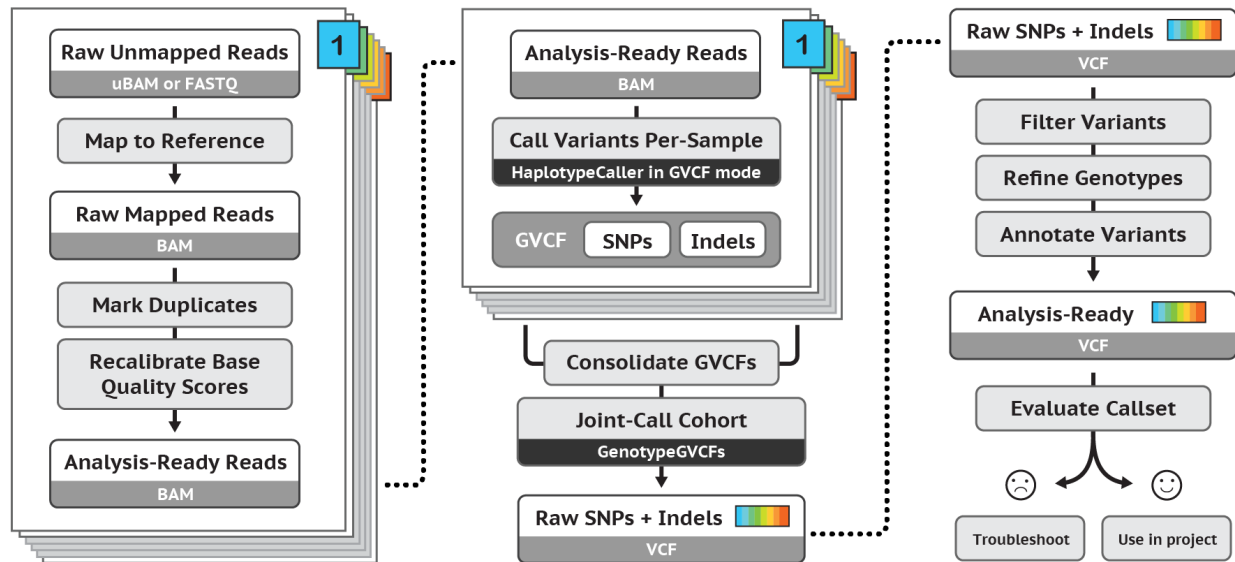


Figure 3-1: Recommended bioinformatics pipeline for DNA analysis



https://qcb.ucla.edu/wp-content/uploads/sites/14/2016/03/GATKwr12-2-Marking_duplicates.pdf

```
%bash

cd gyak08

# duplum readok eltávolítása

# https://broadinstitute.github.io/picard/index.html

PIKARD='/home/bioinfo/tools/picard/build/libs/picard.jar'

java -jar $PIKARD MarkDuplicates \
  INPUT=illesztes01.bam \
  OUTPUT=illesztes01_deduplikalt.bam \
  METRICS_FILE=duplum_metrics.txt \
  VALIDATION_STRINGENCY=LENIENT \
  ASSUME_SORTED=true \
  REMOVE_DUPLICATES=true

# a GATK-hoz kellene:
# minden readhez rendelnünk kell egy új csoportazonosítót
java -jar $PIKARD AddOrReplaceReadGroups INPUT=illesztes01_deduplikalt.bam \
  OUTPUT=illesztes01_deduplikalt_grp.bam RGID=4 RGLB=lib1 RGPL=illumina RGPU=unit1 \
  RGSM=20
# érdekes módon, ha az előző sortörést alkalmazzuk, akkor nem fut le :(

# a referencia fasta-fájlhoz létre kell hozni egy dictionary-t
java -jar $PIKARD CreateSequenceDictionary R=KM034562v1.fa O=KM034562v1.dict

# samtools dict KM034562v1.fa > KM034562v1.dict
```

```
10:40:23.070 INFO NativeLibraryLoader - Loading libgkl_compression.so from jar:file:/
  home/bioinfo/tools/picard/build/libs/picard.jar!/com/intel/gkl/native/libgkl_
  compression.so
[Tue Apr 10 10:40:23 CEST 2018] MarkDuplicates INPUT=[illesztes01.bam]
  OUTPUT=illesztes01_deduplikalt.bam METRICS_FILE=duplum_metrics.txt REMOVE_
  DUPLICATES=true ASSUME_SORTED=true VALIDATION_STRINGENCY=LENIENT MAX_SEQUENCES_
  FOR_DISK_READ_ENDS_MAP=50000 MAX_FILE_HANDLES_FOR_READ_ENDS_MAP=8000 SORTING_
  COLLECTION_SIZE_RATIO=0.25 TAG_DUPLICATE_SET_MEMBERS=false REMOVE_SEQUENCING_
  DUPLICATES=false TAGGING_POLICY=DontTag CLEAR_DT=true ADD_PG_TAG_TO_READS=true
  DUPLICATE_SCORING_STRATEGY=SUM_OF_BASE_QUALITIES PROGRAM_RECORD_ID=MarkDuplicates
  PROGRAM_GROUP_NAME=MarkDuplicates READ_NAME_REGEX=<optimized capture of last three
  ':' separated fields as numeric values> OPTICAL_DUPLICATE_PIXEL_DISTANCE=100 MAX_
  (continues on next page)
```

(folytatás az előző oldalról)

```
[Tue Apr 10 10:40:23 CEST 2018] Executing as sn@sn-OptiPlex-3010 on Linux 4.13.0-38-
↳generic amd64; OpenJDK 64-Bit Server VM 1.8.0_162-8u162-b12-0ubuntu0.16.04.2-b12;
↳Deflater: Intel; Inflater: Intel; Provider GCS is not available; Picard version: 2.
↳18.2-SNAPSHOT
INFO      2018-04-10 10:40:23      MarkDuplicates Start of doWork freeMemory:
↳151835592; totalMemory: 157286400; maxMemory: 1836580864
INFO      2018-04-10 10:40:23      MarkDuplicates Reading input file and
↳constructing read end information.
INFO      2018-04-10 10:40:23      MarkDuplicates Will retain up to 6654278 data
↳points before spilling to disk.
WARNING   2018-04-10 10:40:23      AbstractOpticalDuplicateFinderCommandLineProgram
↳
↳A field field parsed out of a read name was expected to contain an integer
↳and did not. Read name: SRR1553500.92. Cause: String 'SRR1553500.92' did not start
↳with a parsable number.
INFO      2018-04-10 10:40:23      MarkDuplicates Read 6304 records. 0 pairs never
↳matched.
INFO      2018-04-10 10:40:23      MarkDuplicates After buildSortedReadEndLists
↳freeMemory: 191005096; totalMemory: 250085376; maxMemory: 1836580864
INFO      2018-04-10 10:40:23      MarkDuplicates Will retain up to 57393152
↳duplicate indices before spilling to disk.
INFO      2018-04-10 10:40:23      MarkDuplicates Traversing read pair information
↳and detecting duplicates.
INFO      2018-04-10 10:40:23      MarkDuplicates Traversing fragment information
↳and detecting duplicates.
INFO      2018-04-10 10:40:23      MarkDuplicates Sorting list of duplicate records.
INFO      2018-04-10 10:40:23      MarkDuplicates After generateDuplicateIndexes
↳freeMemory: 249788208; totalMemory: 714080256; maxMemory: 1836580864
INFO      2018-04-10 10:40:23      MarkDuplicates Marking 2 records as duplicates.
INFO      2018-04-10 10:40:23      MarkDuplicates Found 0 optical duplicate
↳clusters.
INFO      2018-04-10 10:40:23      MarkDuplicates Reads are assumed to be ordered
↳by: coordinate
INFO      2018-04-10 10:40:23      MarkDuplicates Before output close freeMemory:
↳703788928; totalMemory: 709361664; maxMemory: 1836580864
INFO      2018-04-10 10:40:23      MarkDuplicates After output close freeMemory:
↳708583000; totalMemory: 714080256; maxMemory: 1836580864
[Tue Apr 10 10:40:23 CEST 2018] picard.sam.markduplicates.MarkDuplicates done.
↳Elapsed time: 0.01 minutes.
Runtime.totalMemory()=714080256
10:40:24.453 INFO NativeLibraryLoader - Loading libgkl_compression.so from jar:file:/
↳home/bioinfo/tools/picard/build/libs/picard.jar!/com/intel/gkl/native/libgkl_
↳compression.so
[Tue Apr 10 10:40:24 CEST 2018] AddOrReplaceReadGroups INPUT=illesztes01_deduplikalt.
↳bam OUTPUT=illesztes01_deduplikalt_grp.bam RGID=4 RGLB=lib1 RGPL=illumina
↳RGPU=unit1 RGSM=20 VERBOSITY=INFO QUIET=false VALIDATION_STRINGENCY=STRICT
↳COMPRESSION_LEVEL=5 MAX_RECORDS_IN_RAM=500000 CREATE_INDEX=false CREATE_MD5_
↳FILE=false GA4GH_CLIENT_SECRETS=client_secrets.json USE_JDK_DEFLATER=false USE_JDK_
↳INFLATER=false
[Tue Apr 10 10:40:24 CEST 2018] Executing as sn@sn-OptiPlex-3010 on Linux 4.13.0-38-
↳generic amd64; OpenJDK 64-Bit Server VM 1.8.0_162-8u162-b12-0ubuntu0.16.04.2-b12;
↳Deflater: Intel; Inflater: Intel; Provider GCS is not available; Picard version: 2.
↳18.2-SNAPSHOT
INFO      2018-04-10 10:40:24      AddOrReplaceReadGroups Created read-group ID=4
↳PL=illumina LB=lib1 SM=20

[Tue Apr 10 10:40:24 CEST 2018] picard.sam.AddOrReplaceReadGroups done. Elapsed time:
↳0.00 minutes.
```

(continues on next page)

(folytatás az előző oldalról)

```
Runtime.totalMemory()=124780544
10:40:25.237 INFO NativeLibraryLoader - Loading libgkl_compression.so from jar:file:/
↳home/bioinfo/tools/picard/build/libs/picard.jar!/com/intel/gkl/native/libgkl_
↳compression.so
[Tue Apr 10 10:40:25 CEST 2018] CreateSequenceDictionary OUTPUT=KM034562v1.dict_
↳REFERENCE=KM034562v1.fa TRUNCATE_NAMES_AT_WHITESPACE=true NUM_
↳SEQUENCES=2147483647 VERBOSITY=INFO QUIET=false VALIDATION_STRINGENCY=STRICT_
↳COMPRESSION_LEVEL=5 MAX_RECORDS_IN_RAM=500000 CREATE_INDEX=false CREATE_MD5_
↳FILE=false GA4GH_CLIENT_SECRETS=client_secrets.json USE_JDK_DEFLATER=false USE_JDK_
↳INFLATER=false
[Tue Apr 10 10:40:25 CEST 2018] Executing as sn@sn-OptiPlex-3010 on Linux 4.13.0-38-
↳generic amd64; OpenJDK 64-Bit Server VM 1.8.0_162-8u162-b12-0ubuntu0.16.04.2-b12;_
↳Deflater: Intel; Inflater: Intel; Provider GCS is not available; Picard version: 2.
↳18.2-SNAPSHOT
[Tue Apr 10 10:40:25 CEST 2018] picard.sam.CreateSequenceDictionary done. Elapsed_
↳time: 0.00 minutes.
Runtime.totalMemory()=124780544
```

```
%%bash

cd gyak08

# https://fathom.info/mirador/ebola/datarelease/

# wget https://raw.githubusercontent.com/mirador/ebola-data/master/sources/vcf/SNP-
↳2014.vcf

wget https://github.com/mirador/ebola-data/releases/download/1.3/ebola-raw.zip
unzip ebola-raw.zip

ls -lh ebola-raw/vcf
```

```
Archive: ebola-raw.zip
  creating: ebola-raw/
  creating: ebola-raw/csv/
  inflating: ebola-raw/csv/CaseNotification_schieffelin.csv
  inflating: ebola-raw/csv/DemographicsFromSim_schieffelin.csv
  inflating: ebola-raw/csv/FinalPiccoloData_schieffelin-FinalSummary1.csv
  inflating: ebola-raw/csv/MasterDataListandEBOVResults.csv
  creating: ebola-raw/vcf/
  inflating: ebola-raw/vcf/clusters.tsv
  inflating: ebola-raw/vcf/iSNV-all.vcf
  inflating: ebola-raw/vcf/SNP-2014.vcf
  creating: ebola-raw/xls/
  inflating: ebola-raw/xls/CaseNotification_schieffelin.xlsx
  creating: __MACOSX/
  creating: __MACOSX/ebola-raw/
  creating: __MACOSX/ebola-raw/xls/
  inflating: __MACOSX/ebola-raw/xls/._CaseNotification_schieffelin.xlsx
  inflating: ebola-raw/xls/DemographicsFromSim_schieffelin.xlsx
  inflating: ebola-raw/xls/FinalPiccoloData_schieffelin.xlsx
  inflating: ebola-raw/xls/info.docx
  inflating: __MACOSX/ebola-raw/xls/._info.docx
  inflating: ebola-raw/xls/MasterDataListandEBOVResults.xlsx
  inflating: __MACOSX/ebola-raw/xls/._MasterDataListandEBOVResults.xlsx
total 192K
```

(continues on next page)

(folytatás az előző oldalról)

```

-rw-r--r-- 1 sn sn 2.2K Jan 15 2015 clusters.tsv
-rw-r--r-- 1 sn sn 166K Jan 15 2015 iSNV-all.vcf
-rw-r--r-- 1 sn sn 18K Jan 15 2015 SNP-2014.vcf

```

```

--2018-04-10 10:49:11-- https://github.com/mirador/ebola-data/releases/download/1.3/
→ ebola-raw.zip
Resolving github.com (github.com)... 192.30.253.112, 192.30.253.113
Connecting to github.com (github.com)|192.30.253.112|:443... connected.
HTTP request sent, awaiting response... 302 Found
Location: https://github-production-release-asset-2e65be.s3.amazonaws.com/27739369/
→ 84259d3e-a558-11e4-9e26-219a9a054b51?X-Amz-Algorithm=AWS4-HMAC-SHA256&X-Amz-
→ Credential=AKIAIWNJYAX4CSVEH53A%2F20180410%2Fus-east-1%2Fs3%2Faws4_request&X-Amz-
→ Date=20180410T084830Z&X-Amz-Expires=300&X-Amz-
→ Signature=9e8aa99c39e5f290566ff523e19dff28e5f3a7b39c737954049dbe2de7ec2e0c&X-Amz-
→ SignedHeaders=host&actor_id=0&response-content-disposition=attachment%3B%20filename
→ %3Debola-raw.zip&response-content-type=application%2Foctet-stream [following]
--2018-04-10 10:49:12-- https://github-production-release-asset-2e65be.s3.amazonaws.
→ com/27739369/84259d3e-a558-11e4-9e26-219a9a054b51?X-Amz-Algorithm=AWS4-HMAC-SHA256&
→ X-Amz-Credential=AKIAIWNJYAX4CSVEH53A%2F20180410%2Fus-east-1%2Fs3%2Faws4_request&X-
→ Amz-Date=20180410T084830Z&X-Amz-Expires=300&X-Amz-
→ Signature=9e8aa99c39e5f290566ff523e19dff28e5f3a7b39c737954049dbe2de7ec2e0c&X-Amz-
→ SignedHeaders=host&actor_id=0&response-content-disposition=attachment%3B%20filename
→ %3Debola-raw.zip&response-content-type=application%2Foctet-stream
Resolving github-production-release-asset-2e65be.s3.amazonaws.com (github-production-
→ release-asset-2e65be.s3.amazonaws.com)... 52.216.97.27
Connecting to github-production-release-asset-2e65be.s3.amazonaws.com (github-
→ production-release-asset-2e65be.s3.amazonaws.com)|52.216.97.27|:443... connected.
HTTP request sent, awaiting response... 200 OK
Length: 534670 (522K) [application/octet-stream]
Saving to: 'ebola-raw.zip'

  0K ..... 9% 230K 2s
 50K ..... 19% 228K 2s
100K ..... 28% 1.40M 1s
150K ..... 38% 626K 1s
200K ..... 47% 515K 1s
250K ..... 57% 3.08M 0s
300K ..... 67% 10.3M 0s
350K ..... 76% 518K 0s
400K ..... 86% 3.80M 0s
450K ..... 95% 11.4M 0s
500K ..... 100% 17.1M=0.8s

2018-04-10 10:49:13 (666 KB/s) - 'ebola-raw.zip' saved [534670/534670]

```

```

%%bash

cd gyak08

bgzip -c ebola-raw/vcf/SNP-2014.vcf > ismertSNPk.vcf.gz
tabix -p vcf ismertSNPk.vcf.gz

```

3.3.1 Base Quality Score Recalibration (BQSR)

https://software.broadinstitute.org/gatk/documentation/tooldocs/current/org_broadinstitute_hellbender_tools_

walkers_bqsr_BaseRecalibrator.php

```
%%bash

cd gyak08

export PATH=${PATH}:/home/bioinfo/tools/gatk

gatk BaseRecalibrator \
  -R KM034562v1.fa \
  -I illeszt01_deduplikalt_grp.bam \
  --known-sites ismertSNPk.vcf.gz \
  -O rekalkibracios.table
```

Using GATK jar /home/bioinfo/tools/gatk/gatk-package-4.0.3.0-local.jar
Running:

```
java -Dsamjdk.use_async_io_read_samtools=false -Dsamjdk.use_async_io_
↪write_samtools=true -Dsamjdk.use_async_io_write_tribble=false -Dsamjdk.
↪compression_level=2 -jar /home/bioinfo/tools/gatk/gatk-package-4.0.3.0-
↪local.jar BaseRecalibrator -R KM034562v1.fa -I illeszt01_deduplikalt_grp.
↪bam --known-sites ismertSNPk.vcf.gz -O rekalkibracios.table
10:53:03.990 INFO NativeLibraryLoader - Loading libgkl_compression.so from
↪jar:file:/home/bioinfo/tools/gatk/gatk-package-4.0.3.0-local.jar!/com/intel/
gkl/native/libgkl_compression.so
10:53:04.112 INFO BaseRecalibrator - -----
↪-----
10:53:04.112 INFO BaseRecalibrator - The Genome Analysis Toolkit (GATK) v4.0.
↪3.0
10:53:04.112 INFO BaseRecalibrator - For support and documentation go to
↪https://software.broadinstitute.org/gatk/
10:53:04.113 INFO BaseRecalibrator - Executing as sn@sn-OptiPlex-3010 on
↪Linux v4.13.0-38-generic amd64
10:53:04.113 INFO BaseRecalibrator - Java runtime: OpenJDK 64-Bit Server VM
↪v1.8.0_162-8u162-b12-0ubuntu0.16.04.2-b12
10:53:04.113 INFO BaseRecalibrator - Start Date/Time: April 10, 2018
↪10:53:03 AM CEST
10:53:04.113 INFO BaseRecalibrator - -----
↪-----
10:53:04.113 INFO BaseRecalibrator - -----
↪-----
10:53:04.114 INFO BaseRecalibrator - HTSJDK Version: 2.14.3
10:53:04.114 INFO BaseRecalibrator - Picard Version: 2.17.2
10:53:04.114 INFO BaseRecalibrator - HTSJDK Defaults.COMPRESSION_LEVEL : 2
10:53:04.114 INFO BaseRecalibrator - HTSJDK Defaults.USE_ASYNC_IO_READ_FOR_
↪SAMTOOLS : false
10:53:04.114 INFO BaseRecalibrator - HTSJDK Defaults.USE_ASYNC_IO_WRITE_FOR_
↪SAMTOOLS : true
10:53:04.114 INFO BaseRecalibrator - HTSJDK Defaults.USE_ASYNC_IO_WRITE_FOR_
↪TRIBBLE : false
10:53:04.114 INFO BaseRecalibrator - Deflater: IntelDeflater
10:53:04.114 INFO BaseRecalibrator - Inflater: IntelInflater
10:53:04.114 INFO BaseRecalibrator - GCS max retries/reopens: 20
10:53:04.114 INFO BaseRecalibrator - Using google-cloud-java patch
↪6d11bef1c81f885c26b2b56c8616b7a705171e4f from https://github.com/droazen/
google-cloud-java/tree/dr_all_nio_fixes
```

```

10:53:04.114 INFO   BaseRecalibrator - Initializing engine
10:53:04.452 INFO   FeatureManager - Using codec VCFCodec to read file_
↳file:///home/sn/gyak08/ismertSNPk.vcf.gz
10:53:04.463 INFO   BaseRecalibrator - Shutting down engine
[April 10, 2018 10:53:04 AM CEST] org.broadinstitute.hellbender.tools.walkers.
↳bqsr.BaseRecalibrator done. Elapsed time: 0.01 minutes.
Runtime.totalMemory()=268959744

```

```

A USER ERROR has occurred: Input files reference and features have_
↳incompatible contigs: No overlapping contigs found.
  reference contigs = [KM034562v1]
  features contigs = [KM034562]

```

```

Set the system property GATK_STACKTRACE_ON_USER_EXCEPTION (--java-options '-
↳DGATK_STACKTRACE_ON_USER_EXCEPTION=true') to print the stack trace.

```

```
## !! TERMINÁL !!
```

```
cd gyak08
```

```
vim ebola-raw/vcf/SNP-2014.vcf
```

```
%%bash
```

```
cd gyak08
```

```
bgzip -c ebola-raw/vcf/SNP-2014.vcf > ismertSNPk.vcf.gz
tabix -p vcf ismertSNPk.vcf.gz
```

```
%%bash
```

```
cd gyak08
```

```
export PATH=${PATH}:/home/bioinfo/tools/gatk
```

```
# Base Quality Score Recalibration (BQSR)
```

```
gatk BaseRecalibrator \
  -R KM034562v1.fa \
  -I illeszt01_deduplikalt_grp.bam \
  --known-sites ismertSNPk.vcf.gz \
  -O rekalkibracios.table
```

```
#Apply a linear base quality recalibration model trained with the BaseRecalibrator_
↳tool
```

```
gatk ApplyBQSR \
  -R KM034562v1.fa \
  -I illeszt01_deduplikalt_grp.bam \
  -bqsr rekalkibracios.table \
  -O illeszt01_deduplikalt_grp_bqsr.bam
```

```
Tool returned:
```

```
6302
```

```

Using GATK jar /home/bioinfo/tools/gatk/gatk-package-4.0.3.0-local.jar
Running:
    java -Dsamjdk.use_async_io_read_samtools=false -Dsamjdk.use_async_io_write_
↳ samtools=true -Dsamjdk.use_async_io_write_tribble=false -Dsamjdk.compression_
↳ level=2 -jar /home/bioinfo/tools/gatk/gatk-package-4.0.3.0-local.jar_
↳ BaseRecalibrator -R KM034562v1.fa -I illeszt01_deduplikalt_grp.bam --known-sites_
↳ ismertSNPk.vcf.gz -O rekalkulacios.table
11:02:38.202 INFO NativeLibraryLoader - Loading libgkl_compression.so from jar:file:/
↳ home/bioinfo/tools/gatk/gatk-package-4.0.3.0-local.jar!/com/intel/gkl/native/libgkl_
↳ compression.so
11:02:38.311 INFO BaseRecalibrator - -----
↳ -----
11:02:38.311 INFO BaseRecalibrator - The Genome Analysis Toolkit (GATK) v4.0.3.0
11:02:38.311 INFO BaseRecalibrator - For support and documentation go to https://
↳ software.broadinstitute.org/gatk/
11:02:38.311 INFO BaseRecalibrator - Executing as sn@sn-OptiPlex-3010 on Linux v4.13.
↳ 0-38-generic amd64
11:02:38.311 INFO BaseRecalibrator - Java runtime: OpenJDK 64-Bit Server VM v1.8.0_
↳ 162-8u162-b12-0ubuntu0.16.04.2-b12
11:02:38.311 INFO BaseRecalibrator - Start Date/Time: April 10, 2018 11:02:38 AM CEST
11:02:38.312 INFO BaseRecalibrator - -----
↳ -----
11:02:38.312 INFO BaseRecalibrator - -----
↳ -----
11:02:38.312 INFO BaseRecalibrator - HTSJDK Version: 2.14.3
11:02:38.312 INFO BaseRecalibrator - Picard Version: 2.17.2
11:02:38.312 INFO BaseRecalibrator - HTSJDK Defaults.COMPRESSION_LEVEL : 2
11:02:38.312 INFO BaseRecalibrator - HTSJDK Defaults.USE_ASYNC_IO_READ_FOR_SAMTOOLS_
↳ : false
11:02:38.312 INFO BaseRecalibrator - HTSJDK Defaults.USE_ASYNC_IO_WRITE_FOR_SAMTOOLS_
↳ : true
11:02:38.312 INFO BaseRecalibrator - HTSJDK Defaults.USE_ASYNC_IO_WRITE_FOR_TRIBBLE_
↳ : false
11:02:38.312 INFO BaseRecalibrator - Deflater: IntelDeflater
11:02:38.312 INFO BaseRecalibrator - Inflater: IntelInflater
11:02:38.312 INFO BaseRecalibrator - GCS max retries/reopens: 20
11:02:38.312 INFO BaseRecalibrator - Using google-cloud-java patch_
↳ 6d11bef1c81f885c26b2b56c8616b7a705171e4f from https://github.com/droazen/google-
↳ cloud-java/tree/dr_all_nio_fixes
11:02:38.312 INFO BaseRecalibrator - Initializing engine
11:02:38.628 INFO FeatureManager - Using codec VCFCodec to read file file:///home/sn/
↳ gyak08/ismertSNPk.vcf.gz
11:02:38.639 INFO BaseRecalibrator - Done initializing engine
11:02:38.642 INFO BaseRecalibrationEngine - The covariates being used here:
11:02:38.642 INFO BaseRecalibrationEngine - ReadGroupCovariate
11:02:38.642 INFO BaseRecalibrationEngine - QualityScoreCovariate
11:02:38.642 INFO BaseRecalibrationEngine - ContextCovariate
11:02:38.642 INFO BaseRecalibrationEngine - CycleCovariate
11:02:38.643 INFO ProgressMeter - Starting traversal
11:02:38.643 INFO ProgressMeter - Current Locus Elapsed Minutes Reads_
↳ Processed Reads/Minute
11:02:39.090 INFO BaseRecalibrator - No reads filtered by:_
↳ ((((((MappingQualityNotZeroReadFilter AND MappingQualityAvailableReadFilter) AND_
↳ MappedReadFilter) AND NotSecondaryAlignmentReadFilter) AND NotDuplicateReadFilter)_
↳ AND PassesVendorQualityCheckReadFilter) AND WellformedReadFilter)
11:02:39.091 INFO ProgressMeter - KM034562v1:18001 0.0 _
↳ 6302 845906.0

```

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(folytatás az előző oldalról)

```

11:02:39.091 INFO ProgressMeter - Traversal complete. Processed 6302 total reads in
↳0.0 minutes.
11:02:39.163 INFO BaseRecalibrator - Calculating quantized quality scores...
11:02:39.177 INFO BaseRecalibrator - Writing recalibration report...
11:02:39.982 INFO BaseRecalibrator - ...done!
11:02:39.982 INFO BaseRecalibrator - Shutting down engine
[April 10, 2018 11:02:39 AM CEST] org.broadinstitute.hellbender.tools.walkers.bqsr.
↳BaseRecalibrator done. Elapsed time: 0.03 minutes.
Runtime.totalMemory()=276299776
Using GATK jar /home/bioinfo/tools/gatk/gatk-package-4.0.3.0-local.jar
Running:
    java -Dsamjdk.use_async_io_read_samtools=false -Dsamjdk.use_async_io_write_
↳samtools=true -Dsamjdk.use_async_io_write_tribble=false -Dsamjdk.compression_
↳level=2 -jar /home/bioinfo/tools/gatk/gatk-package-4.0.3.0-local.jar ApplyBQSR -R_
↳KM034562v1.fa -I illeszt01_deduplikalt_grp.bam -bqsr rekalkibracios.table -O_
↳illeszt01_deduplikalt_grp_bqsr.bam
11:02:41.832 INFO NativeLibraryLoader - Loading libgkl_compression.so from jar:file:/
↳home/bioinfo/tools/gatk/gatk-package-4.0.3.0-local.jar!/com/intel/gkl/native/libgkl_
↳compression.so
11:02:41.931 INFO ApplyBQSR - -----
↳-----
11:02:41.932 INFO ApplyBQSR - The Genome Analysis Toolkit (GATK) v4.0.3.0
11:02:41.932 INFO ApplyBQSR - For support and documentation go to https://software.
↳broadinstitute.org/gatk/
11:02:41.932 INFO ApplyBQSR - Executing as sn@sn-OptiPlex-3010 on Linux v4.13.0-38-
↳generic amd64
11:02:41.932 INFO ApplyBQSR - Java runtime: OpenJDK 64-Bit Server VM v1.8.0_162-
↳8u162-b12-0ubuntu0.16.04.2-b12
11:02:41.932 INFO ApplyBQSR - Start Date/Time: April 10, 2018 11:02:41 AM CEST
11:02:41.932 INFO ApplyBQSR - -----
↳-----
11:02:41.932 INFO ApplyBQSR - -----
↳-----
11:02:41.933 INFO ApplyBQSR - HTSJDK Version: 2.14.3
11:02:41.933 INFO ApplyBQSR - Picard Version: 2.17.2
11:02:41.933 INFO ApplyBQSR - HTSJDK Defaults.COMPRESSION_LEVEL : 2
11:02:41.933 INFO ApplyBQSR - HTSJDK Defaults.USE_ASYNC_IO_READ_FOR_SAMTOOLS : false
11:02:41.933 INFO ApplyBQSR - HTSJDK Defaults.USE_ASYNC_IO_WRITE_FOR_SAMTOOLS : true
11:02:41.933 INFO ApplyBQSR - HTSJDK Defaults.USE_ASYNC_IO_WRITE_FOR_TRIBBLE : false
11:02:41.933 INFO ApplyBQSR - Deflater: IntelDeflater
11:02:41.933 INFO ApplyBQSR - Inflater: IntelInflater
11:02:41.933 INFO ApplyBQSR - GCS max retries/reopens: 20
11:02:41.933 INFO ApplyBQSR - Using google-cloud-java patch_
↳6d11bef1c81f885c26b2b56c8616b7a705171e4f from https://github.com/droazen/google-
↳cloud-java/tree/dr_all_nio_fixes
11:02:41.933 INFO ApplyBQSR - Initializing engine
11:02:42.243 INFO ApplyBQSR - Done initializing engine
11:02:42.254 INFO ProgressMeter - Starting traversal
11:02:42.255 INFO ProgressMeter -          Current Locus   Elapsed Minutes       Reads_
↳Processed      Reads/Minute
11:02:43.339 INFO ApplyBQSR - No reads filtered by: WellformedReadFilter
11:02:43.340 INFO ProgressMeter -          KM034562v1:18001           0.0
↳          6302          348819.2
11:02:43.340 INFO ProgressMeter - Traversal complete. Processed 6302 total reads in_
↳0.0 minutes.
11:02:43.391 INFO ApplyBQSR - Shutting down engine
[April 10, 2018 11:02:43 AM CEST] org.broadinstitute.hellbender.tools.walkers.bqsr.
↳ApplyBQSR done. Elapsed time: 0.03 minutes.

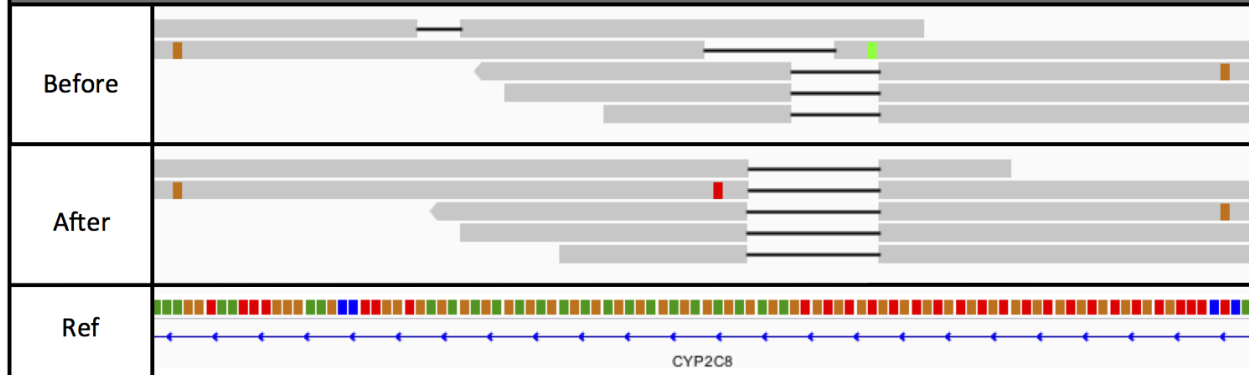
```

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```
Runtime.totalMemory()=259522560
```

Indel realignment optimizes per locus for variant concordance. @stiller February 2016

In the example, deletions at three different positions, represented by the black horizontal bar, become concordant after indel realignment. Only realigned reads are shown before and after for the 100 bp region starting at 10:96,825,853. Viewed in IGV with soft-clips hidden.



```
%bash

cd gyak08

export PATH=${PATH}:/home/bioinfo/tools/gatk

# Variant calling
gatk HaplotypeCaller \
  -R KM034562v1.fa \
  -I illeszt01_deduplikalt_grp_bqsr.bam \
  -O sznipek_GATK.vcf \
  -bamout illeszt01_deduplikalt_grp_bqsr_GATK.bam
```

```
Using GATK jar /home/bioinfo/tools/gatk/gatk-package-4.0.3.0-local.jar
Running:
  java -Dsamjdk.use_async_io_read_samtools=false -Dsamjdk.use_async_io_write_
  ↳ samtools=true -Dsamjdk.use_async_io_write_tribble=false -Dsamjdk.compression_
  ↳ level=2 -jar /home/bioinfo/tools/gatk/gatk-package-4.0.3.0-local.jar
  ↳ HaplotypeCaller -R KM034562v1.fa -I illeszt01_deduplikalt_grp_bqsr.bam -O sznipek_
  ↳ GATK.vcf -bamout illeszt01_deduplikalt_grp_bqsr_GATK.bam
11:08:06.998 INFO NativeLibraryLoader - Loading libgkl_compression.so from jar:file:/
  ↳ home/bioinfo/tools/gatk/gatk-package-4.0.3.0-local.jar!/com/intel/gkl/native/libgkl_
  ↳ compression.so
11:08:07.159 INFO HaplotypeCaller - -----
  ↳ -----
11:08:07.160 INFO HaplotypeCaller - The Genome Analysis Toolkit (GATK) v4.0.3.0
11:08:07.160 INFO HaplotypeCaller - For support and documentation go to https://
  ↳ software.broadinstitute.org/gatk/
11:08:07.160 INFO HaplotypeCaller - Executing as sn@sn-OptiPlex-3010 on Linux v4.13.
  ↳ 0-38-generic amd64
11:08:07.160 INFO HaplotypeCaller - Java runtime: OpenJDK 64-Bit Server VM v1.8.0_
  ↳ 162-8u162-b12-0ubuntu0.16.04.2-b12
11:08:07.161 INFO HaplotypeCaller - Start Date/Time: April 10, 2018 11:08:06 AM CEST
11:08:07.161 INFO HaplotypeCaller - -----
  ↳ -----
11:08:07.161 INFO HaplotypeCaller - -----
  ↳ -----
```

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(folytatás az előző oldalról)

```

11:08:07.162 INFO HaplotypeCaller - HTSJDK Version: 2.14.3
11:08:07.162 INFO HaplotypeCaller - Picard Version: 2.17.2
11:08:07.162 INFO HaplotypeCaller - HTSJDK Defaults.COMPRESSION_LEVEL : 2
11:08:07.162 INFO HaplotypeCaller - HTSJDK Defaults.USE_ASYNC_IO_READ_FOR_SAMTOOLS :
↳ false
11:08:07.162 INFO HaplotypeCaller - HTSJDK Defaults.USE_ASYNC_IO_WRITE_FOR_SAMTOOLS
↳ : true
11:08:07.162 INFO HaplotypeCaller - HTSJDK Defaults.USE_ASYNC_IO_WRITE_FOR_TRIBBLE :
↳ false
11:08:07.162 INFO HaplotypeCaller - Deflater: IntelDeflater
11:08:07.162 INFO HaplotypeCaller - Inflater: IntelInflater
11:08:07.162 INFO HaplotypeCaller - GCS max retries/reopens: 20
11:08:07.162 INFO HaplotypeCaller - Using google-cloud-java patch
↳ 6d11bef1c81f885c26b2b56c8616b7a705171e4f from https://github.com/droazen/google-
↳ cloud-java/tree/dr_all_nio_fixes
11:08:07.162 INFO HaplotypeCaller - Initializing engine
11:08:07.484 INFO HaplotypeCaller - Done initializing engine
11:08:07.487 INFO HaplotypeCallerEngine - Disabling physical phasing, which is
↳ supported only for reference-model confidence output
11:08:08.003 INFO NativeLibraryLoader - Loading libgkl_utils.so from jar:file:/home/
↳ bioinfo/tools/gatk/gatk-package-4.0.3.0-local.jar!/com/intel/gkl/native/libgkl_
↳ utils.so
11:08:08.004 INFO NativeLibraryLoader - Loading libgkl_pairhmm_omp.so from jar:file:/
↳ home/bioinfo/tools/gatk/gatk-package-4.0.3.0-local.jar!/com/intel/gkl/native/libgkl_
↳ pairhmm_omp.so
11:08:08.046 WARN IntelPairHmm - Flush-to-zero (FTZ) is enabled when running PairHMM
11:08:08.046 INFO IntelPairHmm - Available threads: 4
11:08:08.046 INFO IntelPairHmm - Requested threads: 4
11:08:08.047 INFO PairHMM - Using the OpenMP multi-threaded AVX-accelerated native
↳ PairHMM implementation
11:08:08.069 INFO ProgressMeter - Starting traversal
11:08:08.069 INFO ProgressMeter -          Current Locus  Elapsed Minutes      Regions
↳ Processed  Regions/Minute
11:08:09.300 INFO HaplotypeCaller - No reads filtered by:
↳ (((((((MappingQualityReadFilter AND MappingQualityAvailableReadFilter) AND
↳ MappedReadFilter) AND NotSecondaryAlignmentReadFilter) AND NotDuplicateReadFilter)
↳ AND PassesVendorQualityCheckReadFilter) AND
↳ NonZeroReferenceLengthAlignmentReadFilter) AND GoodCigarReadFilter) AND
↳ WellformedReadFilter)
11:08:09.300 INFO ProgressMeter -          KM034562v1:17105          0.0
↳          78          3801.8
11:08:09.300 INFO ProgressMeter - Traversal complete. Processed 78 total regions in
↳ 0.0 minutes.
11:08:09.302 INFO VectorLoglessPairHMM - Time spent in setup for JNI call : 0.
↳ 0011534140000000002
11:08:09.302 INFO PairHMM - Total compute time in PairHMM computeLogLikelihoods() :
↳ 0.026732288000000003
11:08:09.302 INFO SmithWatermanAligner - Total compute time in java Smith-Waterman :
↳ 0.04 sec
11:08:09.380 INFO HaplotypeCaller - Shutting down engine
[April 10, 2018 11:08:09 AM CEST] org.broadinstitute.hellbender.tools.walkers.
↳ haplotypcaller.HaplotypeCaller done. Elapsed time: 0.04 minutes.
Runtime.totalMemory()=397934592

```

```

# R
library(Gviz)

```

(continues on next page)

```
library(seqinr)
library(Biostrings)

options(ucscChromosomeNames=FALSE)

setwd('gyak08')

# a 15599. pozíció ábrázolása

poz = 15599
kezdet = poz - 20
veg = poz + 20

bam.fajlom = 'illesztes01_deduplikalt_grp_bqsr.bam'
illesztes.track = AlignmentsTrack(bam.fajlom, start=kezdet, end=veg)

tengely.track = GenomeAxisTrack()

referencia = read.fasta('KM034562v1.fa', as.string=TRUE, seqonly=TRUE)
referencia.szekvencia = referencia[[1]]
referencia.szekvencia = DNAStringSet(referencia.szekvencia)
names(referencia.szekvencia) = 'KM034562v1'

szekvencia.track = SequenceTrack(referencia.szekvencia)

plotTracks(
  list(tengely.track, illesztes.track, szekvencia.track),
  type=c('coverage', 'pileup'),
  chromosome='KM034562v1',
  from=kezdet,
  to=veg
)
```

```
Loading required package: S4Vectors
Loading required package: stats4
Loading required package: BiocGenerics
Loading required package: parallel

Attaching package: 'BiocGenerics'

The following objects are masked from 'package:parallel':

  clusterApply, clusterApplyLB, clusterCall, clusterEvalQ,
  clusterExport, clusterMap, parApply, parCapply, parLapply,
  parLapplyLB, parRapply, parSapply, parSapplyLB

The following objects are masked from 'package:stats':

  IQR, mad, sd, var, xtabs

The following objects are masked from 'package:base':

  anyDuplicated, append, as.data.frame, cbind, colMeans, colnames,
  colSums, do.call, duplicated, eval, evalq, Filter, Find, get, grep,
  grepl, intersect, is.unsorted, lapply, lengths, Map, mapply, match,
  mget, order, paste, pmax, pmax.int, pmin, pmin.int, Position, rank,
```

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(folytatás az előző oldalról)

```
rbind, Reduce, rowMeans, rownames, rowSums, sapply, setdiff, sort,  
table, tapply, union, unique, unsplit, which, which.max, which.min
```

```
Attaching package: 'S4Vectors'
```

```
The following object is masked from 'package:base':
```

```
  expand.grid
```

```
Loading required package: IRanges
```

```
Loading required package: GenomicRanges
```

```
Loading required package: GenomeInfoDb
```

```
Loading required package: grid
```

```
Loading required package: XVector
```

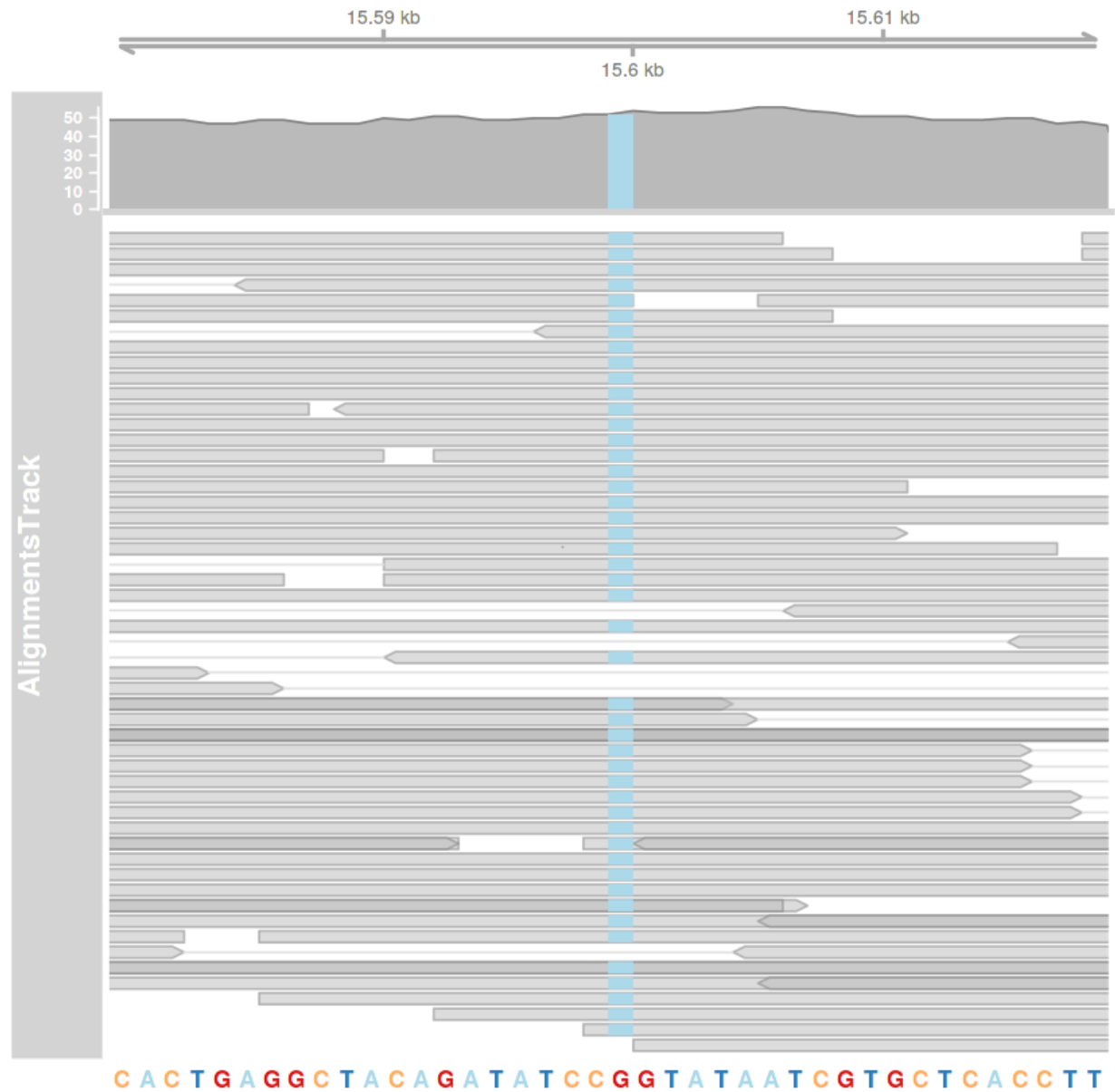
```
Attaching package: 'Biostrings'
```

```
The following object is masked from 'package:seqinr':
```

```
  translate
```

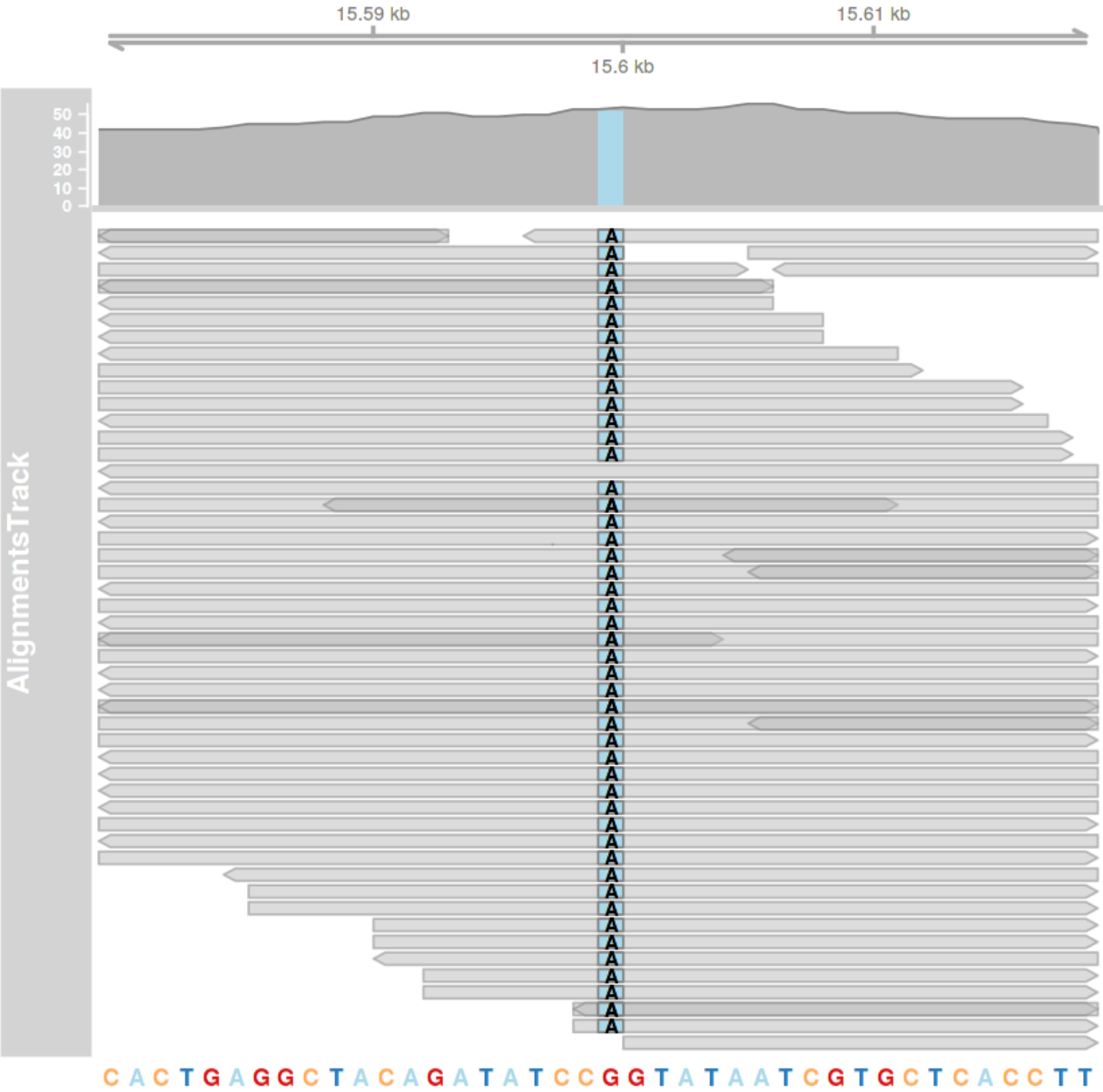
```
The following object is masked from 'package:base':
```

```
  strsplit
```



```
bam.fajlom = 'illesztes01_deduplikalt_grp_bqsr_GATK.bam'
illesztes.track = AlignmentsTrack(bam.fajlom, start=kezdet, end=veg)

plotTracks(
  list(tengely.track, illesztes.track, szekvencia.track),
  type=c('coverage', 'pileup'),
  chromosome='KM034562v1',
  from=kezdet,
  to=veg
)
```



4. fejezet

Transzkripció faktorok

A sejt működés, génexpresszió szabályozásában központi szerepet játszanak a transzkripció faktorok (TF). Ezeknek a DNS-hez való kötődése központi mozzanat a transzkripció folyamatában. A TF-ek nukleinsavhoz való kapcsolódása meghatározott szekvenciákon (*transcription factor binding site*, *TFBS*) történhet meg. Azonban azt is tudjuk, hogy ugyanazon TF nem feltétlenül teljesen azonos TFBS-ekhez kapcsolódik a DNS különböző szakaszain. Ezeket a nem teljesen egységes bázissorrendű szekvenciákat motif-nak nevezik a szakirodalomban.

A **MEF2** transzkripció faktor esetén például az ismert kötohelyek szekvenciája (Wasserman & Sandelin, 2004) az alábbi táblázatban látható:

TFBS														
1	G	A	C	C	A	A	A	T	A	A	G	G	C	A
2	G	A	C	C	A	A	A	T	A	A	G	G	C	A
3	T	G	A	C	T	A	T	A	A	A	A	G	G	A
4	T	G	A	C	T	A	T	A	A	A	A	G	G	A
5	T	G	C	C	A	A	A	A	G	T	G	G	T	C
6	C	A	A	C	T	A	T	C	T	T	G	G	G	C
7	C	A	A	C	T	A	T	C	T	T	G	G	G	C
8	C	T	C	C	T	T	A	C	A	T	G	G	G	C
i:	1	2	3	4	5	6	7	8	9	10	11	12	13	14

Ismert transzkripció faktorok további lehetséges kötődési helyének meghatározásával (predikciójával) foglalkozó kutatásokban alkalmazott bioinformatikai elemzések ezekből az ismert TFBS-szekvenciákból indulnak ki. Kézenfekvő megközelítés lenne, hogy az ismert TFBS-szekvenciák konszenzusát használják a predikcióhoz.

```
library(Biostrings)

TFBSs.seqs = c('GACCAAATAAGGCA', 'GACCAAATAAGGCA', 'TGACTATAAAAGGA', 'TGACTATAAAAGGA',
               'TGCCAAAAGTGGTC', 'CAACTATCTTGGGC', 'CAACTATCTTGGGC', 'CTCCTTACATGGGC')

TFBSs = DNAStringSet(TFBSs.seqs)

consensusString(TFBSs)
```

A konszenzus-szekvencia a nyolc kiindulási szekvencia információ tartalmát ugyan surította egyetlen IUPAC-kódolású szekvenciába, de egyúttal jelentősen csökkentette is az általa tárolt információt. Így növelve a kötési hely predikciójának megbízhatóságát. Pontosabb modellt jelent, ha a szekvencia minden pozíciójára vonatkozó nukleotidgyakorisági mátrixot (*position frequency matrix*, *PFM*) használunk:

```
consensusMatrix(TFBSSs, baseOnly=TRUE)
```

A `consensusMatrix()`-függvény által létrehozott PFM elso négy sora tartalmazza az A, C, G, T bázisokra vonatkozó gyakorisági értékeket.

```
pfm.count = consensusMatrix(TFBSSs, baseOnly=T)[1:4,]
```

A TFBSTools-csomag (Tan & Lenhard, 2016) tartalmaz speciális objek tumokat és függvényeket, amelyek segítségével könnyen dolgozhatunk a TFBS-elemzésekben.

```
library(TFBSTools)

pfm = PFMatrix(name='MEF2', profileMatrix=pfm.count)

pfm
```

```
An object of class PFMatrix
ID: Unknown
Name: MEF2
Matrix Class: Unknown
strand: +
Tags:
list()
Background:
  A    C    G    T
0.25 0.25 0.25 0.25
Matrix:
  [,1] [,2] [,3] [,4] [,5] [,6] [,7] [,8] [,9] [,10] [,11] [,12] [,13] [,14]
A     0     4     4     0     3     7     4     3     5     4     2     0     0     4
C     3     0     4     8     0     0     0     3     0     0     0     0     2     4
G     2     3     0     0     0     0     0     0     1     0     6     8     5     0
T     3     1     0     0     5     1     4     2     2     4     0     0     1     0
```

Vannak esetek, amikor a PFM-et nem gyakorisági értékekkel használják, hanem az egyes pozíciókban előforduló nukleotidok valószínűségével:

$$p(b, i) = \frac{f_{b,i} + s(b)}{N + \sum_{b' \in \{A,C,G,T\}} s(b')},$$

ahol $f_{b,i}$ a b nukleotid gyakorisága az i pozícióban; N a szekvenciák száma; $p(b, i)$ a b nukleotid i pozícióban való előfordulásának korrigált valószínűsége; $s(b)$ ún. pseudocount-függvény. Az átszámítást az alábbiak szerint végezhetjük:

```
pseudocount = sqrt(length(TFBSSs.seqs))
pfm.prob = toPWM(pfm, type='prob', pseudocounts=pseudocount)
pfm.prob
```

```
An object of class PWMMatrix
ID: Unknown
Name: MEF2
Matrix Class: Unknown
strand: +
Pseudocounts: 2.828427
Tags:
list()
Background:
```

(continues on next page)

(folytatás az előző oldalról)

```

      A      C      G      T
0.25 0.25 0.25 0.25
Matrix:
      [,1]      [,2]      [,3]      [,4]      [,5]      [,6]      [,7]
A 0.06530097 0.43469903 0.43469903 0.06530097 0.34234952 0.71174758 0.43469903
C 0.34234952 0.06530097 0.43469903 0.80409709 0.06530097 0.06530097 0.06530097
G 0.25000000 0.34234952 0.06530097 0.06530097 0.06530097 0.06530097 0.06530097
T 0.34234952 0.15765048 0.06530097 0.06530097 0.52704855 0.15765048 0.43469903
      [,8]      [,9]      [,10]      [,11]      [,12]      [,13]      [,14]
A 0.34234952 0.52704855 0.43469903 0.25000000 0.06530097 0.06530097 0.43469903
C 0.34234952 0.06530097 0.06530097 0.06530097 0.06530097 0.25000000 0.43469903
G 0.06530097 0.15765048 0.06530097 0.61939806 0.80409709 0.52704855 0.06530097
T 0.25000000 0.25000000 0.43469903 0.06530097 0.06530097 0.15765048 0.06530097

```

Az eredményből látható, hogy a `PFMatrix` típusú objektumból egy `PWMatrix`-objektum jött létre a `toPWM()`-függvénnyel, aminek a `pseudocounts` argumentumának Wasserman & Sandelin (2004) ajánlásának megfelelően a TFBS szekvenciák számának négyzetgyökét adtuk meg. A pseudocount értéke ettől eltérő is lehet. Gyakori, hogy a PFM-et ún. *position weight matrix*-ként (PWM) használják, amit neveznek *position-specific scoring matrix*-nak (PSSM) is. Annak ellenére, hogy ebben a csomagban ezt az eredményként mátrixot `PWMatrix`-nak nevezik, ez valójában még egy PFM. A PWM adott nukleotid, adott pozícióbeli megfigyelt és a várható háttér-valószínűségének $\log_2$ transzformált hányadosa:

$$W_{b,i} = \frac{\log p(b,i)}{\log p(b)}$$

ahol $p(b)$ a b nukleotid előfordulásának háttér-valószínűsége; $p(b,i)$ a b nukleotid i pozícióbeli előfordulásának korrigált valószínűsége; $W_{b,i}$ a b nukleotid i pozícióbeli PWM-értéke. Az előző `toPWM()`-függvény `type='log2probratio'` beállításával hozhatjuk létre:

```

pwm = toPWM(pfm, type='log2probratio', pseudocounts=pseudocount)
pwm

```

An object of class `PWMatrix`

ID: Unknown

Name: MEF2

Matrix Class: Unknown

strand: +

Pseudocounts: 2.828427

Tags:

`list()`

Background:

```

      A      C      G      T
0.25 0.25 0.25 0.25

```

Matrix:

```

      [,1]      [,2]      [,3]      [,4]      [,5]      [,6]      [,7]
A -1.9367518 0.7980888 0.7980888 -1.936752 0.4535419 1.5094376 0.7980888
C 0.4535419 -1.9367518 0.7980888 1.685442 -1.9367518 -1.9367518 -1.9367518
G 0.0000000 0.4535419 -1.9367518 -1.936752 -1.9367518 -1.9367518 -1.9367518
T 0.4535419 -0.6651985 -1.9367518 -1.936752 1.0760078 -0.6651985 0.7980888
      [,8]      [,9]      [,10]      [,11]      [,12]      [,13]      [,14]
A 0.4535419 1.0760078 0.7980888 0.000000 -1.936752 -1.9367518 0.7980888
C 0.4535419 -1.9367518 -1.9367518 -1.936752 -1.936752 0.0000000 0.7980888

```

(continues on next page)

(folytatás az előző oldalról)

```
G -1.9367518 -0.6651985 -1.9367518 1.308939 1.685442 1.0760078 -1.9367518
T 0.0000000 0.0000000 0.7980888 -1.936752 -1.936752 -0.6651985 -1.9367518
```

A PWM alapján, adott szekvenciára kiszámolható egy összetett pontszám (*site score*), ami nagy számú és reprezentatív TFBS szekvencia esetén arányos a kötődési energiával (King & Roth, 2003).

$$S = \sum_{i=1}^w W_{l_i, i},$$

ahol S a szekvencia PWM-pontszáma; l_i az i pozícióban lévő nukleotid; w a PWM szélessége. Például a fenti PWM alapján a TTACATAAGTAGTC szekvenciára számított pontszám:

```
m = Matrix(pwm)
iseq = 'TTACATAAGTAGTC'
site.score = c()

for(i in 1:nchar(iseq)){
  s = substr(iseq, i, i)
  site.score = c(site.score, m[s, i])
}
site.score
```

```
sum(site.score)
```

A szekvencia összesített pontszámának abszolút értéke nem sokat mond arról, hogy a többi, már ismert kötohely szekvenciájához hogyan viszonyul. Ezért az a szokás, hogy a PWM alapján számított legkisebb és legnagyobb összesített szekvencia pontszám alapján létrehozott eloszláshoz hasonlítják a vizsgált szekvencia összpontszámát. A példában szereplo PWM esetén az 5.26-os érték azt jelenti, hogy az összes lehetséges szekvencia 78%-a kisebb összesített pontszámmal rendelkezik. A PWM további fontos jellemzője az információtartalom profil (information content profile, IC, Schneider et al. (1986)). Az IC-t bitekben fejezik ki és a DNS-szekvencia esetében az értéke 0 és 2 bit között változhat. Egy olyan i pozícióban, ahol mindegyik nukleotid egyforma valószínűséggel fordulhat elő az $IC = 0$, míg egy olyan i pozícióban, ahol egyetlen nukleotid fordulhat elő az $IC = 2$ (Bembom, 2017).

$$IC_i = 2 + \sum_b p_{b,i} \log_2 p_{b,i},$$

ahol IC_i az i pozíció információ tartalma; a $p_{b,i}$ a b nukleotid i pozícióbeli korrigált valószínűsége. Azok a pozíciók, amelyek erosen konzervatívák és így kis toleranciával rendelkeznek a helyettesítésre vonatkozóan, magas IC -értékűek, míg a nagy helyettesítési toleranciájú pozíciók alacsony IC -értékűek (Bembom, 2017). A PFMATRIX-ből ICMATRIXOT hozhatunk létre a toICM()-függvénnyel (A pseudocounts argumentumnak itt azért adtunk 0 értéket, hogy a Wasserman & Sandelin (2004) által bemutatott ábrát rekonstruáljuk):

```
icm = toICM(pfm, pseudocounts=0)
icm
```

```
An object of class ICMATRIX
ID: Unknown
Name: MEF2
Matrix Class: Unknown
strand: +
Pseudocounts: 0
Schneider correction: FALSE
Tags:
list()
Background:
  A    C    G    T
0.25 0.25 0.25 0.25
Matrix:
```

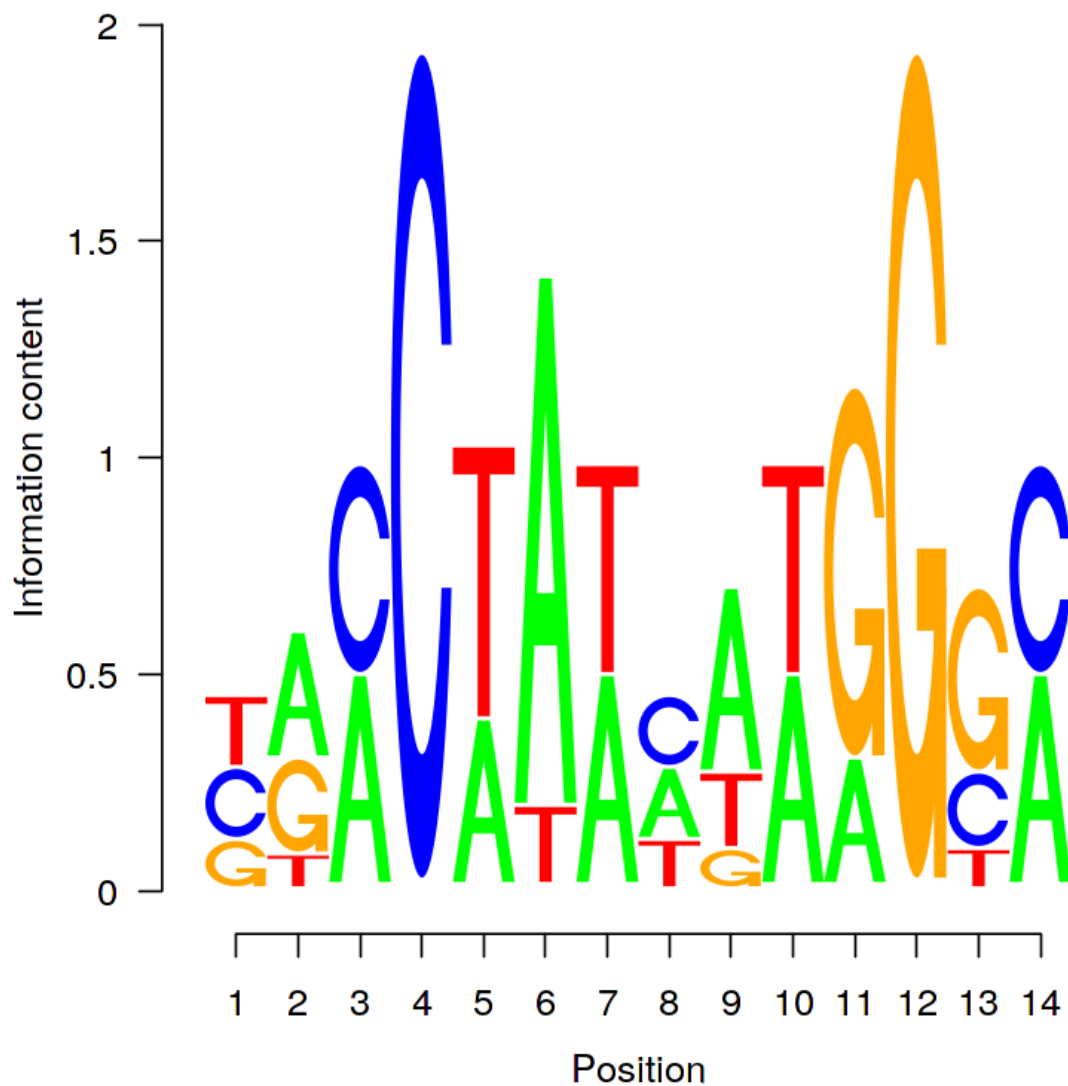
(continues on next page)

(folytatás az előző oldalról)

	[,1]	[,2]	[,3]	[,4]	[,5]	[,6]	[,7]	[,8]	[,9]
A	0.0000000	0.29718047	0.5	0	0.3920872	1.2743811	0.5	0.1645207	0.43825316
C	0.1645207	0.00000000	0.5	2	0.0000000	0.0000000	0.0	0.1645207	0.00000000
G	0.1096805	0.22288535	0.0	0	0.0000000	0.0000000	0.0	0.0000000	0.08765063
T	0.1645207	0.07429512	0.0	0	0.6534787	0.1820544	0.5	0.1096805	0.17530126
	[,10]	[,11]	[,12]	[,13]	[,14]				
A	0.5	0.2971805	0	0.00000000	0.5				
C	0.0	0.0000000	0	0.17530126	0.5				
G	0.0	0.8915414	2	0.43825316	0.0				
T	0.5	0.0000000	0	0.08765063	0.0				

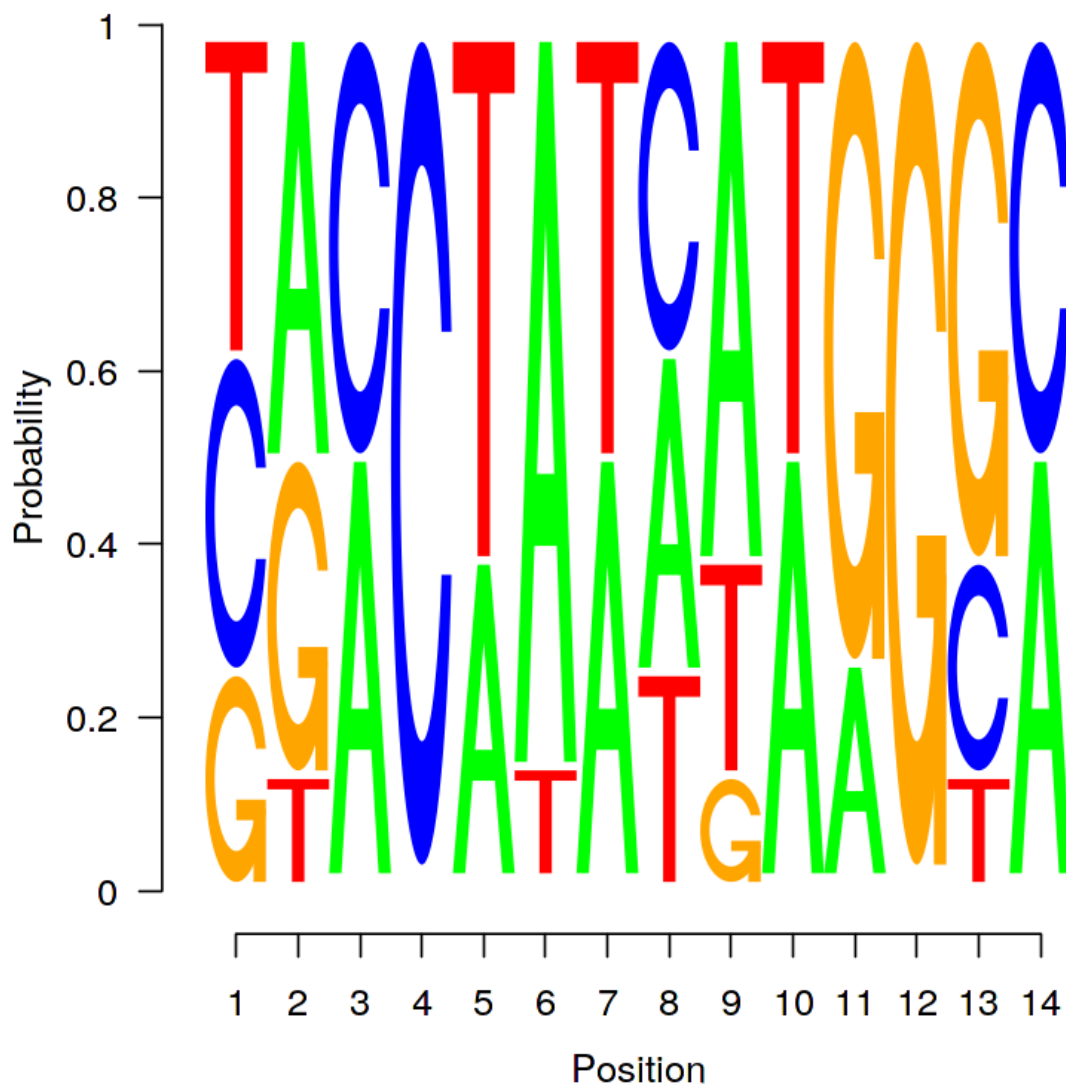
A PWM-ek grafikus reprezentációjának általánosan használt eszköze az ún. sequence logo (Schneider & Stephens, 1990). A logók a szekvencia mindegyik pozíciójára a négy nukleotidot jelölo betuhalmot tartalmaz. Az oszlop magassága a pozíció IC-jével, míg az egyes betuk magassága a nukleotid adott pozícióbeli relatív gyakoriságával arányos. A seqLogo()-függvénnyel létrehozhatunk szekvencialogokat. Ha a függvény ic.scale argumentumának TRUE értéket adunk, akkor a nukleotidoknak megfelelő oszlopok magassága az IC-vel arányos lesz.

```
seqLogo(icm, ic.scale=T)
```



Ha ugyanennek az argumentumnak FALSE értéket adunk, akkor a logo oszlopai egyforma magasságúak lesznek és a betűk mérete a nukleotidok előfordulásának valószínűségét jelzi.

```
seqLogo(icm, ic.scale=F)
```



4.1 Motif-adatbázisok

4.1.1 MotifDb

Az ismert motif-ok jelenleg nem állnak rendelkezésre egyetlen szabad felhasználású forrásból, ezért gyűjtötték össze a különböző források, különböző liszenszelésű adatait a MotifDb-csomagba (Shannon, 2017). Az adatbázisból elérhető motífkra vonatkozó leíró információkat az alábbiak szerint kérdezhetjük le.

```
library(MotifDb)

MotifDb
```

```
See system.file("LICENSE", package="MotifDb") for use restrictions.
```

```
MotifDb object of length 8369
| Created from downloaded public sources: 2013-Aug-30
| 8369 position frequency matrices from 13 sources:
|   cispb_1.02: 874
|   FlyFactorSurvey: 614
|   HOCOMOCov10: 1066
|   HOMER: 332
|   hPDI: 437
|   JASPAR_2014: 592
|   JASPAR_CORE: 459
|   jasper2016: 1209
|   jolma2013: 843
|   ScerTF: 196
|   stamlab: 683
|   SwissRegulon: 684
|   UniPROBE: 380
| 52 organism/s
|   Hsapiens: 4094
|   Mmusculus: 1251
|   Dmelanogaster: 1147
|   Scerevisiae: 876
|   Athaliana: 351
|   Celegans: 67
|   other: 583
Scerevisiae-cispb_1.02-M0001_1.02
Scerevisiae-cispb_1.02-M0002_1.02
Scerevisiae-cispb_1.02-M0003_1.02
Csativa-cispb_1.02-M0004_1.02
Athaliana-cispb_1.02-M0005_1.02
...
Mmusculus-UniPROBE-Zfp740.UP00022
Mmusculus-UniPROBE-Zic1.UP00102
Mmusculus-UniPROBE-Zic2.UP00057
Mmusculus-UniPROBE-Zic3.UP00006
Mmusculus-UniPROBE-Zscan4.UP00026
```

Az adatbázisban elérhető mezők listája:

```
colnames(values(MotifDb))
```

A motífkok többféle eljárással kérdezhetők le az adatbázisból, a legegyszerűbb a query()-függvény használata, pl. az összes humán motífk kiolvasása:

```
query(MotifDb, 'Hsapiens')
```

```
MotifDb object of length 4094
| Created from downloaded public sources: 2013-Aug-30
| 4094 position frequency matrices from 10 sources:
|   cispb_1.02: 313
|   HOCOMOCov10: 640
|   hPDI: 437
|   JASPAR_2014: 117
|   JASPAR_CORE: 66
|   jasper2016: 442
```

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```
|           jolma2013: 710
|           stamlab: 683
|           SwissRegulon: 684
|           UniPROBE: 2
| 1 organism/s
|           Hsapiens: 4094
Hsapiens-jolma2013-BCL6B
Hsapiens-jolma2013-CTCF
Hsapiens-jolma2013-EGR1
Hsapiens-jolma2013-EGR1-2
Hsapiens-jolma2013-EGR2
...
Hsapiens-SwissRegulon-ZNF784.SwissRegulon
Hsapiens-SwissRegulon-ZNF8.SwissRegulon
Hsapiens-SwissRegulon-ZSCAN4.SwissRegulon
Hsapiens-UniPROBE-Sox4.UP00401
Hsapiens-UniPROBE-Oct_1.UP00399
```

Ha több szempontot is szeretnénk érvényesíteni a lekérdezésben, akkor egymásba ágyazott query()-ket használhatunk:

```
mot1 = query(query(query(MotifDb, 'Hsapiens'), 'JASPAR_2014'), 'MEF2')
mot1
```

```
MotifDb object of length 2
| Created from downloaded public sources: 2013-Aug-30
| 2 position frequency matrices from 1 source:
|           JASPAR_2014: 2
| 1 organism/s
|           Hsapiens: 2
Hsapiens-JASPAR_2014-MEF2C-MA0497.1
Hsapiens-JASPAR_2014-MEF2A-MA0052.2
```

A kiválasztott motifhoz tartozó mátrix(ok) az alábbi szerint olvasható(k) ki a mot1-objektumból:

```
as.list(mot1)
```

A mátrixokat az adatbázis PFM formában tárolja, mégpedig úgy, hogy a mátrix mindegyik oszlopához, vagyis a motif szekvenciájának pozíciójához tartozó értékeket egyre normálja. Amennyiben a mátrix létrehozásához felhasznált szekvenciák száma ismert, akkor azt a sequenceCount mezoból olvashatjuk ki:

```
as.matrix(values(mot1))
```

Ennek felhasználásával előállítható pl. az első eredeti gyakorisági mátrix (PFM):

```
pfm1 = round(as.numeric(values(mot1)$sequenceCount[1])*as.list(mot1)[[1]])
pfm1
```

4.1.2 JASPAR2014, JASPAR2016

A TFBSTools-könyvtár objektumaival való munkához létrehoztak JASPAR3 adatbázisokat (Tan, 2014, 2015). A JASPAR 2016 a 2014-hez képest több TF-kötő profilt tartalmaz, a korábbi adatokat frissítették benne, illetve 130 TFFM is van már benne (Mathelier et al., 2016). A 2014-es adatbázisból az összes motif lekérdezése:

```
library(JASPAR2014)

opts = list()
getMatrixSet(JASPAR2014, opts)
```

```
PFMatrixList of length 593
names(593): MA0004.1 MA0006.1 MA0008.1 MA0009.1 ... MA0599.1 MA0600.1 MA0113.2
```

A 2016-os adatbázisban elérhető motifok:

```
library(JASPAR2016)

getMatrixSet(JASPAR2016, opts)
```

```
PFMatrixList of length 1082
names(1082): MA0004.1 MA0006.1 MA0010.1 MA0011.1 ... MA1096.1 MA1097.1 MA1098.1
```

A TFBSTools-könyvtár `getMatrixSet()`-függvény második paraméterével állíthatjuk be a lekérdezési szempontokat, így a korábban használt motif-ot az alábbi szerint olvashatjuk ki:

```
opts = list(species=9606, name='MEF2A', all_versions=TRUE)
gres = getMatrixSet(JASPAR2016, opts)
gres
```

```
PFMatrixList of length 3
names(3): MA0052.1 MA0052.2 MA0052.3
```

Mivel a lekérdezés eredménye egy lista, ezért annak elemeit így írathatjuk ki:

```
gres[[1]]
```

```
An object of class PFMatrix
ID: MA0052.1
Name: MEF2A
Matrix Class: Other Alpha-Helix
strand: +
Tags:
$comment
[1] "-"

$family
[1] "MADS"

$medline
[1] "1748287"

$pazar_tf_id
[1] "TF0000034"

$tax_group
[1] "vertebrates"

$tfe_id
[1] "145"

$type
```

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```
[1] "SELEX"

$collection
[1] "CORE"

$species
      9606
"Homo sapiens"

$acc
[1] "EAX02249"

Background:
      A      C      G      T
0.25 0.25 0.25 0.25
Matrix:
  [,1] [,2] [,3] [,4] [,5] [,6] [,7] [,8] [,9] [,10]
A     1     0     57     2     9     6     37     2     56     6
C    50     0     1     1     0     0     0     0     0     0
G     0     0     0     0     0     0     0     0     2    50
T     7    58     0    55    49    52    21    56     0     2
```

A JASPAR2014-csomagban van egy JASPAR2014SitesSeqs-objektum is, aminek a segítségével egyszerűen lekérdezheto a motifok kiindulási szekvenciái:

```
JASPAR2014SitesSeqs$MA0052.1
```

```
A DNAStringSet instance of length 58
width seq
[1] 26 ATGTGGGCTATTTATAGAAATTCAG
[2] 26 GGAGTCGCCTCTTAAGTATTTATAGA
[3] 26 AATGCGCGATGCACTATTTATAGTTC
[4] 27 CTATTTATAGCTAGGACGAGTCGTTCC
[5] 26 ACGCTTATTAAGTCTATTTATAGCCT
...
[54] 26 GAAATTCTGATTATATTTAGACTCC
[55] 26 ATTTAACCCGAGTTACTTATAACTGC
[56] 25 GTTGACGTGATGCTATTTTAGACA
[57] 26 TGTACTATTTTAGTCCGAGTACTGT
[58] 26 ATCGGAAGGAAGTTGATCTATTTATA
```

names	MA0052	MEF2A	
MA0052	MEF2A	1	
MA0052	MEF2A	2	
MA0052	MEF2A	3	
MA0052	MEF2A	4	
MA0052	MEF2A	5	
MA0052	MEF2A	54	
MA0052	MEF2A	55	
MA0052	MEF2A	56	
MA0052	MEF2A	57	
MA0052	MEF2A	58	

4.2 Motifok, szekvenciák összehasonlítása

PFM-eket összehasonlíthatjuk a TFBSTools-könyvtár PFMSimilarity()-függvényével (Sandelin et al., 2003):

```
pfmORIG = qres[[1]]
PFMSimilarity(pfmORIG, pfmORIG)
```

A score itt látható 100%-os egyezés (relScore) esetén a szekvencia hosszának kétszerese. Ha egy apró módosítást végzünk az egyik mátrixon, akkor az alábbi eredményt kapjuk:

```
tmp = Matrix(pfmORIG)
tmp[2,2] = 1000
```

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```
tmp[4,2] = 22
pfmALT = PFMatrix(profileMatrix = tmp)
PFMSimilarity(pfmORIG, pfmALT)
```

Ugyanezzel a függvénnyel összehasonlíthatunk egy PFM-et és egy IUPAC-szekvenciát is, pl. a PFM-et és annak konszenzus szekvenciáját:

```
library(seqLogo)
pwmORIG = toPWM(pfmORIG, type='prob')
pwmALT = toPWM(pfmALT, type='prob')
seq = makePWM(Matrix(pwmORIG))@consensus
seq
```

```
PFMSimilarity(pfmORIG, seq)
```

PWM-ek összehasonlítására a TFBSTools-könyvtár PWMSimilarity()-függvényével három különböző módszert használhatunk. Normalizált euklideszi távolság:

```
PWMSimilarity(pwmORIG, pwmALT, method='Euclidean')
```

A statisztika értéke 0 és 1 között változhat, a 0 teljes egyezőséget, az 1 teljes eltérést jelent.

Pearson-korreláció:

```
PWMSimilarity(pwmORIG, pwmALT, method='Pearson')
```

A statisztika értéke -1 és 1 között változhat, az 1 teljes egyezőséget, a 1 teljes eltérést jelent.

Kullback-Leibler eltérés (Linhart et al., 2008):

```
PWMSimilarity(pwmORIG, pwmALT, method='KL')
```

A két PWM eloszlásának eltérését vizsgáljuk ezzel a mértékkel, értéke 0 és 1 között változhat, a 0 eros hasonlóságot, az 1 nagy eltérést jelez.

4.3 PWM keresése szekvenciában

A searchSeq()-függvénnyel vizsgálhatjuk, hogy a PWM-ünk mely DNS-szakaszra illeszkedik. Ha a fenti példa konszenzusszekvenciáját beillesztjük egy véletlen szekvenciába, az alábbi eredményt kapjuk:

```
set.seed(20)
rnd.seq.str = paste(sample(c('A', 'C', 'G', 'T'), 30, replace=TRUE), collapse='')
seq.str = paste(substr(rnd.seq.str, 1, 10), seq, substr(rnd.seq.str, 11, 30), sep='')
seq.str
```

```
seq.dns = DNString(seq.str)
siteset = searchSeq(pwmORIG, seq.dns, seqname='teszt', strand='*', min.score=0.8)
siteset
```

```
An object of class SiteSet with 2 site sequences
  seqname source feature start end   score strand frame
1  teszt   TFBS    TFBS    11  20 8.877551      +    .
2  teszt   TFBS    TFBS    11  20 7.414966      -    .
```

(continues on next page)

(folytatás az előző oldalról)

```

                                attributes
1 TF=MEF2A;class=Other Alpha-Helix;sequence=CTATTTATAG
2 TF=MEF2A;class=Other Alpha-Helix;sequence=CTATAAATAG

```

A min.score-argumentumban meghatározhatjuk, hogy a PWM és a vizsgált szekvencia között milyen mértéku eltérés fogadható még el, 0 1 vagy 0 100% közötti érték lehet. A PWM-ból létrehozható szekvenciák minimális és maximális összesített pontszámából számított eloszlásnak az illeszkedő szakaszok szurására használt percentilis határértéket jelenti.

A függvény eredménye egy SiteSet-objektum, azonban, ha az első argumentuma nem PWMMatrix, hanem PWMMatrixList, akkor SiteSetList-objektumot kapunk vissza.

```

gres.pwm = toPWM(gres, type='prob')
siteset = searchSeq(gres.pwm, seq.dns, seqname='teszt', strand='*', min.score=0.8)
siteset

```

```

SiteSetList of length 3
names(3): MA0052.1 MA0052.2 MA0052.3

```

A PWM szekvenciával való összehasonlítására használhatjuk a Biostrings-csomag függvényeit is:

```

pwm.B = PWM(Matrix(pfmORIG))
pwm.B

```

```

res.B = matchPWM(pwm.B, seq.dns, with.score=TRUE, min.score=0.8)
res.B

```

```

Views on a 40-letter DNAString subject
subject: TTCGTTAACCCTATTTATAGGTAGACCACTCACACATTAG
views:
      start end width
[1]    11  20    10 [CTATTTATAG]

```

A Biostrings-csomagra épülően a PWMEnrich-csomag (Stojnic & Diez, 2015) további lehetőségeket nyújt PWM-ek szekvenciákra való illeszkedésének vizsgálatában. A csomag függvényeivel többek között azt vizsgálhatjuk (a Cloverhez hasonlóan (<https://zlab.bu.edu/clover/>), hogy egy adott szekvenciában mely motív-ok vannak felülreprezentálva. Ahogy korábban láttuk a motív-oknak valamely szekvenciára való kötődését használhatjuk a TFBS predikciójára. Azonban a fent bemutatott módszer, illetve a legtöbb, a szakirodalomban fellelhető eljárás esetén meg kell határozunk valamilyen a motív és a szekvencia hasonlóságára vonatkozó határértéket. Ahogy Frith et al. (2004) bemutatja, ha ez az érték alacsony, akkor sok nem „valódi” TF-et azonosíthatunk, mint olyat, ami a kérdéses szekvenciához kötődhet. Ha pedig túl magas, akkor „valóban” kötődő TF-eket veszíthetünk el. Azonkívül ezek az eljárások nem kezelik azt, ha egy TF az adott szekvencián egynél több TFBS-el rendelkezik, illetve azt sem, hogy a teljes genomban vagy egy-egy kromoszómán milyen valószínűséggel kötődhet. Frith et al. (2004) által bemutatott megközelítésben a TF-ekre vonatkozóan egy nagyobb genomszakaszra, vagy a teljes genomra meghatározzuk, hogy milyen ún. nyers pontszámmal (raw score) kötődnek. Majd a vizsgált szekvenciára is meghatározzuk ezt a pontszámot. Így a háttér ismeretében becsülhető, hogy milyen valószínűséggel kötődhet a TF a vizsgált szekvencián olyan (vagy annál nagyobb) nyers pontszámmal, amelyet számoltunk rá. Frith et al. (2004) szerint a $p < 0.01$ valószínűséggel kötődő TF-ek esetén alaposan feltételezhető, hogy az adott szakaszon felülreprezentáltak (over-represented), míg ha a $p > 0.99$, akkor alulreprezentáltak (under-represented). Továbbá, ha ilyen felül- vagy alulreprezentáltságot tapasztalunk, akkor annak funkcionális szerepe lehet. Mondjuk, hogy egy a humán 8. kromoszómán lévő szekvenciára kötődő TF-eket szeretnénk azonosítani. Ráadásul nem az összes ismert TF-re vagyunk kíváncsiak, hanem csak néhányra. Eloszor a TF-eket kell megfelelő formátumba alakítani:

```
library(TFBSTools)
library(JASPAR2016)

opts = list(
  species=9606,
  name=c('AR', 'ETV1', 'FOXA1', 'GATA2', 'HOXB13', 'NKX3-1'),
  all_versions=TRUE
)

TF.set = getMatrixSet(JASPAR2016, opts)
lst = as.list(TF.set)
TF.lst = lapply(lst, Matrix)
names(TF.lst) = paste(as.character(lapply(lst, name)), names(TF.lst), sep='_')
```

A 8. humán kromoszómára lesz szükségünk a háttér létrehozásához, azonban mivel a szekvencia tartalmaz N-eket, ezeket ki kell törölnünk a továbbiakban használt függvényekhez. Ezt – többek között – tehetjük az alábbiak szerint:

```
library(BSgenome.Hsapiens.UCSC.hg19)

chr8 = as.character(BSgenome.Hsapiens.UCSC.hg19$chr8)
lst = strsplit(chr8, 'N')
lst = lst[[1]]
lst = lst[which(lapply(lst, nchar)!=0)]
chr8 = DNAStringSet(unlist(lst))
```

A PWMEnrich-könyvtár makePWMLognBackground()-függvényével hozhatunk létre saját lognormális háttér eloszlást valamely szekvenciá(k)ra, itt a 8. kromoszómára:

```
library(PWMEnrich)

bg.logn = makeBackground(motifs=TF.lst, bg.seq=chr8, type='logn', algorithm='human')
```

A motifok szekvencián belüli alul- vagy felülreprezentáltságát ezek után az alábbiak szerint vizsgálhatjuk:

```
myseq = DNAString('TGC GTTCTCTTGGAGACTCTCTATTGCCCCCTCTCTTTTACAG')
res = motifEnrichment(myseq, bg.logn)
rpt = sequenceReport(res, 1)
rpt
```

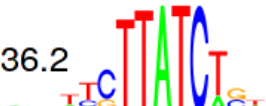
Scanning sequence 1 / 1

Calculating motif enrichment scores ...

```
An object of class 'MotifEnrichmentReport':
  rank      target      id      raw.score  p.value
1     1  FOXA1_MA0148.1  FOXA1_MA0148.1 0.3001255927 0.3072201
2     2      AR_MA0007.2      AR_MA0007.2 0.0400636241 0.3281074
3     3  FOXA1_MA0148.2  FOXA1_MA0148.2 0.2398628307 0.3330050
4     4  FOXA1_MA0148.3  FOXA1_MA0148.3 0.0020333381 0.5656657
5     5  GATA2_MA0036.2  GATA2_MA0036.2 0.0002374392 0.6262785
6     6 NKX3-1_MA0124.1 NKX3-1_MA0124.1 0.0250463314 0.7531180
7     7  ETV1_MA0761.1  ETV1_MA0761.1 0.0002927674 0.9671194
8     8 HOXB13_MA0901.1 HOXB13_MA0901.1 0.0005775918 0.9709271
9     9  GATA2_MA0036.1  GATA2_MA0036.1 0.0426125957 0.9991309
```

Az eredményeket a plot()-függvénnyel vizualizálhatjuk is. A listából és az ábráról az látható, hogy egyedül a GATA2_MA0036.1 tekinthető alulreprezentáltnak.

```
plot (rpt)
```

Rank	Target	PWM	Motif ID	Raw score	P-value
1	FOXA1_MA0148.1		FOXA1_MA0148.1	0.3	0.307
2	AR_MA0007.2		AR_MA0007.2	0.0401	0.328
3	FOXA1_MA0148.2		FOXA1_MA0148.2	0.24	0.333
4	FOXA1_MA0148.3		FOXA1_MA0148.3	0.00203	0.566
5	GATA2_MA0036.2		GATA2_MA0036.2	0.000237	0.626
6	NKX3-1_MA0124.1		NKX3-1_MA0124.1	0.025	0.753
7	ETV1_MA0761.1		ETV1_MA0761.1	0.000293	0.967
8	HOXB13_MA0901.1		HOXB13_MA0901.1	0.000578	0.971
9	GATA2_MA0036.1		GATA2_MA0036.1	0.0426	0.999

4.4 „de novo” motif-azonosítás

Új motifok keresésére a TFBSTools-csomag is tartalmaz függvényt (runMEME), ami a MEME-t (<http://meme-suite.org/>) használja. Ehhez képest újabb és talán hatékonyabb módszert fejlesztett Li (2009), amit az rGADEM-csomag (Droit et al., 2014) segítségével alkalmazhatunk az R-en belül. A vizsgálatokból (pl. ChIP-seq) származó szekvenciákat az alábbiak szerint elemezhetjük motif-tartalmukra vonatkozóan.

Az elemzésekhez szükségünk van a szekvenciákra, amelyeket az itt bemutatott példában FASTA-fájlban tárolunk, az állomány első három sora így néz ki:

```
>FOXA1 _ 1
CTACAGCTGTTTCCTTGTCATCAGCCTGGGGTGGGTAGTATTTTATCTTAAACGATGCCTGTTTGTCTTACTCTGAGCTAGTCTTAGTGTAAAGAGTCACCTT
TCTATGTACATAGAAACCGTTTGTCTTTATTGACAGAAGCCCTGGAGATGGGTCCCCATGTGACTGTAGGGTTCCTGAAACCTGGCAGGCCACTCTGCTT
>FOXA1 _ 10
TTATTCTGATGTGGTTTTGCGGTTATACAGTAAGCAGCACTGCTTATGTGGACATGGTGTACTTTTCAGTTTCTGAAAGTGAGTCATGTTGACTTTCTCTT
AGGAGTAAGAGTGACCCTTGCTTAAAGGACAACATGACTAGAAGGAAGAAACACCAGAGGCCACCAGGACCAGAATGTTTACCAATGTAGGCAGTCACCTA
>FOXA1 _ 11
AAAGGAGAAACACAGCCAAATAATAAAACAATATCTTCTGTAAAGTAAAGAGTACACCCCTGTTTACCTGGTCGCCACTGTTTATTCTGAAAGACTACACTA
AGCAAATACTGAGCCTGACAGCTAGGCTGGAGGGGAGGGGTCTCTAGGCCACAAAGGTGCAAAGCCCTCTTTCAGATCCATCTCCACCATTTCCTTCAGT
```

Az R-ben így olvassunk be a szekvenciákat és alakítjuk át Biostrings objektummá:

```
library(rGADEM)

fasta.file = system.file('extdata/Test_100.fasta', package='rGADEM')
seqs = readDNAStringSet(fasta.file, 'fasta')
seqs
```

```
A DNAStringSet instance of length 49
      width seq                                     names
[1]    202 CTACAGCTGTTTCCTTGTCATCAG...ACCTGGCAGGCCACTCTGCTTG FOXA1 _ 1
[2]    202 TTATTCTGATGTGGTTTTGCGGT...TTACCAATGTAGGCAGTCACTA FOXA1 _ 10
[3]    202 AAAGGAGAAACACAGCCAAATAA...ATCTCCACCATTTCCTTCAGG FOXA1 _ 11
[4]    202 TGTACCCCCCAATATTTTCATGA...AACACTGAGCCTGGCATTCCAA FOXA1 _ 12
[5]    202 TTTAAGACTGCCACCTGAAATCA...AGAAGACGGGTTGAGCGAGTCA FOXA1 _ 13
...
[45]    202 ATTAGTTTCATGCCAGGCAGGGTT...GTGGTTTGGAAACACCCTCATG FOXA1 _ 5
[46]    202 CCAGAGCCACCACAGCCAGGCCT...CCTGAAACACCTGCTGTTTAA FOXA1 _ 6
[47]    202 CAGATCAGAGCCTGGGAGCGGGC...AGGGAAGGGAGCTGTGGGAGGA FOXA1 _ 7
[48]    202 GGAATGTGATTTACCCAGATAAA...AAACATGTCCACATGGAACCTG FOXA1 _ 8
[49]    202 GGCTTTTCCAAGAACAATAGTGT...TAAAGTCGGGTTGGTCTTGGC FOXA1 _ 9
```

Továbbá szükség van a referenciaként szolgáló genomra:

```
library(BSgenome.Hsapiens.UCSC.hg19)

Hsapiens
```

```
Human genome:
# organism: Homo sapiens (Human)
# provider: UCSC
# provider version: hg19
# release date: Feb. 2009
# release name: Genome Reference Consortium GRCh37
# 93 sequences:
#   chr1          chr2          chr3
#   chr4          chr5          chr6
#   chr7          chr8          chr9
#   chr10         chr11         chr12
#   chr13         chr14         chr15
#   ...          ...          ...
#   chrUn_g1000235 chrUn_g1000236 chrUn_g1000237
#   chrUn_g1000238 chrUn_g1000239 chrUn_g1000240
#   chrUn_g1000241 chrUn_g1000242 chrUn_g1000243
#   chrUn_g1000244 chrUn_g1000245 chrUn_g1000246
#   chrUn_g1000247 chrUn_g1000248 chrUn_g1000249
```

(continues on next page)

(folytatás az előző oldalról)

```
# (use 'seqnames()' to see all the sequence names, use the '$' or '[' operator
# to access a given sequence)
```

A két forrásállományt a GADEM-függvénnyel elemezhetjük:

```
gadem.res = GADEM(seqs, genome=Hsapiens)
```

```
top 3 4, 5-mers: 10 28 52
top 3 4, 5-mers: 8 24 44
```

```
gadem.res
```

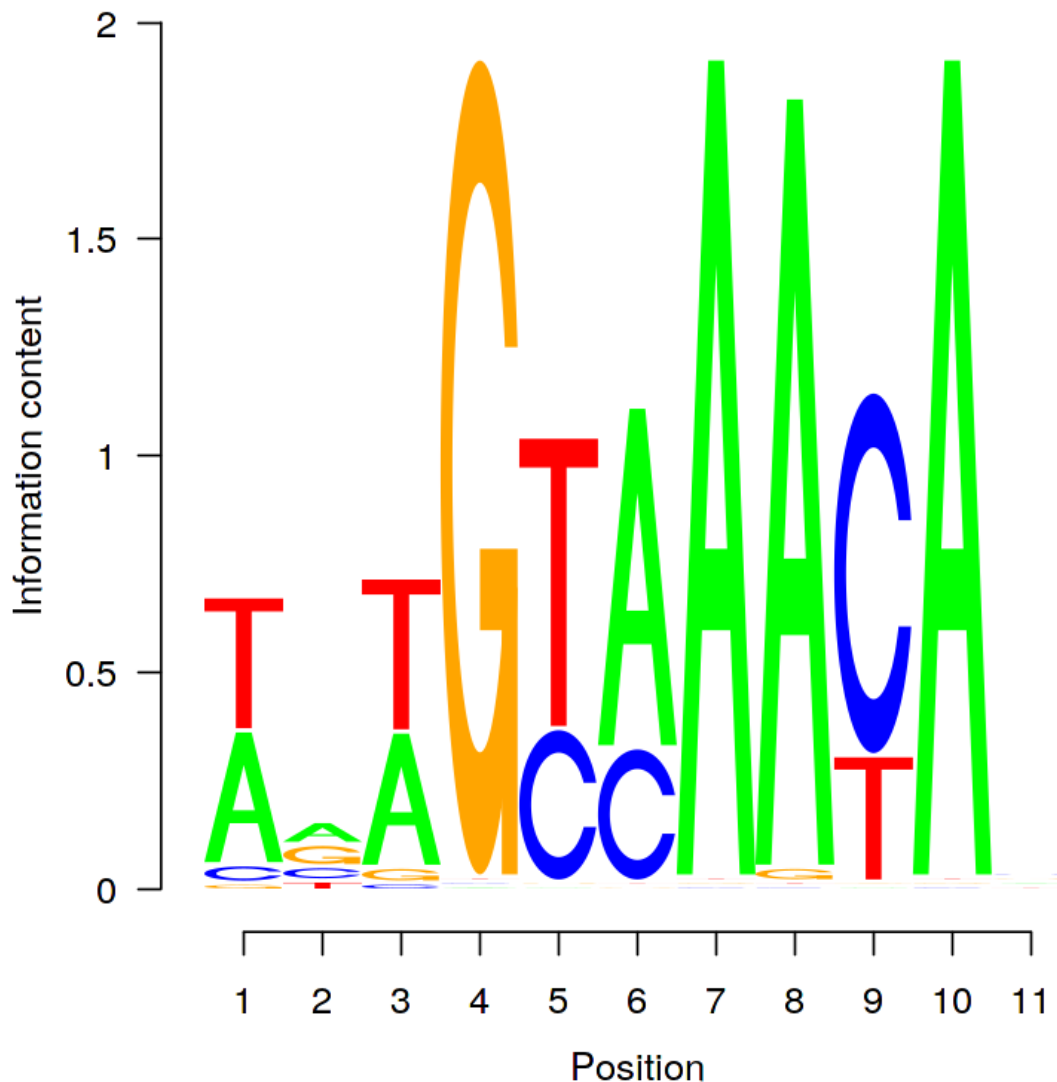
```
Object of class 'gadem'
This object has the following slots:
motifs,pwm,consensus,align,name,seq,chr,start,end,strand,seqID,pos,pval,fastaHeader
```

A számítások eredményeként az „új motifra” vonatkozóan létrejött objektumból többek között kiolvasható a PWM:

```
getPWM(gadem.res)
```

A gadem.res-objektumból közvetlenül létrehozhatunk szekvencialogót az rGADEM-könyvtár saját plot()-függvényével, azonban ez nem túl rugalmas, így az ábrázolásban inkább érdemes a seqLogo-könyvtár függvényét használni:

```
pwm.m = getPWM(gadem.res)[[1]]
seqLogo::seqLogo(pwm.m)
```



4.4.1 motifStack

Több motif együttes ábrázolására hozták létre a motifStack-csomagot (Ou & Zhu, 2017). A csomag függvényei nagy rugalmasságot nyújtanak a vizualizációra, ezek közül egy egyszerűbb példa azt mutatja be, hogy egymással valamilyen szintű hasonlóságot mutató motifokat hogyan tudjuk megjeleníteni:

```
opts = list(species=9606, name=c('GATA2', 'PHOX2A', 'HOXD13'), all_versions=T)
qres = getMatrixSet(JASPAR2016, opts)

library(motifStack)

pfm.lst = list()
for(i in length(qres):1){
```

(continues on next page)

4.5 TFBS-en belüli SNP-k hatásának predikciója

Ahogy a genomban általánosságban, úgy a TF-kötőhelyeken is bekövetkeznek szekvenciális változások. Ennek ismeretében könnyen merül fel az a kérdés, hogy ezek a változások hogyan befolyásolják a génexpressziót? A kérdés megválaszolásához kapcsolódó motifbreakR-csomag (Coetzee et al., 2015) segítséget nyújt annak eldöntésében, hogy a polimorfizmus/mutációkörüli szekvencia jó kötést biztosít-e, illetve, hogy a megváltozott allél információvesztéssel, vagy -nyereséggel jár-e.

4.5.1 Polimorfizmus adatok

Szekvencia polimorfizmusokra, variánsokra vonatkozóan számos adatbázis áll rendelkezésre. Ilyen pl. az NCBI dbSNP-je, amelyből a Bioconductor-on elérhetünk számos verziót:

```
library(BSgenome)

available.SNPs()
```

Míg az SNPlocs-állományok csak SNP-ket (single nucleotide-polymorphism) tartalmaznak, addig az XtraSNPlocs-állományokban további molekuláris változatok (pl. in-del, multinucleotide-polymorphism) találhatók. Az SNPlocs-állományokból több módon lekérdezhetők SNP-k, pl. azonosító alapján:

```
library(SNPlocs.Hsapiens.dbSNP150.GRCh38)

snpsById(SNPlocs.Hsapiens.dbSNP150.GRCh38, 'rs7837328', ifnotfound='drop')
```

```
GPos object with 1 position and 2 metadata columns:
  seqnames      pos strand | RefSNP_id alleles_as_ambig
      <Rle> <integer> <Rle> | <character>      <character>
[1]          8 127410882      * |   rs7837328              R
-----
seqinfo: 25 sequences (1 circular) from GRCh38.p7 genome
```

Az rs7837328 azonosítójú SNP a lekérdezés szerint a 8. kromoszómán helyezkedik el, és vagy A vagy G lehet. Az SNP-azonosítót használhatjuk az rs eltag nélkül is, akár karakterként, akár egész számként. Lekérdezhetjük azt is, hogy egy adott szekvenciaszakasz tartalmaz-e ismert SNP-t. Ehhez először létre kell hoznunk egy GRanges-objektumot amit kereshetünk a SNP-adatbázisban:

```
gr = GRanges(
  seqnames=c('8'),
  ranges=IRanges(127410880, 127410885),
  strand=c('*')
)

snpsByOverlaps(SNPlocs.Hsapiens.dbSNP150.GRCh38, ranges=gr)
```

```
GPos object with 1 position and 2 metadata columns:
  seqnames      pos strand | RefSNP_id alleles_as_ambig
      <Rle> <integer> <Rle> | <character>      <character>
[1]          8 127410882      * |   rs7837328              R
-----
seqinfo: 25 sequences (1 circular) from GRCh38.p7 genome
```

Az XtraSNPlocs-állományokból hasonlóan olvashatunk ki adatokat. A 8. kromoszómán azonosított variánsok pl.:

```
q = snpsBySeqname(
  XtraSNPlocs.Hsapiens.dbSNP144.GRCh38,
  'ch8',
  columns=c('RefSNP_id', 'snpClass')
)
q
```

```
GRanges object with 606507 ranges and 2 metadata columns:
      seqnames      ranges strand |   RefSNP_id snpClass
      <Rle>        <IRanges> <Rle> | <character> <factor>
[1]      ch8      [61481, 61482]   + | rs200678772   in-del
[2]      ch8      [61793, 61793]   + | rs752636065   in-del
[3]      ch8      [70901, 70901]   + | rs201602045   in-del
[4]      ch8      [70950, 70950]   + | rs767986677   in-del
[5]      ch8      [73088, 73088]   + | rs750775551   in-del
...      ...      ...           ... | ...         ...
[606503] ch8 [145076006, 145076005] + | rs145524841   in-del
[606504] ch8 [145076464, 145076463] + | rs199775790   in-del
[606505] ch8 [145077216, 145077215] + | rs200565575   in-del
[606506] ch8 [145078174, 145078173] + | rs574365224   in-del
[606507] ch8 [145078175, 145078174] + | rs539896939   in-del
-----
seqinfo: 25 sequences (1 circular) from GRCh38.p2 genome
```

A variánsok típusainak megoszlása a 8. kromoszómán:

```
table(elementMetadata(q)$snpClass)
```

```

      in-del      heterozygous
      596293      0
      microsatellite      named-locus
      215      0
      no-variation      mixed
      0      0
multinucleotide-polymorphism
      9999
```

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4.7 Session info

```
sessionInfo()
```

```
R version 3.4.3 (2017-11-30)
Platform: x86_64-pc-linux-gnu (64-bit)
Running under: Ubuntu 16.04.4 LTS

Matrix products: default
BLAS: /usr/local/lib/R/lib/libRblas.so
LAPACK: /usr/local/lib/R/lib/libRlapack.so

locale:
```

(continues on next page)

(folytatás az előző oldalról)

```

[1] LC_CTYPE=en_US.UTF-8      LC_NUMERIC=C
[3] LC_TIME=hu_HU.UTF-8       LC_COLLATE=en_US.UTF-8
[5] LC_MONETARY=hu_HU.UTF-8   LC_MESSAGES=en_US.UTF-8
[7] LC_PAPER=hu_HU.UTF-8      LC_NAME=C
[9] LC_ADDRESS=C              LC_TELEPHONE=C
[11] LC_MEASUREMENT=hu_HU.UTF-8 LC_IDENTIFICATION=C

attached base packages:
[1] grid      stats4    parallel  stats      graphics  grDevices  utils
[8] datasets  methods  base

other attached packages:
[1] XtraSNPlocs.Hsapiens.dbSNP144.GRCh38_0.99.12
[2] rGADEM_2.26.0
[3] PWMEnrich_4.14.0
[4] BSgenome.Hsapiens.UCSC.hg19_1.4.0
[5] seqLogo_1.44.0
[6] JASPAR2016_1.6.0
[7] JASPAR2014_1.14.0
[8] MotifDb_1.20.0
[9] TFBSTools_1.16.0
[10] SNPlocs.Hsapiens.dbSNP150.GRCh38_0.99.20
[11] BSgenome_1.46.0
[12] rtracklayer_1.38.3
[13] Biostrings_2.46.0
[14] XVector_0.18.0
[15] GenomicRanges_1.30.3
[16] GenomeInfoDb_1.14.0
[17] IRanges_2.12.0
[18] S4Vectors_0.16.0
[19] BiocGenerics_0.24.0

loaded via a namespace (and not attached):
[1] httr_1.3.1                Biobase_2.38.0
[3] VGAM_1.0-5                bit64_0.9-7
[5] jsonlite_1.5              splines_3.4.3
[7] R.utils_2.6.0             gtools_3.5.0
[9] blob_1.1.1                GenomeInfoDbData_1.0.0
[11] Rsamtools_1.30.0          DirichletMultinomial_1.20.0
[13] pillar_1.2.1              RSQLite_2.1.0
[15] lattice_0.20-35           uuid_0.1-2
[17] digest_0.6.15             colorspace_1.3-2
[19] Matrix_1.2-13             R.oo_1.21.0
[21] plyr_1.8.4                XML_3.98-1.10
[23] pkgconfig_2.0.1           zlibbioc_1.24.0
[25] xtable_1.8-2              GO.db_3.5.0
[27] scales_0.5.0              gdata_2.18.0
[29] BiocParallel_1.12.0       tibble_1.4.2
[31] annotate_1.56.2           KEGGREST_1.18.1
[33] ggplot2_2.2.1             SummarizedExperiment_1.8.1
[35] repr_0.12.0               TFMPvalue_0.0.6
[37] lazyeval_0.2.1           splitstackshape_1.4.4
[39] magrittr_1.5              crayon_1.3.4
[41] memoise_1.1.0             powerlaw_0.70.1
[43] evaluate_0.10.1           R.methodsS3_1.7.1
[45] CNEr_1.14.0              BiocInstaller_1.28.0
[47] data.table_1.10.4-3       tools_3.4.3

```

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(folytatás az előző oldalról)

```
[49] hms_0.4.2                matrixStats_0.53.1
[51] stringr_1.3.0             munsell_0.4.3
[53] DelayedArray_0.4.1        AnnotationDbi_1.40.0
[55] compiler_3.4.3            evd_2.3-2
[57] caTools_1.17.1            rlang_0.2.0
[59] RCurl_1.95-4.10           pbdZMQ_0.3-2
[61] IRkernel_0.8.12.9000      bitops_1.0-6
[63] gtable_0.2.0              DBI_0.8
[65] reshape2_1.4.3            R6_2.2.2
[67] GenomicAlignments_1.14.2  bit_1.1-12
[69] readr_1.1.1               stringi_1.1.7
[71] IRdisplay_0.4.4           Rcpp_0.12.16
[73] png_0.1-7
```

5. fejezet

Indexek és táblázatok

- genindex
- modindex
- search