mageri Documentation

Release 1.0.0

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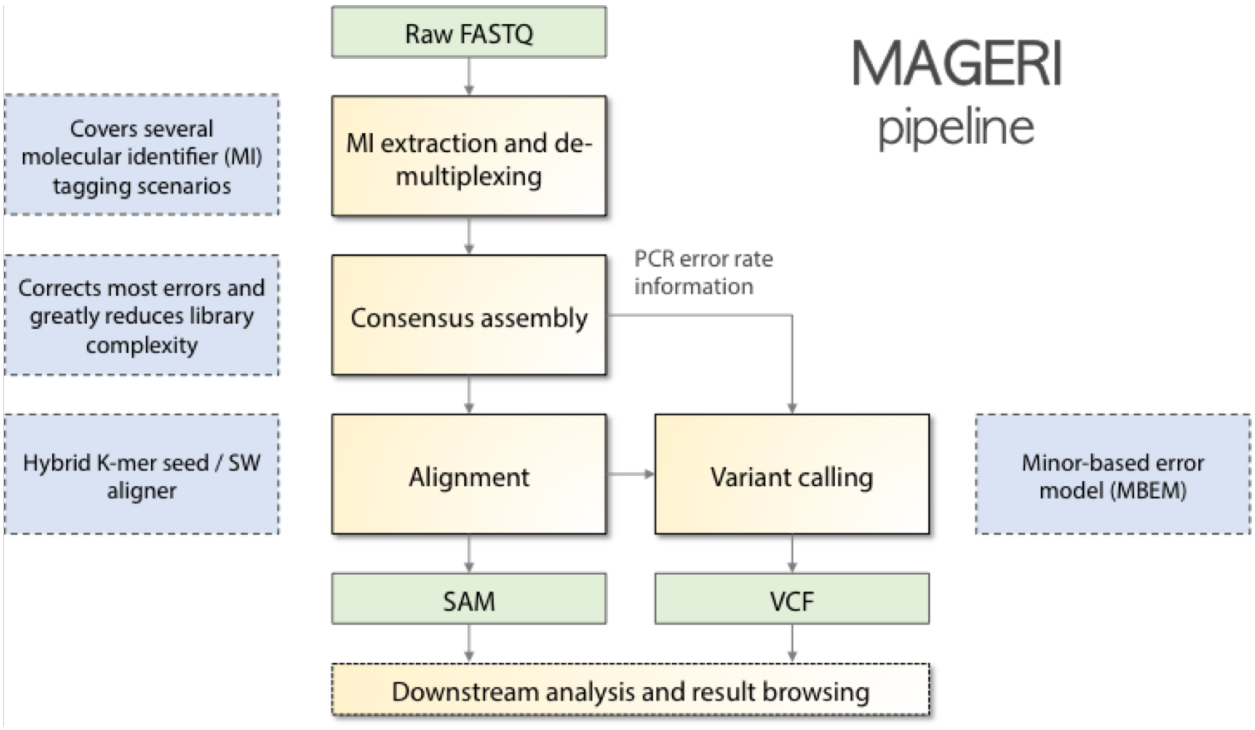
May 08, 2017

Contents



MAGERI is an all-in-one software for analysis of targeted genome re-sequencing data for libraries prepared with novel unique molecular identifier tagging technology. Starting from raw sequencing reads, MAGERI extracts UMI sequences, performes primer and adapter matching and trimming, assembles molecular consensuses, alignes them to reference sequences and calls variants. MAGERI output is provided in conventional SAM and VCF formats, so it can be browsed and post-processed by the majority of conventional bioinformatics software.

CHAPTER 1

Terminology

- UMI - unique molecular identifier, a short (4-20bp) degenerate nucleotide sequence, that is attach to cDNA/DNA molecules in order to trace them throughout the entire experiment.
- Sample barcode - a short specific nucleotide sequence used to mark cDNA/DNA molecules that correspond to a given sample in a pooled sequencing library
- MIG - molecular identifier group, a set of reads or read pairs that have an identical UMI sequence
- MIG consensus - the consensus sequence of MIG, that is, the consensus of multiple alignment of all reads in a given MIG
- CQS - consensus quality score, calculated as the fraction of reads matching the consensus sequence at a given position. Can be scaled to `[2, 40]` range to fit Phred33 quality representation.
- Major variant (aka dominant variant, supermutant) - a sequence variant that is present in MIG consensus, but doesn't match the reference sequence
- Minor variant - a sequence variant that differs from the consensus sequence found in one or more reads within a given MIG

CHAPTER 2

Table of contents

Installation and running

MAGERI is distributed in a form of executable JAR file and requires Java v1.8 to run. Source code and binaries can be found in corresponding repository. The latest release can be found here. MAGERI can be executed by running

```
java -Xmx32G -jar mageri.jar [arguments]
```

The `-Xmx32G` option sets the memory usage limit that should be enough for most datasets. The list of arguments is given by `-h` option and described below as well.

option	argument	description
`-R1`	fastq[.gz]	First read file.
`-R2`	fastq[.gz]	Second read file [optional].
`--platform`	string	Platform: Illumina, IonTorrent or Roche454 [default = Illumina].
`--library-type`	string	Library prep start, SS (single-stranded, linear PCR) or DS (double-stranded) [default=SS].
`--project-name`	string	Project name [optional].
`--sample-name`	string	Sample name [optional].
`-O`	path	Path to output folder [default = current folder].
`-M1`	metadata file	De-multiplexing, primer trimming and barcode extraction using specified metadata file. See *Multiplex (M1)*
`-M2`	metadata file	Primer trimming and barcode extraction using specified metadata file. See *Primer (M2)*
`-M3`	mask1[:mask2]	Positional UMI extraction. See *Positional (M3)*
`-M4`		Preprocessed data, UMI information is stored in header. See *Header (M4)*
`--references`	FASTA file	File with reference sequences. See *Sequences*
`--bed`	BED file	Genomic coordinates of reference sequences [optional]. See *Coordinates*
`--contigs`	metadata file	File with contig names, lengths, and assembly id [optional]. See *Contigs*

Important: One of `M1-4` options should be specified. While `--bed` and `--contigs` parameters are optional, if not specified, resulting SAM and VCF files could not be directly annotated and visualized using data from corresponding

genome assembly.

Input

MAGERI accepts paired or single-end raw sequencing reads in FASTQ format with Phred33 quality encoding, either uncompressed or GZIP-compressed. To run the pipeline, user should specify pre-processing options which tell the software how to extract UMI sequences and sample barcodes in case the latter are incorporated into the adapter sequence. Additionally, genomic information for reference sequences can be provided to be used during mapping and VCF/SAM file generation.

Note: Genomic information bundle for genes from Cancer Gene Census (*hg38* genome assembly) is available here. This bundle contains all exons with +/-50 base flanks. Additional instructions to create genomic information bundle for your own list of genes are given below.

Pre-processing

Four de-multiplexing modes are available in MAGERI to handle the majority of possible library designs.

Multiplex (M1)

In this mode it is assumed that samples are multiplex with barcodes in adapter sequences that additionally cary a UMI tag. In this case tab-delimited barcodes table should be provided, e.g.:

sample_name	master_first	master_adapter	slave_adapter
Sample1	0	NNNNNNNNNNNNNctctcATGC	GATTTttcaNNNNNNNNNNNNN
Sample2	1	NNNNNNNNNNNNNtgaaATAGC	GCATgaga-gaNNNNNNNNNNNNN
Sample3	1	NNNNNNNNNNNNNt-gaaTAGCA	GCATgaga-gaNNNNNNNNNNNNN
Sample4	1	NNNNNNNNNNNNNtgaaATAGC	
...			

Samples are de-multiplexed based on master adapter sequence and filtered for matching slave adapter sequence in case it is provided (paired-end data). Master adapter is first searched in both read#1 and read#2. Then the mate read of the master adapter-containing read is reverse-complemented and searched for slave adapter sequence if it is provided. Simply speaking, master and slave adapter sequences should be provided as if they were on the same strand.

After matching and UMI extraction, reads are oriented to be on the same strand and adapter sequences are trimmed. If `master_first` is set to `0` reads are swapped and reverse-complemented. De-multiplexed samples are further analyzed separately.

The following rules apply to master and slave adapter sequence specification:

- Slave adapter sequence could be omitted, master adapter sequence should be unique for each sample.
- Adaptor sequence can contain any IUPAC DNA letters.
- Upper and lower case letters mark seed (exact match) and fuzzy-search region parts respectively.
- *N* characters mark UMI region to be extracted.
- Multiple rows could correspond to the same sample

Primer (M2)

This is a variant of `-M1` mode that extracts UMIs and removes primer sequences, but processes all reads together as if they were coming from the same sample, e.g.

region_name	master_first	left_primer	right_primer
ARAF_E7_F	1	NNNNNNNNNNNNNactgtGACCCGGAgcact	cacaGGGCAGAGggtagag
BRAF_E15_F	1	NNNNNNNNNNNNNcataaTGCTTGCTctgatagga	ggagTGGGTCCCatcagttt
...			

Positional (M3)

This mode is specified by one or two masks which are used to scan the first read and the reverse complement of the second read. As always, *N* characters are used to specify UMI positions. Nucleotides (including ambiguity codes) require exact match, while *n* characters are used to specify offset. For example

- `-M3 NNNNN` will use first 5 bases of read#1 as UMI.
- `-M3 nnnNNNNN` will use bases from 4 to 8 of read#1 as UMI.
- `-M3 nnnNNNNNatgc` will scan read#1 for *nnnNNNNNatgc*, *nnNNNNNatgc*, *nNNNNNatgc* and *NNNNNatgc* until matching the *atgc* string.
- `-M3 NNNNN:NNNNN` will extract first 5 bases of read#1 and last 5 bases of reverse complement of read#2.

Warning: This mode should be used with care for non-oriented reads, as only one read pair orientation will be scanned.

Header (M4)

If this mode is specified, it is assumed that FASTQ files contain *UMI:NNN:QQQ* entry in read headers, separated by tab or space from other header entries. Here *NNN* are UMI nucleotides and *QQQ* are corresponding quality Phred scores.

Note: In case working with a large set of primers/adapters, it is common to misspecify several of them. It is advised to first manually check for primer extraction efficiency and troubleshoot incorrect ones. To do so for both `M1` and `M2` cases, run MAGERI in `M1` mode and tell it to take only a fraction of reads, say 10000, with `--limit 10000` and inspect resulting `*.checkout.txt` output file to see if any of the primer sequences were not extracted. To figure out real primer sequences one can run in the `M3` mode specifying only UMI positions and then check resulting SAM files in IGV to get sequences of corresponding regions. Those sequences can then be manually checked against the primer set to correct errors in primer sequences.

Genomic information

Sequences

MAGERI requires a reference file in FASTA format to run the alignment and variant calling. Note that by default more than 30% of MIG consensus sequence should align to targeted region, so ideally adapter/primer trimming

is recommended. In case of targeted capture (e.g. exome sequencing), upstream and downstream regions (`+/- readlength/2` bases) of exons should be included. Typical reference FASTA file should look like

```
>HER3_E2
CGGCGATGCTGAGAACCAATACCAGACACTGTACAAGCTCTACGAGAGGTGTGAGGTGGTGATGGGGAACCTTGAGATTGTGCTCACGGGAC
>HER3_E3
CTATGTCCTCGTGGCCATGAATGAATTCTCTACTCTACCATTGCCCAACCTCCGCGTGGTGCGAGGGACCCAGGTCTACGATGGGAAGTTTGCCATCTTCC
>HER3_E6
TTCTCTCCTTCCATAGTGACCAAGACCATCTGTGCTCCTCAGTGTAATGGTCACTGCTTTGGGCCCAACCCCAACCAGTGCTGCCATGATGAGTGTGCCGC
>HER3_E7
CCACAGCCTCTTGTCTACAACAAGCTAACTTTCCAGCTGGAACCCAATCCCCACACCAAGTATCAGTATGGAGGAGTTTGTGTAGCCAGCTGTCCCCGTAA
>HER3_E8
TCATCTCTAATGGTGTCCTCCTCCTCTTCCCTAGATAACTTTGTGGTGGATCAAACATCCTGTGTCAGGGCCTGTCCTCCTGACAAGATGGAAGTAGATAA
>HER3_E9
GGGAACAGGCTCTGGGAGCCGCTTCCAGACTGTGGACTCGAGCAACATTGATGGATTTGTGAACTGCACCAAGATCCTGGGCAACCTGGACTTTCTGATCA
>HER3_E21
TACAGGGAATGTACTACCTTGAGGAACATGGTATGGTGCATAGAAACCTGGCTGCCCGAAACGTGCTACTCAAGTCACCCAGTCAGGTTCAGGTGGCAGAT
>HER3_E23
TTCCTGCAACAGGTGTGACAGTTTGGGAGTTGATGACCTTCGGGGCAGAGCCCTATGCAGGGCTACGATTGGCTGAAGTACCAGACCTGCTAGAGAAGGGC
```

Coordinates

In order to make output feasible for post-analysis and visualization, a BED file containing genomic coordinates of references should be included. For the example FASTA file above it should be

```
#chr start   end     name    unused  strand
chr12        56477568        56477659        HER3_E2 0       +
chr12        56478798        56478924        HER3_E3 0       +
chr12        56481562        56481701        HER3_E6 0       +
chr12        56481849        56481966        HER3_E7 0       +
chr12        56482292        56482447        HER3_E8 0       +
chr12        56482538        56482639        HER3_E9 0       +
chr12        56491563        56491703        HER3_E21        0       +
chr12        56492530        56492673        HER3_E23        0       +
```

Note: FASTA entries that do not have corresponding BED rows will be skipped from SAM and VCF output.

Note: The most straightforward way (in my experience) to generate FASTA and BED files is to use ENSEMBL Biomart. Specify your gene identifiers in the `Filters` section and choose `Sequences` as output mode. Don't forget to manually add flanking bases count (in case you specify them) to BED file as they're not accounted for in Biomart output. Importantly, ENSEMBL coordinates are 1-based, while BED format is 0-based, so adjust appropriately by subtracting 1 from start coordinate in BED.

A step-by-step instruction on getting your references from Ensembl BioMart is given below:

- Go to Martview.
- In the dataset section select **Ensembl genes 84** and choose **Homo sapiens genes**.
- In the filter section and **Gene** subsection, input your gene IDs to **external references ID** textbox and select appropriate nomenclature (e.g. **HGNC symbol** when using gene symbols).
- In attributes section select **Sequences** and untick all header information features.

- Under the **SEQUENCES** menu select **Exon sequences** and input the 5' and 3' flank base count, e.g. 50 (it should be the same for both 5' and 3' flank). This is to ensure that reads produced by exome capture techniques are fully mapped.
- Select the following features (order matters here): **Associated Gene Name**, **Ensembl Exon ID**, **Chromosome Name**, **Exon Chr Start (bp)**, **Exon Chr End (bp)** and **Strand**.
- Go to results, select **unique results only** and download the resulting FASTA file (save it as `biomart_refs.fa`).
- Download and run the following script (requires Groovy to be installed): `groovy ExtractBedFormBiomartRefs.groovy biomart_refs.fa refs.fa refs.bed 50`. The last argument specifies the flank size.
- You can now supply resulting `refs.fa`, `refs.bed` and this contigs file when running the pipeline.

Contigs

Genome assembly metadata file is required to create SAM and VCF file headers, here is an example tab-delimited table for *hg19* genome assembly

```
#chrom      assembly        length
chr12       hg19    133851895
```

Again, contig names (*chr12*,...) and coordinates in BED file should be concordant with assembly metadata file.

Note: If assembly for a given contig is named *PRIVATE*, corresponding results will be skipped SAM and VCF output (but not from internal MAGERI output files).

Output

MAGERI generates multiple internal output files summarizing the results of each pipeline step:

1. `*.checkout.txt` - de-multiplexing and UMI extraction yield
2. `*.umi.histogram.txt` - MIG size distribution
3. `*.assemble.txt` - MIG consensus assembly efficiency; `*.assemble.R1/2.fastq.gz` - assembled consensus sequences in FASTQ format with CQS quality scores
4. `*.mapper.txt` - MIG consensus mapping statistics for each reference
5. `*.variant.caller.txt` - tab-delimited file with variant calls (in original reference coordinates, not genomic ones)

Those files are useful for analysis quality control, for example, `*.checkout.txt` should be monitored to ensure correct primer specificaiton and `*.umi.histogram.txt` should contain a clear peak that can be thresholded with `5+` reads per UMI to check if library prep yields optimal starting molecule coverage.

Additionally, mapping and variant calling results are provided in SAM and VCF formats

Example SAM output:

```
@HD     VN:1.0  SO:unsorted     GO:query
@SQ     SN:chr1 LN:249250621    AS:hg19
@SQ     SN:chr2 LN:243199373    AS:hg19
@SQ     SN:chr3 LN:198022430    AS:hg19
```

```
@SQ     SN:chr4 LN:191154276    AS:hg19
@SQ     SN:chr5 LN:180915260    AS:hg19
@SQ     SN:chr6 LN:171115067    AS:hg19
@SQ     SN:chr7 LN:159138663    AS:hg19
@SQ     SN:chr8 LN:146364022    AS:hg19
@SQ     SN:chr9 LN:141213431    AS:hg19
@SQ     SN:chr10        LN:135534747    AS:hg19
@SQ     SN:chr11        LN:135006516    AS:hg19
@SQ     SN:chr12        LN:133851895    AS:hg19
@SQ     SN:chr13        LN:115169878    AS:hg19
@SQ     SN:chr14        LN:107349540    AS:hg19
@SQ     SN:chr15        LN:102531392    AS:hg19
@SQ     SN:chr16        LN:90354753     AS:hg19
@SQ     SN:chr17        LN:81195210     AS:hg19
@SQ     SN:chr18        LN:78077248     AS:hg19
@SQ     SN:chr19        LN:59128983     AS:hg19
@SQ     SN:chr20        LN:63025520     AS:hg19
@SQ     SN:chr21        LN:48129895     AS:hg19
@SQ     SN:chr22        LN:51304566     AS:hg19
@SQ     SN:chrX LN:155270560    AS:hg19
@SQ     SN:chrY LN:59373566     AS:hg19
@RG     ID:3    SM:h1-1 PU:h1-1 LB:p126-1       PL:ILLUMINA
@PG     ID:mageri    VN:1.0.0  CL:mageri-1.0.0.jar -I project-1.json -O output/
TGTATATCCCCTGA  16      chr1    115258663       30      20S131M22S      *       0
↪  0
↪AGGTCAGCGGGCTACCACTGGGCCTCACCTCTATGGTGGGATCATATTCATCTACAAAGTGGTTCTGGATTAGCTGGATTGTCAGTGCGCTTTTCCCAA
↪
↪GHGGHHHHHHHHHHHHHHHHHHHHHHHHIHIHHHHHHHHHHHHHHIHHHHHIHIHIHHIHHHHHIIIHHIHHHHIHHHHHHHHHHHHHHHHHHHHH
↪       RG:Z:3
GTGTAATTAAATGA  0       chr2    209113093       28      22S103M21S      *       0
↪  0
↪CATTATTGCCAACATGACTTACTTGATCCCCATAAGCATGACGACCTATGATGATAGGTTTTACCCATCCACTCACAAGCCGGGGGATATTTTTGCAGA
↪
↪HHHIHHHHHIHIHIIHHHIIHIIHIHIIHHHGIIIHIHHHHIHHHHHHHHHHHHHHIHHIIIHHHHHIHHHHHHHIHHHHHHHHHHIIIHIIHHIHH
↪      RG:Z:3
```

Example VCF output:

```
##fileformat=VCFv4.0
##fileDate=Tue Jun 02 05:30:36 GMT+03:00 2015
##source=mageri-1.0.0
##reference=file:///data/misha/P126/meta/refs.fa
##contig=<ID=chr1,assembly=hg19,length=249250621>
##contig=<ID=chr2,assembly=hg19,length=243199373>
##contig=<ID=chr3,assembly=hg19,length=198022430>
##contig=<ID=chr4,assembly=hg19,length=191154276>
##contig=<ID=chr5,assembly=hg19,length=180915260>
##contig=<ID=chr6,assembly=hg19,length=171115067>
##contig=<ID=chr7,assembly=hg19,length=159138663>
##contig=<ID=chr8,assembly=hg19,length=146364022>
##contig=<ID=chr9,assembly=hg19,length=141213431>
##contig=<ID=chr10,assembly=hg19,length=135534747>
##contig=<ID=chr11,assembly=hg19,length=135006516>
##contig=<ID=chr12,assembly=hg19,length=133851895>
##contig=<ID=chr13,assembly=hg19,length=115169878>
##contig=<ID=chr14,assembly=hg19,length=107349540>
##contig=<ID=chr15,assembly=hg19,length=102531392>
##contig=<ID=chr16,assembly=hg19,length=90354753>
```

```
##contig=<ID=chr17,assembly=hg19,length=81195210>
##contig=<ID=chr18,assembly=hg19,length=78077248>
##contig=<ID=chr19,assembly=hg19,length=59128983>
##contig=<ID=chr20,assembly=hg19,length=63025520>
##contig=<ID=chr21,assembly=hg19,length=48129895>
##contig=<ID=chr22,assembly=hg19,length=51304566>
##contig=<ID=chrX,assembly=hg19,length=155270560>
##contig=<ID=chrY,assembly=hg19,length=59373566>
##phasing=none
##INFO=<ID=DP,Number=1,Type=Integer,Description="Total Depth">
##INFO=<ID=AF,Number=.,Type=Float,Description="Allele Frequency">
##INFO=<ID=AA,Number=1,Type=String,Description="Ancestral Allele">
##INFO=<ID=CQ,Number=1,Type=Integer,Description="Assembly quality">
##FILTER=<ID=q20,Description="Quality below 20">
##FILTER=<ID=si10000,Description="Singleton, frequency below 10000">
##FILTER=<ID=c100,Description="Coverage below 100">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##INFO=<ID=DP,Number=1,Type=Integer,Description="MIG Depth">
#CHROM  POS     ID      REF     ALT     QUAL    FILTER  INFO    FORMAT  p126-1.h1-1
chr1    115252206       .       G       A       16      q20     DP=307;AF=0.
↪0032573289;AA=G;CQ=39.0     GT:DP   0/1:307
chr1    115258758       .       C       T       383     .       DP=542;AF=0.040590405;
↪AA=C;CQ=39.0       GT:DP   0/1:542
```

Those files can be further used in downstream analysis. For example, SAM files can be viewed in IGV browser, while VCF files can be annotated with SnpEff. It is always recommended to inspect variant calls and alignment data in IGV to ensure there are no alignment artefacts.

Example

A toy example dataset can be downloaded from here. Unpack it and run the following command:

```
java -jar mageri.jar -M2 primers.txt --references refs.fa -R1 example_R1.fastq.gz -R2␣
↪example_R2.fastq.gz out/
```

The resulting VCF file should contain `31:T>C`, `88:T>C` and `89:T>C` variants. Next, SAM files can be converted to BAM format, sorted and indexed using samtools. Indexed BAM files can be browsed in Integrative Genome Viewer using `refs.fa` as user-provided genome. Manual inspection of alignments should reveal that mutations at positions 31 and 88 are linked:

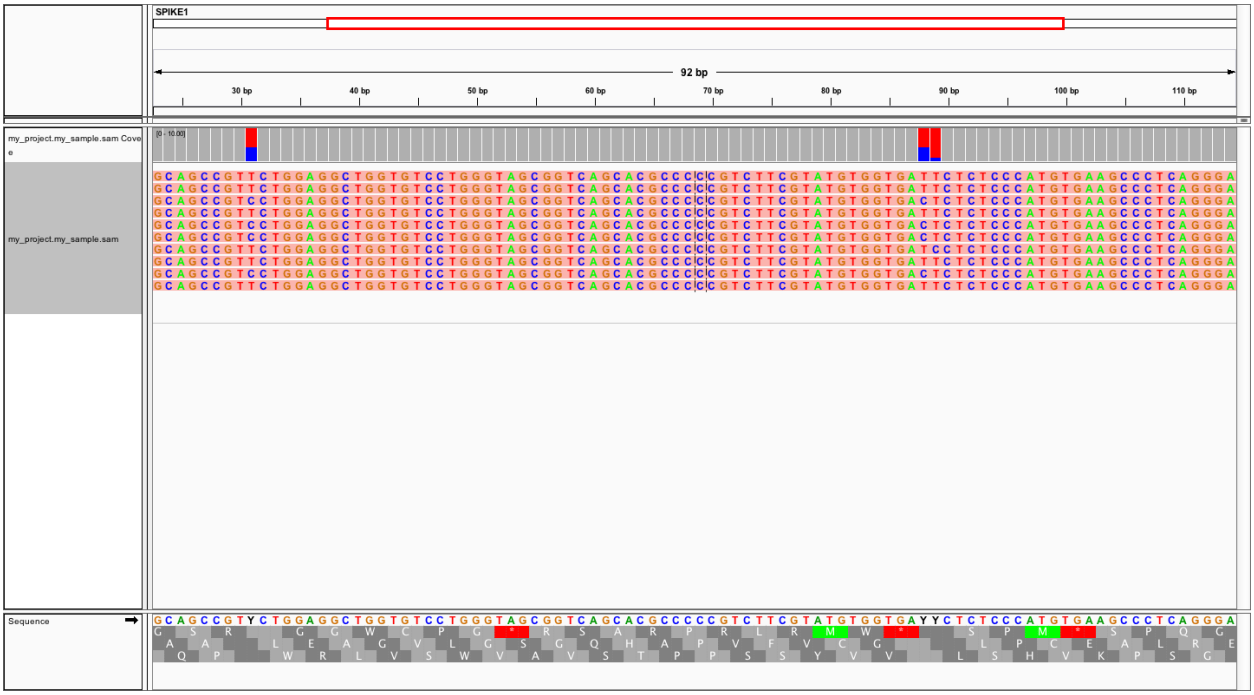
In order to evaluate MAGERI software and learn its capabilities I recommend checking out the mageri-paper repository containing real-world datasets, metadata and shell scripts that can be used to run MAGERI analysis.

Advanced

Presets

Importing and exporting parameters

By default MAGERI runs with pre-configured and optimized parameters, so change them only if you know what you are doing. The parameter config can be changed by exporting, modifying and re-importing corresponding XML file:




```
java -jar mageri.jar --export-preset my_preset.xml
gedit my_preset.xml
...
java -Xmx64G -jar mageri.jar --import-preset my_preset.xml [arguments]
```

Note that the `--platform` and `--library-type` arguments can be specified to alter the default preset. The content of the default XML config file is given below:

```
<?xml version="1.0" encoding="UTF-8"?>
<MageriPresets>
  <version>1.0.1-SNAPSHOT</version>
  <platform>ILLUMINA</platform>
  <libraryType>SS</libraryType>
  <DemultiplexParameters>
    <orientedReads>false</orientedReads>
    <maxTruncations>2</maxTruncations>
    <maxGoodQualMMRatio>0.05</maxGoodQualMMRatio>
    <maxLowQualityMMRatio>0.1</maxLowQualityMMRatio>
    <lowQualityThreshold>20</lowQualityThreshold>
  </DemultiplexParameters>
  <PreprocessorParameters>
    <umiQualThreshold>10</umiQualThreshold>
    <goodQualityThreshold>30</goodQualityThreshold>
    <trimAdapters>true</trimAdapters>
    <minUmiMismatchRatio>20.0</minUmiMismatchRatio>
    <forceOverseq>false</forceOverseq>
    <defaultOverseq>5</defaultOverseq>
  </PreprocessorParameters>
  <AssemblerParameters>
    <offsetRange>4</offsetRange>
    <anchorRegion>8</anchorRegion>
    <maxMMs>4</maxMMs>
    <maxConsequentMMs>0</maxConsequentMMs>
```

```
    <maxDroppedReadsRatio>0.3</maxDroppedReadsRatio>
    <maxDroppedReadsRatioAfterRescue>0.0</maxDroppedReadsRatioAfterRescue>
    <maxTrimmedConsensusBasesRatio>0.3</maxTrimmedConsensusBasesRatio>
    <minMatchedBasesInRealignedReadRatio>0.0</minMatchedBasesInRealignedReadRatio>
    <pcrMinorTestPValue>0.001</pcrMinorTestPValue>
    <cqsRescue>false</cqsRescue>
    <qualityTrimming>true</qualityTrimming>
    <greedyExtend>true</greedyExtend>
  </AssemblerParameters>
  <ReferenceLibraryParameters>
    <splitLargeReferences>true</splitLargeReferences>
    <maxReferenceLength>1000</maxReferenceLength>
    <readLength>100</readLength>
  </ReferenceLibraryParameters>
  <ConsensusAlignerParameters>
    <k>11</k>
    <matchReward>1</matchReward>
    <mismatchPenalty>-3</mismatchPenalty>
    <gapOpenPenalty>-6</gapOpenPenalty>
    <gapExtendPenalty>-1</gapExtendPenalty>
    <minIdentityRatio>0.9</minIdentityRatio>
    <minAlignedQueryRelativeSpan>0.7</minAlignedQueryRelativeSpan>
    <muationCqsThreshold>30</muationCqsThreshold>
    <useSpacedKmers>true</useSpacedKmers>
  </ConsensusAlignerParameters>
  <VariantCallerParameters>
    <noIndels>false</noIndels>
    <qualityThreshold>20</qualityThreshold>
    <singletonFrequencyThreshold>10000</singletonFrequencyThreshold>
    <coverageThreshold>100</coverageThreshold>
    <errorModelType>MinorBased</errorModelType>
    <modelOrder>1.0</modelOrder>
    <modelCycles>20.0</modelCycles>
    <modelEfficiency>1.8</modelEfficiency>
    <modelCoverageThreshold>100</modelCoverageThreshold>
    <modelMinorCountThreshold>10</modelMinorCountThreshold>
    <substitutionErrorRateMatrix>0.0,1.0E-6,1.0E-6,1.0E-6;1.0E-6,0.0,1.0E-6,1.0E-6;1.
↪0E-6,1.0E-6,0.0,1.0E-6;1.0E-6,1.0E-6,1.0E-6,0.0</substitutionErrorRateMatrix>
  </VariantCallerParameters>
</MageriPresets>
```

Presets are also available for 454 and IonTorrent platforms that are characterized by indel errors at homopolymer regions and therefore require a more robust algorithm for consensus assembly, and therefore different `<AssemblerParameters>` preset:

```
java -jar mageri.jar --platform roche454 --export-preset my_preset.xml
```

Will have the following difference:

```
...
<platform>ROCHE454</platform>
...
<AssemblerParameters>
  <offsetRange>4</offsetRange>
  <anchorRegion>8</anchorRegion>
  <maxMMs>4</maxMMs>
  <maxConsequentMMs>2</maxConsequentMMs>
  <maxDroppedReadsRatio>0.7</maxDroppedReadsRatio>
```

```
  <maxDroppedReadsRatioAfterRescue>0.3</maxDroppedReadsRatioAfterRescue>
  <maxTrimmedConsensusBasesRatio>0.3</maxTrimmedConsensusBasesRatio>
  <minMatchedBasesInRealignedReadRatio>0.5</minMatchedBasesInRealignedReadRatio>
  <pcrMinorTestPValue>0.001</pcrMinorTestPValue>
  <cqsRescue>true</cqsRescue>
  <qualityTrimming>true</qualityTrimming>
  <greedyExtend>false</greedyExtend>
</AssemblerParameters>
...
```

Parameter descriptions

Below goes the description of each parameter that can be changed in configuration XML file.

Preset

- `version` version of software that generated this preset via `--export-preset`.
- `platform` name of the platform for which the preset was generated, specified with `--platform` option during `--export-preset`. Allowed values are `illumina`, `roche454` and `iontorrent`. The preset affects consensus assembler parameters.
- `libraryType` type of library, single-stranded (`SS`) or double-stranded (`DS`), specified with `--library-type` option during `--export-preset`. This affects the consensus assembler and minor-based error model parameters.

De-multiplexing

- `orientedReads` if set to `false` will search both read orientations for UMI in `M1` and `M2` cases, otherwise will search only the original read
- `maxTruncations` maximum number of non-seed nucleotides that fall out the read boundaries (`M1` and `M2` mode)
- `maxGoodQualMMRatio` maximum number of allowd non-seed mismatches with quality greater or equal to `lowQualityThreshold` (`M1` and `M2` mode)
- `maxLowQualityMMRatio` maximum number of allowd non-seed mismatches with quality less than `lowQualityThreshold` (`M1` and `M2` mode)
- `lowQualityThreshold` used in primer/adapter matching see above

Pre-processing

- `umiQualThreshold` UMIs that have at least one base with quality less than that threshold will be discarded
- `goodQualityThreshold` quality threshold used to mask nucleotides for minor-based error model (MBEM) used in variant caller
- `trimAdapters` specifies whether to trim found primer/adapter sequences
- `minUmiMismatchRatio` minimum ratio of reads associated with parent and child UMI sequences, used to filter errors in UMI sequence
- `forceOverseq` specifies whether to enforce `defaultOverseq` threshold or to estimate one from MIG size histogram
- `defaultOverseq` threshold for number of reads in MIGs, used to filter unusable, erroneous and artefact UMIs

Consensus assembly

- `offsetRange` read offsets (from `-offsetRange` to `+offsetRange`) to try when aligning reads
- `anchorRegion` halfsize of region used to compare reads during alignemnt
- `maxMMs` maximum number of mismatches in `anchorRegion`, reads having more that `maxMMs` mismatches in any offset will be dropped
- `maxConsequentMMs` maximum number of consequent mismatches between read and consensus during CQS rescue (see `cqsRescue` below). Reads that fail this filter are likely to contain an indel and are re-aligned to consensus using Smith-Waterman algorithm.
- `maxDroppedReadsRatio` maximum ratio of reads dropped for a consensus to be discarded
- `maxDroppedReadsRatioAfterRescue` maximum ratio of reads dropped after CQS rescue (see `cqsRescue` below) for a consensus to be discarded
- `maxTrimmedConsensusBasesRatio` maximum ratio of bases trimmed from consensus due to poor CQS (see `qualityTrimming` below) for a consensus to be discarded
- `minMatchedBasesInRealignedReadRatio` minimum fraction of matching bases during read re-alignment (see `cqsRescue` below) for a read to be dropped
- `pcrMinorTestPValue` P-value threshold used during PCR-induced minor error calling (see `minor calling`)
- `cqsRescue` perform consensus quality score (CQS) rescue for indel-heavy reads
- `qualityTrimming` trim consensus bases with low consensus quality score which is proportional to the ratio of major base and total base count
- `greedyExtend` specifies whether to compute the initial PWM for maximal span of reads, uses average span if set to `false`

Reference library

- `splitLargeReferences` split references larger than `maxReferenceLength` into partitions to speed up the consensus alignment and decrease its memory footprint.
- `maxReferenceLength` maximum length of reference, beyond which reference will be partitioned
- `readLength` estimate of max read length. In case reference is split, its paritions will contain overlapping regions to ensure that each read coming from a given reference will be fully contained in at least one of its paritions.

Consensus alignment

- `k` k-mer size used by reference mapping algorithm
- `matchReward` match reward used by local alignment algorithm
- `mismatchPenalty` mismatch penalty used by local alignment algorithm
- `gapOpenPenalty` gap open penalty used by local alignment algorithm
- `gapExtendPenalty` gap extend penalty used by local alignment algorithm
- `minIdentityRatio` minimal local alignment identity (accounting for substitutions only) used for filtering
- `minAlignedQueryRelativeSpan` minimal relative span of query sequence that are aligned to reference, used for filtering
- `muationCqsThreshold` consensus quality threshold used to filter unreliable major mutations
- `useSpacedKmers` if set to `true` will use k-mers with the central base set to `N`. This strategy (introduced in Vidjil software) can greatly improve mapping sensitivity while having the same specificity.

Variant calling

- `noIndels` if set to `true` will not attempt to call indel variants
- `qualityThreshold` variant error model quality threshold, used in FILTER field of output VCF file
- `singletonFrequencyThreshold` threshold for ratio between signleton errors and their parent molecules (filters extremely rare errors introduced during UMI attachment), used in FILTER field of output VCF file
- `coverageThreshold` threhsold for variant coverage (number of MIGs), used in FILTER field of output VCF file
- `errorModelType` error model type: `MinorBased` infer error rate from minor PCR errors that are deduced during consensus assembly (see Minor-Based Error Model aka MBEM), `RawData` compute error rate from average variant quality Phred score, or `Custom` that uses error rates defined in `substitutionErrorRateMatrix`
- `modelOrder` order of minor-based error model (MBEM), 1 for signle-stranded and 2 for double-stranded library
- `modelCycles` effective number of PCR cycles used by MBEM
- `modelEfficiency` PCR efficiency value used by MBEM
- `modelCoverageThreshold` coverage threshold that is used in MBEM to decide whether to use error rate inferred at a given position or global error rate for a given substution type (e.g. `A>G`)
- `modelMinorCountThreshold` total number of inferred PCR minors at a given position that is used in MBEM to decide whether to use error rate inferred at a given position or global error rate for a given substution type (e.g. `A>G`)
- `substitutionErrorRateMatrix` a flat representation of substitution error rate matrix: `0,A>G,A>C,A>T;G>A,0,G>C,G>T;C>A,C>G,0,C>T;T>A,T>G,T>C,0`. Used if `Custom` error model is selected.

The parameters you are likely to change under certain conditions:

- `goodQualityThreshold` in case reads are of poor sequencing quality (e.g. MiSeq 300+300 reads)
- `readLength` when analyzing data generated by 454, IonTorrent platfroms and MiSeq long reads
- `forceOverseq` and `defaultOverseq` in case MIG size histogram shows irregular behavior or `5+` reads per UMI coverage cannot be reached
- `mismatchPenalty`, `minIdentityRatio` and `minAlignedQueryRelativeSpan` in case of a complex library and high number of artefact alignments; Note that a good solution to this problem is to introduce additional references (e.g. pseudogenes) if your reference set doesn't cover everything that can be amplified with your primers
- `errorModelType` and `substitutionErrorRateMatrix` .. we plan to publish a comprehensive set of error models inferred for different polymerases

Batch processing

MAGERI can be configured to run for multiple input files using a metadata config in JSON format. An example of metadata file is given below:

```
{
  "project": "project_name",
  "references": "metadata/refs.fa",
  "bed": "metadata/refs.bed",
  "contigs": "metadata/contigs.txt",
  "structure": [
    {
     "byindex": [
```

```
        {
          "index": "group_name",
          "r1": "R1.fastq.gz",
          "r2": "R2.fastq.gz",
          "submultiplex": {
           "file": "metadata/adapters.txt"
          }
        }
      ]
    },
    {
      "tabular": {
        "file": "metadata/index1.txt",
        "primer": {
          "file": "metadata/primers.txt"
        }
      }
    },
    {
      "tabular": {
        "file": "metadata/index2.txt",
        "positional": {
          "mask1": "nnNNNNNNNNNNNN"
        }
      }
    },
    {
      "tabular": {
        "file": "metadata/index3.txt",
        "preprocessed": {}
      }
    }
  ]
}
```

Here the `byindex` and `tabular` entries specify a sample group with corresponding FASTQ files or index file, a tab-delimited table with `sample_name\tfastq_R1\tfastq_R2` structure. The `submultiplex`, `primer`, `positional` and `preprocessed` entries correspond to `M1-4` demultiplexing rules described above.

After the `input.json` and `metadata/*` files are prepared the entire pipeline can be run as follows:

```
java -Xmx32G -jar mageri.jar -I input.json -O output/
```

Example JSON files and metadata can be found here.