bio$_{b}itsDocumentation$

Release 1.4.0

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Contents

Various bioinformatics scripts

All documentation is hosted at http://bio-bits.readthedocs.org/en/latest

CHAPTER 1

TODO

- Include existing scripts

Contents:

Installation

It is recommended to install into a virtualenv. If you know what you are doing and don't want to install into virtualenv, then you can skip right to step 3

1. Setup Virtualenv

   It is assumed you have virtualenv already installed. If not see https://virtualenv.pypa.io/en/latest/installation.html

   ```
   virtualenv env
   ```

2. Activate virtualenv

   ```
   . env/bin/activate
   ```

3. Install dependencies

   ```
   pip install -r requirements.txt
   ```

   For python 2.6 you will need to also install some additional packages

   ```
   pip install -r requirements-py26.txt
   ```

4. Install bio_bits

   ```
   python setup.py install
   ```

Scripts

rename_fasta

Many times you find you have a fasta file where the identifiers are all wrong and you want to rename them all via some mapping file.

Take the example where you have the following fasta file(example.fasta):

```
>id1
ATGC
>id2
ATGC
>id3
ATGC
```

You want to rename each identifier(id1, id2, id3) based on a mapping you have. In a file called renamelist.csv you would have the following:

```
#From,To
id1,samplename1
id2,samplename2
id3,samplename3
```

Then to rename your fasta without replacing the original file you have two options:

1. Rename without replacing original file

   ```
   rename_fasta renamelist.csv example.fasta > renamedfasta.fasta
   ```

2. Rename replacing original file's contents

   ```
   reanme_fasta renamelist.csv example.fasta --inplace
   ```

Rename Mapping File Syntax

The file you specify as the rename map file is a simple comma separated text file.

The following rules apply to the format:

- The first entry is the identifier to find in the supplied fasta file.
- The second entry is what to replace the found identifier with
- Any line beginning with a pound sign(#) will be ignored by the renamer

Missing identifiers that are in fasta but not rename file

In the case where your fasta file contains an identifier that is not in the rename map file you supply, an error will be displayed in the console telling you as such:

```
idwhatever is not in provided mapping
```

beast_checkpoint

beast_checkpoint is a fork of https://gist.github.com/trvrb/5277297 that has been rewritten in python and slightly improved as the ruby script seemed to have a few errors.

It accepts any previously run or terminated beast run and will generate an xml file that essentially starts from the last generated tree/log state.

Since beast is random in nature, there does not appear to be a way to restart the run exactly from the same state that it left off.

Example

We will use the benchmark2.xml file that comes with Beast 1.8 This file is located in:

```
BEASTv1.8.0/examples/Benchmarks/benchmark2.xml
```

First you need to fix the benchmark2.xml because each taxa has a trailing space and that is annoying

```
$> sed 's/ "/"/' benchmark2.xml > beast.xml
```

Now run beast for about half of the iterations and hit CTRL-C to kill it This benchmark is set to run 1,000,000 iterations so around 500,000 you can kill it. Notice we are using a predefined seed

```
$> seed=1234567890
$> mkdir run1
$> cp beast.xml run1/beast.xml
$> beast -seed $seed -beagle_SSE beast.xml
```

Now we will want to re-run beast from that last state. We can use beast_checkpoint to do so by supplying the original xml and the produced trees and log files. We will put the new xml into a new directory since the .trees and .log files would create an error or possibly be overwritten.

NOTE If your fileLog and treeFileLog do not have the same logEvery then when beast exits you may end up with more/less tree states than log states. For now you will have to manually edit the files and ensure that the last tree state matches the last log state.

Todo

Could be possible to get beast_checkpoint to check for that scenario and use the last tree state that matches the last log state

---

```
$> mkdir run2
$> beast_checkpoint beast.xml *.trees *.log > run2/beast.xml
```

Now you can simply just re-run beast on the new xml using the same seed

```
$> cd run2
$> beast -seed $seed -beagle_SSE beast.xml
```

Tracer

If you name your runs sequentially as we did in the example(aka, run1, run2,...) then you can easily load all log files into tracer via the command line as follows

```
tracer run*/*.log
```

LogCombiner

After you have run all your beast checkpointed xml files you will probably want to combine them with logcombiner which comes with beast

beast_wrapper

Beast wrapper is intended as a helper script to run beast. At this point it just runs beast with the same arguments you would normally give to beast from the command line and just adds a estimated time left column to the console output

Example

```
$> beast_wrapper -beagle_SSE my_beast.xml
...
state   Posterior       Prior           Likelihood      rootHeight      my_beast.ucld.
↪mean  location.clock.rate location.nonZeroRates
0   -86527.5880     -6850.8316      -79676.7564     57.6772         1.16103E-3      4.
↪86012         15.0000         -
20000   -29044.3753     -1123.5287      -27920.8466     288.102         3.02471E-4
↪  0.11891         16.0000         0.21 hours/million states   2d 04:29:44
40000   -25517.9525     -979.5343       -24538.4182     211.705         1.35118E-4
↪  0.25060         16.0000         0.25 hours/million states   2d 14:29:24
60000   -24212.1250     -1040.4103      -23171.7147     188.454         1.05572E-4
↪  0.18908         15.0000         0.25 hours/million states   2d 14:29:06
80000   -24097.9354     -1019.8099      -23078.1256     182.242         1.53593E-4
↪  0.12857         16.0000         0.26 hours/million states   2d 16:58:45
100000  -24121.5382     -1105.6545      -23015.8837     178.060         1.26907E-4
↪  0.10367         17.0000         0.27 hours/million states   2d 19:28:22
120000  -23930.6897     -1105.7390      -22824.9507     187.411         1.01885E-4
↪  0.34214         17.0000         0.27 hours/million states   2d 19:28:03
140000  -23869.4856     -1087.1915      -22782.2942     178.535         8.76375E-5
↪  0.26128         18.0000         0.26 hours/million states   2d 16:57:48
```

group_references

group_references splits an alignment file by reference into seperate FASTQ files. group_references takes a SAM or BAM file as input, and can optionally be given an output directory where the FASTQ files will be saved. If not output directory name is provided, the files will be saved in the new folder group_references_out.

```
$> group_references contigs.bam
$> group_references contigs.bam --outdir split_fastqs
```

degen

Find genes where a sequence has degenerate bases.

How-to

Usage: degen.py <fasta> <options>

Options:

--gb-id=<accession_id> Accession id for reference

--gb-file=<gbfile> Local Genbank file for reference

--tab-file=<tabfile> TSV/CSV file for reference with fields name,start,end

Example:

```
degen sequence.fasta --gb-id   12398.91
degen sequence.fasta --gb-file  tests/testinput/sequence.gb
degen sequence.fasta --tab-file tests/testinput/degen.tab
degen sequence.fasta --tab-file tests/testinput/degen.csv
```

Output:

Gene name, degnerate position, degenerate base:

```
anchored capsid protein         85      R
anchored capsid protein         88      Y
membrane glycoprotein precursor 509     R
nonstructural protein NS5       8513    Y
nonstructural protein NS5       8514    Y
nonstructural protein NS5       8515    Y
anchored capsid protein         85      R
anchored capsid protein         88      Y
membrane glycoprotein precursor 509     R
nonstructural protein NS5       8513    Y
nonstructural protein NS5       8514    Y
nonstructural protein NS5       8515    Y
```

Gene/Tab File

degen.tab could look like:

```
genename    start    stop
foo    1    2
bar    9    33
```

The headers do not matter, but the start field must always come before the stop field, so the below example would also be valid:

```
start    GENEName    stop
1    foo    2
9    bar    33
```

or optionally without headers:

```
1    foo    2
9    bar    33
```

alternatively, with commas in place of tabs:

```
name,start,stop
foo,1,2
bar,9,33
```

You can also specify a coding region(CDS) in your file as well:

```
name,start,stop

CDS,3,33
foo,1,2
bar,9,33
```

Genbank File

As downloaded from NCBI's entrez database. Use this option if you don't have internet access.

An example

```
LOCUS       KJ189367               10452 bp ss-RNA     linear   VRL 10-FEB-2014
DEFINITION  Dengue virus 1 isolate DENV-1/PR/BID-V8188/2010, complete genome.
ACCESSION   KJ189367
VERSION     KJ189367.1  GI:582052497
DBLINK      BioProject: PRJNA31235
KEYWORDS    .
SOURCE      Dengue virus 1
  ORGANISM  Dengue virus 1
            Viruses; ssRNA viruses; ssRNA positive-strand viruses, no DNA
            stage; Flaviviridae; Flavivirus; Dengue virus group.
REFERENCE   1  (bases 1 to 10452)
  AUTHORS   Zody,M.C., Newman,R.M., Henn,M., Munoz-Jordan,J., McElroy,K.L.,
            Santiago,G., Poon,T.W., Charlebois,P., Weiner,B., Yang,X.,
            Piper,M.E., Fitzgerald,M., McCowan,C., Young,S., Gargeya,S.,
            Levin,J., Malboeuf,C., Qu,J., Ireland,A., Chapman,S.B., Murphy,C.,
            Wortman,J., Nusbaum,C. and Birren,B.
  CONSRTM   Genome Resources in Dengue Consortium; The Broad Institute Genomics
            Platform; The Broad Institute Genome Sequencing Center for
            Infectious Disease; Centers for Disease Control and Prevention
            Division of Vector Borne Infectious Diseases; CDC Dengue Branch
            Puerto Rico
  TITLE     Direct Submission
  JOURNAL   Submitted (22-JAN-2014) Broad Institute of MIT & Harvard, 7
            Cambridge Center, Cambridge, MA 02142, USA
COMMENT     ##Assembly-Data-START##
            Assembly Method       :: Vicuna v. 1
            Sequencing Technology :: Illumina
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
     source          1..10452
                     /organism="Dengue virus 1"
                     /mol_type="genomic RNA"
                     /isolate="DENV-1/PR/BID-V8188/2010"
                     /isolation_source="cell supernatant"
                     /host="Homo sapiens"
                     /db_xref="taxon:11053"
                     /country="Puerto Rico"
```

```
                     /collection_date="2010"
                     /note="cell passage history: C6/36 1; cohort population:
                     Dengue Surveillance;
                     type: 1"
     5'UTR           1..83
                     /note="indels in UTR have not been validated"
     CDS             84..10262
                     /codon_start=1
                     /product="polyprotein"
                     /protein_id="AHI43750.1"
                     /db_xref="GI:582052498"
                     /translation="MNNQRKKTGRPSFNMLKRARNRVSTGSQLAKRFSKGLLSGQGPM
                     KLVMAFIAFLRFLAIPPTAGILARWSSFKKNGAIKVLRGFKKEISSMLNIMNRRKRSV
                     TMLLMLLPTALAFHLTTRGGEPHMIVSKQERGKSLLFKTSAGVNMCTLIAMDLGELCE
                     DTMTYKCPRITEAEPDDVDCWCNATDTWVTYGTCSQTGEHRREKRSVALAPHVGLGLE
                     TRTETWMSSEGAWKQIQRVETWALRHPGFTVIAFFLAHAIGTSITQKGIIFILLMLVT
                     PSMAMRCVGIGNRDFVEGLSGATWVDVVLEHGSCVTTMAKNKPTLDIELLKTEVTNPA
                     VLRKLCIEAKISNTTTDSRCPTQGEATLVEEQDANFVCRRTFVDRGWGNGCGLFGKGS
                     LLTCAKFKCVTKLEGKIVQYENLKYSVIVTVHTGDQHQVGNETTEHGTIATITPQAPT
                     SEIQLTDYGALTLDCSPRTGLDFNEMVLLTMKEKSWLVHKQWFLDLPLPWTSGASTSQ
                     ETWNRQDLLVTFKTAHAKKQEVVVLGSQEGAMHTALTGATEIQTSGTTTIFAGHLKCR
                     LKMDKLTLKGMSYVMCTGSFKLEKEVAETQHGTVLVQVKYEGTDAPCKIPFSTQDEKG
                     VTQNGRLITANPIVTDKEKPVNIETEPPFGESYIVVGAGEKALKLSWFKRGSSIGKMF
                     EATARGARRMAILGDTAWDFGSIGGVFTSVGKLVHQIFGTAYGVLFSGVSWTMKIGIG
                     ILLTWLGLNSRSTSLSMTCIVVGMVTLYLGVMVQADSGCVINWKGRELKCGSGIFVTN
                     EVHTWTEQYKFQADSPKRLSAAIGKAWEEGVCGIRSATRLENIMWKQISNELNHILLE
                     NDMKFTVVVGDANGILAQGKKMIRPQPMEHKYSWKSWGKAKIIGADIQNTTFIIDGPD
                     TPECPDGQRAWNIWEVEDYGFGVFTTNIWLKLRDSYTQMCDHRLMSAAIKDSKAVHAD
                     MGYWIESEKNETWKLARASFIEVKTCTWPKSHTLWSNGVLESEMIIPKIYGGPISQHN
                     YRPGYFTQTAGPWHLGKLELDFDLCEGTTVVVDEHCGNRGPSLRTTTVTGKIIHEWCC
                     RSCTLPPLRFRGEDGCWYGMEIRPVKEKEENLVRSMVSAGSGEVDSFSLGILCVSIMI
                     EEVMRSRWSRKMLMTGTLAVFLLLIMGQLTWNDLIRLCIMVGANASDRMGMGTTYLAL
                     MATFKMRPMFAVGLLFRRLTSREVLLLTIGLSLVASVELPNSLEELGDGLAMGIMMLK
                     LLTEFQPHQLWTTLLSLTFVKTTLSLDYAWKTTAMALSIVSLFPLCLSTTSQKTTWLP
                     VLLGSFGCKPLTMFLITENKIWGRKSWPLNEGIMAIGIVSILLSSLLKNDVPLAGPLI
                     AGGMLIACYVISGSSADLSLEKAAEVSWEQEAEHSGASHSILVEVQDDGTMKIKDEER
                     DDTLTILLKATLLAVSGVYPMSIPATLFVWYFWQKKKQRSGVLWDTPSPPEVERAVLD
                     NGIYRILQRGLLGRSQVGVGVFQDGVFHTMWHVTRGAVLMYQGKRLEPSWASVKKDLI
                     SYGGGWRFQGSWNTGEEVQVIAVEPGKNPKNVQTTPGTFKTPEGEVGAIALDFKPGTS
                     GSPIVNREGKIVGLYGNGVVTTSGTYVSAIAQAKASQEGPLPEIEDEVFKKRNLTIMD
                     LHPGSGKTRRYLPAIVREAIKRKLRTLILAPTRVVASEMAEALKGMPIRYQTTAVKSE
                     HTGREIVDLMCHATFTMRLLSPVRVPNYNMIIMDEAHFTDPASIAARGYISTRVGMGE
                     AAAIFMTATPPGSVEAFPQSNAVIQDEERDIPERSWNSGYDWITDFPGKTVWFVPSIK
                     SGNDIANCLRKNGKRVIQLSRKTFDTEYQKTKNNDWDYVVTTDISEMGANFRADRVID
                     PRRCLKPVILKDGPERVILAGPMPVTAASAAQRRGRIGRNQNKEGDQYVYMGQPLNND
                     EDHAHWTEAKMLLDNINTPEGIIPALFEPEREKSAAIDGEYRLRGEARKTFVELMRRG
                     DLPVWLSYKVASEGFQYSDRRWCFDGERNNQVLEENMDVEIWTKEGERKKLRPRWLDA
                     RTYSDPLALREFKEFAAGRRSVSGDLILEIGKLPQHLTLRAQNALDNLVMLHNSEQGG
                     KAYRHAMEELPDTIETLMLLALIAVLTGGVTLFFLSGKGLGKTSIGLLCVTASSALLW
                     MASVEPHWIAASIILEFFLMVLLIPEPDRQRTPQDNQLAYVVIGLLFMILTVAANEMG
                     LLETTKKDLGIGYVAAENHQHATMLDVDLHPASAWTLYAVATTVITPMMRHTIENTTA
                     NISLTAIANQAAILMGLDKGWPISKMDIGVPLLALGCYSQVNPLTLTAAVLMLVAHYA
                     IIGPGLQAKATREAQKRTAAGIMKNPTVDGIVAIDLDPVVYDAKFEKQLGQIMLLILC
                     TSQILLMRTTWALCESITLATGPLTTLWEGSPGKFWNTTIAVSMANIFRGSYLAGAGL
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                     KRGETTKHAVSRGTAKLRWFVERNLVKPEGKVIDLGCGRGGWSYYCAGLKKVTEVKGY
                     TKGGPGHEEPIPMATYGWNLVKLHSGKDVFFMPPEKCDTLLCDIGESSPNPTIEEGRT
                     LRVLKMVEPWLRGNQFCIKILNPYMPSVVETLERMQRKHGGMLVRNPLSRNSTHEMYW
```

```
                     VSCGTGNIVSAVNMTSRMLLNRFTMAHRKPTYERDVDLGAGTRHVAVEPEVANLDIIG
                     QRIENIKNEHKSTWHYDEDNPYKTWAYHGSYEVKPSGSASSMVNGVVRLLTKPWDVIP
                     MVTQIAMTDTTPFGQQRVFKEKVDTRTPRAKRGTTQIMEVTAKWLWGFLSRNKKPRIC
                     TREEFTRKVRSNAAIGAVFVDENQWNSAKEAVEDERFWDLVHRERELHKQGKCATCVY
                     NMMGKREKKLGEFGKAKGSRAIWYMWLGARFLEFEALGFMNEDHWFSRENSLSGVEGE
                     GLHKLGYILRDISKIPGGNMYADDTAGWDTRVTEDDLQNEAKITDIMEPEHALLATSI
                     FKLTYQNKVVRVQRPAKNGTVMDVISRRDQRGSGQVGTYGLNTFTNMEVQLIRQMESE
                     GIFLPSELETPNLAERALDWLEKHGAERLKRMAISGDDCVVKPIDDRFATALTALNDM
                     GKVRKDIPQWEPSKGWNDWQQVPFCSHHFHQLIMKDGREIVVPCRNQDELVGRARVSQ
                     GAGWSLRETACLGKSYAQMWQLMYFHRRDLRLAANAICSAVPVDWVPTSRTTWSIHAH
                     HQWMTTEDMLSVWNRVWIDENPWMENKTHVSSWEEVPYLGKREDQWCGSLIGLTARAT
                     WATNIQVAINQVRRLIGNENYLDYMTSMKRFKNESDSEGALW"
     mat_peptide     84..425
                     /product="anchored capsid protein"
     mat_peptide     426..923
                     /product="membrane glycoprotein precursor"
     mat_peptide     924..2408
                     /product="envelope protein"
     mat_peptide     2409..3464
                     /product="nonstructural protein NS1"
     mat_peptide     3465..4118
                     /product="nonstructural protein NS2A"
     mat_peptide     4119..4508
                     /product="nonstructural protein NS2B"
     mat_peptide     4509..6365
                     /product="nonstructural protein NS3"
     mat_peptide     6366..6746
                     /product="nonstructural protein NS4A"
     mat_peptide     6747..6815
                     /product="2K peptide"
     mat_peptide     6816..7562
                     /product="nonstructural protein NS4B"
     mat_peptide     7563..10259
                     /product="nonstructural protein NS5"
     3'UTR           10263..10452
                     /note="indels in UTR have not been validated"
ORIGIN
        1 catctggacc gacaagaaca gtttcgaatc ggaagcttgc ttaacgtagt tctaacagtt
       61 ttttattaga gagcagatct ctgatgaaca accaacggaa aaagacgggt cgaccgtctt
      121 tcaatatgct gaaacgcgcg agaaaccgcg tgtcaactgg ttcacagttg gcgaagagat
      181 tctcaaaagg attgctttca ggccaaggac ccatgaaatt ggtgatggct ttcatagcat
      241 ttctaagatt tctagccata cccccaacag caggaatttt ggctagatgg agctcattca
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      361 acataatgaa caggaggaaa agatccgtga ccatgctcct catgctgctg cccacagccc
      421 tggcgtttca tttgaccaca cgaggggggag agccacacat gatagttagt aagcaggaaa
      481 gaggaaagtc actcttgttt aagacctctg cgggcgtcaa tatgtgcacc ctcattgcga
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      601 cggaaccaga tgacgttgac tgctggtgca atgccacaga cacatgggtg acctatggga
      661 cgtgttctca aaccggcgaa caccgacgag agaaacgttc cgtggcactg gccccacacg
      721 tgggacttgg tctagaaaca agaaccgaaa catggatgtc ctctgaaggc gcctggaaac
      781 aaatacaaag agtggaaact tgggctttga gacacccagg attcacggtg atagcctttt
      841 ttttagcaca tgctatagga acatccatca ctcagaaagg gatcattttc atcttgctga
      901 tgctggtgac accatcaatg gccatgcgat gcgtgggaat aggcaacaga gacttcgttg
      961 aaggactgtc aggagcaacg tgggtggacg tggtactgga gcacggaagc tgcgtcacca
     1021 ccatggcaaa aaataaacca acattggaca ttgaactctt gaagacggag gtcacgaacc
     1081 ctgccgtctt gcgcaaactg tgcattgaag ctaaaatatc aaacaccacc accgattcaa
     1141 gatgtccaac acaaggagag gccacactgg tggaagaaca agacgcgaac tttgtgtgtc
     1201 gccgaacgtt tgtggacaga ggctggggta atggctgcgg actattcgga aagggaagtc
```

```
1261 tattgacgtg tgccaagttc aagtgtgtga caaaactaga aggaaagata gttcaatatg
1321 aaaacctaaa atattcagtg atagtcactg tccacactgg ggaccagcac caggtgggaa
1381 acgagaccac agaacatgga acaattgcaa ccataacacc tcaagctccc acgtcggaaa
1441 tacagctgac cgactacgga gccctcacac tggactgctc acctagaaca gggctggact
1501 ttaatgagat ggtgctattg acaatgaaag aaaaatcatg gcttgtccac aaacaatggt
1561 ttctagactt gccactgcca tggacttcgg gggcttcaac atcccaagag acctggaaca
1621 gacaagattt gctggtcaca ttcaagacag ctcatgcaaa gaaacaggaa gtagtcgtat
1681 tgggatcaca ggaaggagca atgcatactg cgttgactgg ggcgacagaa atccagacgt
1741 caggaacgac aacaatcttc gcaggacacc tgaaatgcag actaaaaatg gataaactga
1801 ccttaaaggg gatgtcatat gtgatgtgca caggctcatt taagctagag aaggaagtgg
1861 ctgagaccca gcatggaact gttctagtgc aggtcaaata tgaaggaaca gacgcgccat
1921 gcaagatccc cttttcgacc caagatgaga aaggagtgac ccagaatggg agattgataa
1981 cagccaatcc catagttact gacaaagaaa aaccagtcaa cattgagaca gaaccacctt
2041 ttggtgagag ctacatcgtg gtaggggcag gcgaaaaagc tttgaaacta agctggttca
2101 agagaggaag cagcataggg aaaatgttcg aagcaaccgc ccgaggagca cgaaggatgg
2161 ctatcctggg agacaccgca tgggacttcg gttctatagg aggagtgttt acatctgtgg
2221 gaaaattggt acaccagatt tttggaaccg catatggggt tctgtttagc ggtgtttctt
2281 ggaccatgaa aataggaata gggattctgc tgacatggtt gggattaaat tcaaggagca
2341 cgtcactttc gatgacgtgc attgtagttg gcatggtcac actgtaccta ggagtcatgg
2401 ttcaagcgga ttcgggatgt gtgatcaact ggaagggcag agaacttaaa tgcggaagtg
2461 gcatttttgt cactaatgaa gtccacactt ggacagagca atacaaattc caggctgact
2521 ccccaaaaag actgtcagca gccattggaa aggcgtggga ggagggcgtg tgtggaattc
2581 gatcagccac gcgtcttgag aacatcatgt ggaagcagat atcaaatgaa ttgaaccaca
2641 ttttacttga gaatgacatg aaattcacag tggttgtagg agatgccaac ggaattttgg
2701 cccaaggaaa aaaaatgatt aggccacaac ccatggaaca caaatactca tggaaaagct
2761 ggggaaaagc taaaatcata ggagcagaca tacaaaatac caccttcatt atcgacggcc
2821 cagacacccc agaatgtcct gatggccaaa gagcatggaa catttgggaa gttgaggact
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3421 tgaaggagaa ggaggagaat ctagttaggt caatggtctc tgcagggtca ggagaagtgg
3481 acagtttttc attaggaata ctatgcgtat caataatgat tgaagaagtg atgagatcca
3541 gatggagtag aaagatgctg atgactggaa cactggctgt cttcctcctt cttataatgg
3601 gacaactgac atggaatgat ctgattaggt tatgcatcat ggtcggagct aacgcttcag
3661 acaggatggg gatgggaaca acgtacctag ccttgatggc tactttcaaa atgagaccaa
3721 tgttcgctgt agggctatta ttccgcagac taacatccag agaagttctt ctcctaacga
3781 ttggattaag cctggtggca tccgtggagc taccaaattc cttggaggag ctaggggatg
3841 gacttgcaat gggtatcatg atgttaaaat tgttgactga atttcagcca caccagttat
3901 ggaccacctt attgtctctg acatttgtca aaacaactct ctcattggat tatgcatgga
3961 aaacaacggc tatggcactg tctatcgtat ctctctttcc tttatgcctg tctacgacct
4021 cccaaaaaac aacatggctt ccggtgctgt taggatcttt tggatgcaaa ccattaacca
4081 tgtttcttat aacagaaaat aaaatctggg gaaggaaaag ttggcccctc aatgaaggaa
4141 ttatggctat tggaatagtc agcattctac taagctcact cctcaaaaat gatgtgccgt
4201 tggccgggcc attaatagct ggaggcatgc taatagcatg ttatgtcata tccggtagct
4261 cagccgattt atcattggag aaagcggctg aagtatcctg ggaacaagaa gcagaacact
4321 ccggtgcctc acacagcata ttagtagagg tccaagatga tggaactatg aaaataaaag
4381 atgaagagag ggatgacaca ctcaccatac tccttaaagc aactttgctg gcagtctcag
4441 gagtgtaccc aatgtcaata ccagcaactc tttttgtgtg gtatttttgg cagaaaaaga
4501 aacagagatc aggagtgtta tgggacacac ccagccctcc ggaagtggaa agagcagttc
4561 ttgataatgg catctataga atcttgcaaa gaggattgtt gggcaggtcc caagtaggag
4621 tgggagtttt ccaagacggc gtgttccaca caatgtggca cgttaccagg ggagctgtcc
4681 ttatgtacca agggaagaga ctggaaccaa gctgggccag tgtgaaaaag gacttgatct
```

```
4741 catatggagg aggttggagg ttccaaggat catggaacac gggagaagaa gtgcaggtaa
4801 tagctgttga accaggaaaa aaccccaaaa atgtacagac aacgccgggc acctttaaga
4861 ctcctgaagg cgaagttgga gccatagctc tagatttcaa acccggcaca tctggatctc
4921 ccatcgtgaa cagagaggga aaaatagtgg gtctgtatgg aaatggagtg gtgacaacaa
4981 gtggaaccta cgtcagtgcc attgcccaag ctaaagcatc acaggaaggg cctctaccag
5041 agattgagga cgaggtattt aagaaaagaa acttaacaat aatggacctg cacccaggat
5101 cagggaaaac aagaagatat cttccagcca tagtccgtga ggccataaaa aggaaactgc
5161 gtacgttaat cctggctccc acaagagttg tcgcctctga aatggcagag gcactcaagg
5221 gaatgccaat aagatatcag acaacagcag tgaagagtga acacacagga agggagatag
5281 ttgacctcat gtgccacgct acttttacca tgcgtctctt atccccagtg agagttccca
5341 attacaacat gatcattatg gatgaagcac attttaccga tccagctagc atagcggcca
5401 gagggtacat ctcaacccga gtgggtatgg gtgaagcagc tgcgatcttt atgacagcca
5461 ctcccccagg atcggtggag gcctttccac agagcaatgc agttatccaa gatgaggaaa
5521 gagacattcc tgagagatca tggaactcag gctacgactg gatcactgac tttccaggta
5581 aaacagtctg gtttgttcca agcattaaat caggaaatga cattgccaac tgtttaagaa
5641 agaacggaaa acgggtaatc caattgagca gaaaaacctt tgacactgag taccagaaaa
5701 caaaaaacaa tgactgggac tatgttgtca caacagacat ttctgaaatg ggggcaaatt
5761 tccgggccga cagggtaata gacccaaggc ggtgcttgaa accggtaata ctaaaagatg
5821 gtccagagcg tgtcattcta gccggaccga tgccagtgac tgcggccagt gctgcccaga
5881 ggagaggaag aattggaagg aaccaaaaca aggaaggtga tcagtatgtt tatatgggac
5941 agcctttaaa taatgatgag gatcacgctc attggacaga agcaaaaatg ctccttgaca
6001 atataaacac accagaaggg atcatcccag ccctttttga gccagagaga gaaaagagtg
6061 cagcaataga cggggagtac agactgcggg gagaagcaag gaaaacgttc gtggagctca
6121 tgagaagagg agatctacca gtttggctat cctacaaagt agcctcagaa ggtttccagt
6181 actccgacag aaggtggtgc tttgatgggg aaaggaacaa ccaggtgttg gaggagaaca
6241 tggacgtgga gatctggaca aaggaaggag aaagaaagaa attgcgacct cgctggttgg
6301 acgccagaac atactctgat ccattggccc tgcgcgagtt taaagagttc gcagcaggaa
6361 gaagaagtgt ctcaggtgac ctgatattgg aaatagggaa acttccacaa catttgacgt
6421 taagagccca gaatgctctg gacaacttgg tcatgttgca caattccgaa caaggaggaa
6481 aagcctacag acatgccatg gaggaactac cagacaccat agaaacattg atgctactag
6541 ctttgatagc tgtgttgact ggtggagtga cgctgttctt cctatcagga aaaggcctag
6601 ggaaaacatc cattggcttg ctctgtgtga cggcctcaag cgcactgtta tggatggcca
6661 gtgtggagcc ccattggata gcggcctcca tcatactaga gttctttttg atggtgctgc
6721 tcattccaga gccagacaga cagcgcactc cacaggacaa ccagctagca tatgtggtga
6781 taggtttgtt attcatgata ctgacagtgg cagccaatga gatgggatta ttggaaacca
6841 caaagaaaga cctggggatt ggctatgtag ccgccgaaaa ccaccaacat gccacaatgc
6901 tggacgtaga cctacaccca gcttcagcct ggaccctcta tgcagtagcc acaacagtca
6961 tcactcccat gatgagacac acaattgaaa atacaacggc aaacatttcc ctgaccgcca
7021 ttgcaaatca ggcagctata ttgatgggac ttgacaaggg atggccaata tcgaagatgg
7081 acataggagt tccacttctc gccttagggt gctattccca ggtgaaccca ttgacactga
7141 cagcggcggt gttgatgtta gtggctcatt atgccataat tggaccagga ctgcaagcaa
7201 aggccactag agaagcccaa aaaaggacag cagccggaat aatgaaaaat ccaaccgtag
7261 acgggattgt tgcaatagac ttggatcctg tggtttatga tgcaaaattt gaaaaacaac
7321 taggccaaat aatgttactg atactttgta catcacagat cctcttgatg cggaccacat
7381 gggccttgtg tgaatccatc acactggcta ctggacccct gaccactctc tgggagggat
7441 ctccaggaaa attctggaat accacaatag cagtgtccat ggcaaatatt ttcaggggaa
7501 gttatctagc aggagcaggt ctggctttct cattgatgaa atctttagga ggaggtagga
7561 gaggcacggg agctcaaggg gaaacactgg gagagaaatg gaaaagacag ttgaaccaac
7621 tgagcaagtc agaattcaac acctacaaaa ggagtgggat tatggaggtg gacagatccg
7681 aagccaaaga gggactgaaa agaggagaaa caaccaaaca tgcagtgtca agaggaacag
7741 ccaaactgag gtggtttgtg gagaggaacc tcgtgaaacc agaaggaaaa gtcatagacc
7801 tcggttgtgg aagaggtggc tggtcatatt attgtgctgg gctgaagaaa gttactgaag
7861 tgaagggata cacaaaagga ggacctggac atgaggaacc tatcccaatg gcgacctatg
7921 gatggaacct agtaaaacta cactctggaa aggatgtatt ttttatgcca cctgagaaat
7981 gtgacactct tctgtgtgat attggtgagt cctctccgaa tccaactata gaagaaggaa
8041 gaacgttacg tgttctaaaa atggtggaac catggctcag aggaaaccaa ttctgcataa
8101 aaatcctaaa tccttacatg ccaagtgtgg tagaaactct ggagcgaatg caaagaaaac
8161 atggagggat gctagtgcga aacccactct caagaaattc tacccatgaa atgtattggg
```

```
     8221 tttcatgtgg aacaggaaac attgtgtcgg cagtgaacat gacatccaga atgttactga
     8281 accgattcac aatggctcac aggaagccaa catatgaaag agacgtggac ttaggcgctg
     8341 gaacaagaca tgtggcagtg gaaccagagg tagccaacct agatatcatt ggccagagga
     8401 tagaaaatat aaaaaatgaa cacaagtcaa catggcatta tgatgaggac aatccataca
     8461 aaacatgggc ctatcatgga tcatatgagg tcaagccatc aggatcagcc tcatctatgg
     8521 tgaatggagt ggtgagattg ctcacgaaac catgggatgt catccccatg gtcacacaaa
     8581 tagctatgac tgataccaca ccctttggac aacagagagt gtttaaagag aaagttgaca
     8641 cgcgcacacc aagagcaaaa cgaggcacaa cacagattat ggaggtgaca gccaagtggt
     8701 tatggggttt cctttccaga aacaaaaaac ccagaatctg cacaagagag gagttcacaa
     8761 gaaaggttag gtcaaacgcg gcaataggag cagtgttcgt tgatgaaaac caatggaact
     8821 cagcaaaaga agcagtggaa gacgaaaggt tttgggatct tgtgcacaga gagagggagc
     8881 ttcataaaca gggaaaatgt gccacgtgtg tctacaacat gatggggaag agagagaaaa
     8941 aattaggaga gtttggaaag gcaaaaggaa gtcgtgcaat atggtacatg tggctgggag
     9001 cacgctttct ggagttcgaa gcccttggtt ttatgaatga agatcactgg tttagtagag
     9061 agaattcact cagtggagtg gaaggagaag gactgcacaa acttggatac atactcagag
     9121 acatatcaaa gattccgggg ggaaatatgt atgcagatga tacagccgga tgggacacaa
     9181 gagtaacaga ggatgacctc cagaatgagg ctaaaatcac tgacatcatg gagcctgaac
     9241 atgctctatt ggctacgtca atttttaagc tgacttatca aaacaaggtg gtgagggtgc
     9301 aaagaccagc aaaaaatgga accgtgatgg atgttatatc cagacgtgat cagagaggga
     9361 gtggacaggt cggaacttat ggcttaaata ctttcaccaa tatggaggtc caactaataa
     9421 gacaaatgga gtctgaggga atctttttac ccagcgaatt ggaaaccccc aacctagctg
     9481 agagggctct tgactggtta gaaaaacatg gcgccgaaag gctgaaacga atggcaatca
     9541 gcggagatga ttgcgtggtg aaaccaattg acgacaggtt cgcaacagcc ttaacagctc
     9601 tgaatgacat gggaaaagta aggaaagaca taccgcagtg ggaaccttca aaaggatgga
     9661 atgattggca gcaagtgcct ttttgttcac accatttcca ccaactgatc atgaaggatg
     9721 ggagggaaat agtggtgcca tgccgcaacc aagatgaact tgtgggcagg gctagagtat
     9781 cacaaggcgc cggatggagc ctgagagaaa ctgcttgcct aggcaagtca tatgcacaaa
     9841 tgtggcagct gatgtacttc cacaggagag acctgagact agcggctaac gctatctgtt
     9901 cagccgtccc agttgattgg gtcccaacca gccgcacaac ctggtcaatc catgcccacc
     9961 accaatggat gacaacagaa gacatgttat cagtgtggaa tagggtttgg atagacgaaa
    10021 acccatggat ggagaacaaa actcatgtat ccagttggga agaagttcca tacctaggaa
    10081 aaagggaaga tcaatggtgt ggatccctga taggcttgac agcgagggcc acctgggcca
    10141 ccaacataca agtagccata aaccaagtga gaaggctcat cgggaatgag aattatttag
    10201 attacatgac atcaatgaag agattcaaga atgagagtga ttccgaagga gcactctggt
    10261 aagtcaacac actcatgaaa taaaggaaaa tagaagatca aacaaagtaa gaagtcaggc
    10321 cagattaagc catagcacgg aaagagctat gctgcctgtg agccccgtcc aaggacgtaa
    10381 aatgaagtca ggccgaaagc cacggattga gcaagccgtg ctgcctgtgg ctccatcgtg
    10441 gggatgtagc tc
//
```

parallel_blast

Parallel blast is a wrapper script around the blast commands as well as diamond. It utilizes GNU Parallel to run the commands in parallel by splitting up the input fasta files and distributes them across multiple subprocesses. If it detects that it is running inside of a PBS or SGE job it will run the job on multiple hosts that may be allocated to the job.

parallel_blast requires that you have gnu parallel installed and in your environments PATH as well as diamond and/or blastn/blastx/blastp.

- diamond
- blast
- GNU parallel

Usage

You can get all the arguments that can be supplied via the following

```
$> parallel_blast --help
```

Examples

For the examles below assume you have an input fasta in the current directory called `input.fasta`

Running blastn

```
$> parallel_blast input.fasta output.blast --ninst 4 --db /path/to/nt \
--blast_exe blastn --task megablast --blast_options "--evalue 0.01"
[cmd] /path/to/parallel -u --pipe --block 10 --recstart > --sshlogin 4/: /path/to/
↪blastn -task megablast -db /path/to/nt -max_target_seqs 10 -outfmt "6 qseqid sseqid␣
↪pident length mismatch gapopen qstart qend sstart send evalue bitscore" -query -
```

Notice how we had to quote the additional `--blast_options`

Running diamond

Diamond v0.7.9 is the version that was tested with parallel_blast. As diamond is still in development the options may change in future versions and parallel_blast may not run them correctly. Please submit a new issue if you find any issues.

```
$> parallel_blast input.fasta out.blast --ninst 4 --db /path/to/diamondnr \
--blast_exe diamond --task blastx --blast_options "--tmpdir dtmp"
[cmd] /path/to/parallel -u --pipe --block 10 --recstart > --cat --sshlogin 1/: /path/
↪to/diamond blastx --threads 4 --db /path/to/diamondnr --query {} --compress 0 -a␣
↪out.blast
```

Notice how even though we specified `--ninst 4` that `--sshlogin 1/:` was used and `--threads 4` was set instead.

Note In recent versions of diamond, diamond outputs a daa binary file instead of a tab separated file. parallel_blast automatically converts the diamond output from daa to tab format for you but leaves the daa file behind(Same name as the output file you specify, but with the extension .daa)

Command that is run

You will notice in the examples above that when you run parallel_blast that it outputs the command that it is running in case you want to copy/paste it and run it yourself sometime.

You might notice that the command does not include all the quoted arguments such as the `--recstart` argument which should be `--recstart ">"` as well as the `--outfmt` which should be quoted as `--outfmt "6 ..."`. If you intend on rerunning the command you will have to add the quotes manually.

Running inside of a PBS or SGE Job

parallel_blast is able to detect if it is running inside of a PBS or SGE job by looking to see if `PBS_NODEFILE` or `PE_HOSTFILE` is set in the environment's variables.

If it finds either of them it will run the job by supplying `--sshlogin` for each host it finds in the file.

`PBS_NODEFILE` and `PE_HOSTFILE` have different syntax so parallel_blast first builds a CPU,NODENAME list from them.

PBS_NODEFILE

This file is parsed and counts how many of each unique host is listed such that the following PBS_NODEFILE:

```
node1.localhost
node2.localhost
node2.localhost
node3.localhost
node3.localhost
node3.localhost
```

would run 1 instance on node1.localhost, 2 instances on node2.localhost and 3 instances on node3.localhost

PE_HOSTFILE

This file is almost in the exact syntax that parallel_blast uses so it is almost a 1-to-1 mapping.

Diamond and multiple hosts

Since diamond utilizes threads much more efficiently than blast, for each unique host in a job only 1 instance is launched but the `-p` option is set to the number of CPUS for each host listed in the `PE_HOSTFILE` or `PBS_NODEFILE`

degen_regions

Finds all degenerate bases in a given fasta input file that may contain multiple sequeces and reports their position as well as the annotated gene name that contains them.

The fasta file must be previously aligned to the query sequence. That is, if you are using a genbank annotation file or having the script download it for you, you should have aligned all your input sequences to that sequence.

The annotation is retrieved via supplied genbank accession, genbank file path or gene tab/csv file.

Usage

You can view the usage of degen_regions via:

```
degen_regions --help
```

Using Genbank Files

If you already have downloaded the genbank annotation file(typically the extension is .gb) you can use the *–gb-file* argument

The following will use the test input fasta file as well as the test input genbank file to find all degenerate bases and will put the output in a tab separated file called output.tsv

```
degen_regions -i tests/Den4_MAAPS_TestData16.fasta -o output.tsv --gb-file tests/
↪testinput/sequence.gb
```

Fetching Genbank Files Automatically

If you want the script to automatically fetch the Genbank annotation file from the internet you can use the *–gb-id* option and specify an accession number.

```
degen_regions -i tests/Den4_MAAPS_TestData16.fasta -o output.tsv --gb-id KJ189367
```

Using tab/csv file of gene annotation info

If you have a tab/csv file of gene annotations you can supply that using the *–tab-file* argument

You can read more about the format of the tab/csv annotation file in the *degen* docs

```
degen_regions -i tests/Den4_MAAPS_TestData16.fasta -o output.tsv --gb-file tests/
↪testinput/sequence.gb
```

Manually specify CDS

You can use the `--cds` argument to set the coding region. This argument should be comma separated such as `start,stop`. Specifying this argument will override any other cds found in the tab file, genbank file or fetched genbank file.

The following would mark all locations as NON-CODING as you are specifying that only position 1 is coding

```
degen_regions -i tests/Den4_MAAPS_TestData16.fasta -o output.tsv --gb-file tests/
↪testinput/sequence.gb --cds 1,1
```

Without Gene Information

The gene information is optional. If it is not provided the output file will not be annotated with the gene information; otherwise, the output will look the same (you will also lose the "non-coding region" flag.)

```
degen_regions -i tests/Den4_MAAPS_TestData16.fasta -o output.tsv
```

Output

The output is a simple tab separated file

```
seq id                                          nt Position    aa position  nt␣
↪composition    aa composition    gene name
----------------------------------------  -------------  -------------  -------------
↪---  ----------------  --------------------------------
721                                               991            331  WCA          ␣
↪     S/T               envelope protein
721                                              1307            436  AYA          ␣
↪     I/T               envelope protein
721                                              1826            609  AYA          ␣
↪     I/T               envelope protein
721                                              1865            622  GRA          ␣
↪     E/G               envelope protein
721                                              7766           2589  ARA          ␣
↪     K/R               nonstructural protein NS5
2055_Den4/AY618992_1/Thailand/2001/Den4_1        1927            643  RAC          ␣
↪     D/N               envelope protein
2055_Den4/AY618992_1/Thailand/2001/Den4_1        2833            945  YCG          ␣
↪     P/S               nonstructural protein NS1
2055_Den4/AY618992_1/Thailand/2001/Den4_1        3565           1189  YAT          ␣
↪     H/Y               nonstructural protein NS2A
2055_Den4/AY618992_1/Thailand/2001/Den4_1        6271           2091  RAA          ␣
↪     E/K               nonstructural protein NS3
2055_Den4/AY618992_1/Thailand/2001/Den4_1        8656           2886  YAT          ␣
↪     H/Y               nonstructural protein NS5
2055_Den4/AY618992_1/Thailand/2001/Den4_1        8998           3000  YAG          ␣
↪     */Q               nonstructural protein NS5
2055_Den4/AY618992_1/Thailand/2001/Den4_1        9811           3271  YCC          ␣
↪     P/S               nonstructural protein NS5
2055_Den4/AY618992_1/Thailand/2001/Den4_1       10542           3515  AGN          ␣
↪     NON-CODING        -
2055_Den4/AY618992_1/Thailand/2001/Den4_1       10543           3515  NNN          ␣
↪     NON-CODING        -
2055_Den4/AY618992_1/Thailand/2001/Den4_1       10541           3514  NNN          ␣
↪     NON-CODING        -
2055_Den4/AY618992_1/Thailand/2001/Den4_1       10539           3514  NNN          ␣
↪     NON-CODING        -
2055_Den4/AY618992_1/Thailand/2001/Den4_1       10546           3516  NNN          ␣
↪     NON-CODING        -
2055_Den4/AY618992_1/Thailand/2001/Den4_1       10544           3515  NNN          ␣
↪     NON-CODING        -
2055_Den4/AY618992_1/Thailand/2001/Den4_1       10542           3515  NNN          ␣
↪     NON-CODING        -
1942_Den4/AY618992_1/Thailand/2001/Den4_1        4540           1514  RTA          ␣
↪     I/V               nonstructural protein NS3
1942_Den4/AY618992_1/Thailand/2001/Den4_1       10177           3393  MCA          ␣
↪     P/T               nonstructural protein NS5
1942_Den4/AY618992_1/Thailand/2001/Den4_1       10546           3516  NNN          ␣
↪     NON-CODING        -
1942_Den4/AY618992_1/Thailand/2001/Den4_1       10544           3515  NNN          ␣
↪     NON-CODING        -
1942_Den4/AY618992_1/Thailand/2001/Den4_1       10542           3515  NNN          ␣
↪     NON-CODING        -
1875_Den4/AY618992_1/Thailand/2001/Den4_1        1514            505  AYG          ␣
↪     M/T               envelope protein
1875_Den4/AY618992_1/Thailand/2001/Den4_1        3056           1019  ARA          ␣
↪     K/R               nonstructural protein NS1
1875_Den4/AY618992_1/Thailand/2001/Den4_1        3058           1020  KCA          ␣
↪     A/S               nonstructural protein NS1
```

```
1875_Den4/AY618992_1/Thailand/2001/Den4_1           3073           1025  WTT
↪     F/I               nonstructural protein NS1
1875_Den4/AY618992_1/Thailand/2001/Den4_1           3491           1164  AYC
↪     I/T               nonstructural protein NS2A
1875_Den4/AY618992_1/Thailand/2001/Den4_1           3895           1299  RTG
↪     M/V               nonstructural protein NS2A
1875_Den4/AY618992_1/Thailand/2001/Den4_1           7445           2482  GYA
↪     A/V               nonstructural protein NS4B
948_Den4/AY618992_1/Thailand/2001/Den4_1            2819            940  ARC
↪     N/S               nonstructural protein NS1
871_Den4/AY618992_1/Thailand/2001/Den4_1            2947            983  RCC
↪     A/T               nonstructural protein NS1
871_Den4/AY618992_1/Thailand/2001/Den4_1            3058           1020  KCA
↪     A/S               nonstructural protein NS1
871_Den4/AY618992_1/Thailand/2001/Den4_1            3073           1025  WTT
↪     F/I               nonstructural protein NS1
871_Den4/AY618992_1/Thailand/2001/Den4_1            3116           1039  GYG
↪     A/V               nonstructural protein NS1
871_Den4/AY618992_1/Thailand/2001/Den4_1            3181           1061  RTW
↪     I/V               nonstructural protein NS1
871_Den4/AY618992_1/Thailand/2001/Den4_1            3179           1060  RTW
↪     I/V               nonstructural protein NS1
871_Den4/AY618992_1/Thailand/2001/Den4_1            3338           1113  ART
↪     N/S               nonstructural protein NS1
871_Den4/AY618992_1/Thailand/2001/Den4_1            3362           1121  ARA
↪     K/R               nonstructural protein NS1
871_Den4/AY618992_1/Thailand/2001/Den4_1            3373           1125  WCR
↪     S/T               nonstructural protein NS1
871_Den4/AY618992_1/Thailand/2001/Den4_1            3371           1124  WCR
↪     S/T               nonstructural protein NS1
871_Den4/AY618992_1/Thailand/2001/Den4_1            4314           1439  ATV
↪     I/M               nonstructural protein NS2B
871_Den4/AY618992_1/Thailand/2001/Den4_1            7045           2349  WCC
↪     S/T               nonstructural protein NS4B
871_Den4/AY618992_1/Thailand/2001/Den4_1           10536           3513  GAW
↪     NON-CODING        -
871_Den4/AY618992_1/Thailand/2001/Den4_1           10537           3513  YCA
↪     NON-CODING        -
947_Den4/AY618992_1/Thailand/2001/Den4_1            2971            991  YTY
↪     F/L               nonstructural protein NS1
947_Den4/AY618992_1/Thailand/2001/Den4_1            2969            990  YTY
↪     F/L               nonstructural protein NS1
947_Den4/AY618992_1/Thailand/2001/Den4_1            6763           2255  YTT
↪     F/L               2K peptide
1793_Den4/AY618992_1/Thailand/2001/Den4_1            223             75  MAG
↪     K/Q               anchored capsid protein
1793_Den4/AY618992_1/Thailand/2001/Den4_1            556            186  RCC
↪     A/T               membrane glycoprotein precursor
1793_Den4/AY618992_1/Thailand/2001/Den4_1            586            196  RGT
↪     G/S               membrane glycoprotein precursor
1793_Den4/AY618992_1/Thailand/2001/Den4_1            613            205  YCA
↪     P/S               membrane glycoprotein precursor
1793_Den4/AY618992_1/Thailand/2001/Den4_1           2875            959  YCG
↪     P/S               nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1           2943            982  AAN
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1           2944            982  NNG
↪     GAPFOUND          nonstructural protein NS1
```

```
1793_Den4/AY618992_1/Thailand/2001/Den4_1           2942            981  NNG
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1           2976            993  ATN
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1           2977            993  NNN
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1           2975            992  NNN
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1           2973            992  NNN
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1           2980            994  NTG
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1           2987            996  ANN
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1           2986            996  ANN
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1           2989            997  NGT
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1           2996            999  TNN
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1           2995            999  TNN
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1           3001           1001  NNN
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1           2999           1000  NNN
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1           2997           1000  NNN
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1           3004           1002  NCC
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1           3073           1025  NTT
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1           3086           1029  ARC
↪     N/S               nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1           3095           1032  CNG
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1           3116           1039  GNG
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1           3144           1049  GAN
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1           3159           1054  GAN
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1           3160           1054  NNC
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1           3158           1053  NNC
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1           3206           1069  GNC
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1           3235           1079  NNN
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1           3233           1078  NNN
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1           3231           1078  NNN
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1           3238           1080  NNN
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1           3236           1079  NNN
↪     GAPFOUND          nonstructural protein NS1
```

```
1793_Den4/AY618992_1/Thailand/2001/Den4_1            3234            1079  NNN
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1            3241            1081  NNN
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1            3239            1080  NNN
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1            3237            1080  NNN
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1            3244            1082  NNN
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1            3242            1081  NNN
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1            3240            1081  NNN
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1            3247            1083  NNN
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1            3245            1082  NNN
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1            3243            1082  NNN
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1            3250            1084  NNN
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1            3248            1083  NNN
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1            3246            1083  NNN
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1            3253            1085  NNN
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1            3251            1084  NNN
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1            3249            1084  NNN
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1            3256            1086  NNN
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1            3254            1085  NNN
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1            3252            1085  NNN
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1            3316            1106  NGG
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1            3337            1113  NAT
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1            3341            1114  GNA
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1            3408            1137  ATN
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1            3412            1138  NTG
↪     GAPFOUND          nonstructural protein NS1
1793_Den4/AY618992_1/Thailand/2001/Den4_1            3493            1165  MCC
↪     P/T               nonstructural protein NS2A
1793_Den4/AY618992_1/Thailand/2001/Den4_1            3509            1170  ANT
↪     GAPFOUND          nonstructural protein NS2A
1793_Den4/AY618992_1/Thailand/2001/Den4_1            3837            1280  TTN
↪     GAPFOUND          nonstructural protein NS2A
1793_Den4/AY618992_1/Thailand/2001/Den4_1            6185            2062  ARG
↪     K/R               nonstructural protein NS3
1793_Den4/AY618992_1/Thailand/2001/Den4_1            6187            2063  RAR
↪     E/K               nonstructural protein NS3
```

```
1793_Den4/AY618992_1/Thailand/2001/Den4_1           6185           2062  RAR
↪     E/K               nonstructural protein NS3
1793_Den4/AY618992_1/Thailand/2001/Den4_1           6614           2205  TYT
↪     F/S               nonstructural protein NS4A
1793_Den4/AY618992_1/Thailand/2001/Den4_1           6650           2217  ARA
↪     K/R               nonstructural protein NS4A
1793_Den4/AY618992_1/Thailand/2001/Den4_1           8630           2877  ART
↪     N/S               nonstructural protein NS5
1793_Den4/AY618992_1/Thailand/2001/Den4_1           8844           2949  AAN
↪     GAPFOUND          nonstructural protein NS5
1793_Den4/AY618992_1/Thailand/2001/Den4_1           9938           3313  AYT
↪     I/T               nonstructural protein NS5
1793_Den4/AY618992_1/Thailand/2001/Den4_1           9941           3314  GRC
↪     D/G               nonstructural protein NS5
1793_Den4/AY618992_1/Thailand/2001/Den4_1          10015           3339  RTT
↪     I/V               nonstructural protein NS5
1793_Den4/AY618992_1/Thailand/2001/Den4_1          10087           3363  NGR
↪     GAPFOUND          nonstructural protein NS5
1793_Den4/AY618992_1/Thailand/2001/Den4_1          10085           3362  NGR
↪     GAPFOUND          nonstructural protein NS5
1901_Den4/AY618992_1/Thailand/2001/Den4_1             15              6  AAN
↪     NON-CODING        5'UTR
1901_Den4/AY618992_1/Thailand/2001/Den4_1            111             38  TTN
↪     GAPFOUND          anchored capsid protein
1901_Den4/AY618992_1/Thailand/2001/Den4_1           2279            760  GYT
↪     A/V               envelope protein
1901_Den4/AY618992_1/Thailand/2001/Den4_1           8798           2933  ARA
↪     K/R               nonstructural protein NS5
1901_Den4/AY618992_1/Thailand/2001/Den4_1          10195           3399  RAG
↪     E/K               nonstructural protein NS5
1901_Den4/AY618992_1/Thailand/2001/Den4_1          10366           3456  RGG
↪     NON-CODING        3'UTR
1934_Den4/AY618992_1/Thailand/2001/Den4_1             15              6  AAN
↪     NON-CODING        5'UTR
1934_Den4/AY618992_1/Thailand/2001/Den4_1            111             38  TTN
↪     GAPFOUND          anchored capsid protein
1934_Den4/AY618992_1/Thailand/2001/Den4_1            998            333  GMT
↪     A/D               envelope protein
1934_Den4/AY618992_1/Thailand/2001/Den4_1           4515           1506  TTM
↪     F/L               nonstructural protein NS3
1934_Den4/AY618992_1/Thailand/2001/Den4_1           8798           2933  ARA
↪     K/R               nonstructural protein NS5
```

plot_muts

Plot mutations either by comparing two sequences or by comparing a bunch of sequences to another sequence.

This command is still under a bit of development so bare with the nuances

Usage

You can view the usage of degen_regions via:

```
plot_muts --help
```

Example comparing multiple sequences against a query sequence

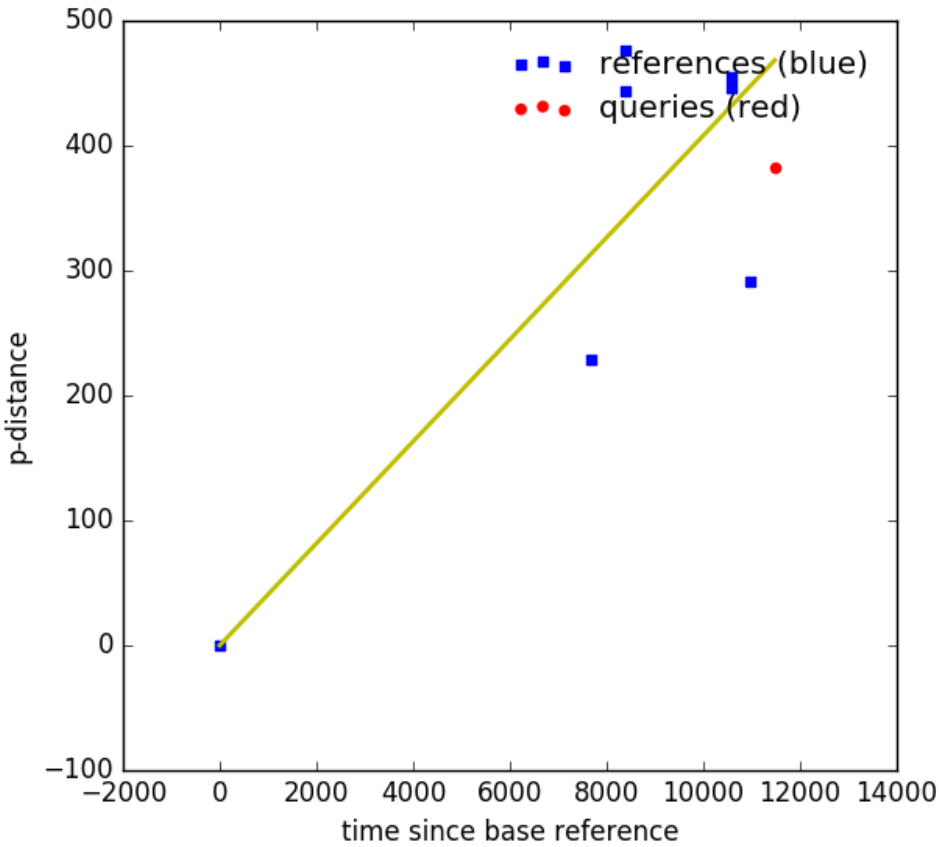
`--refs` is a fasta file containing multiple sequences where the sequence that has the earliest date will be used as the base reference.

`--query` is a fasta file containing a single sequence to be plotted in a different color to see how it compares.

```
plot_muts --refs tests/testinput/refs.fas --query tests/testinput/query.fas --out␣
↪plot.png
```

The `--out` option is optional. If it is not provided, the plot will pop up on the user's screen automatically. If this does not work, try saving the image using `--out` instead.

Example Output



Plot muts also outputs a csv file named after the `--out` with .csv appended

```
name,dates,p-dist
Ref_gi|499073378|gb|KC807176.1|_Houston_virus_strain_V3982_complete_genome____2004,
↪8401,476
Ref_gi|499073386|gb|KC807178.1|_Houston_virus_strain_16757_complete_genome____2010,
↪10592,455
Ref_gi|499073382|gb|KC807177.1|_Houston_virus_strain_16740_complete_genome____2010,
↪10592,446
```

```
Ref_gi|499073374|gb|KC807175.1|_Houston_virus_strain_V3872_complete_genome____2004,
↪8401,444
Query_A12x2520____08_16_2012,11478,383
Ref_gi|557884407|gb|KF522691.1|_Nam_Dinh_virus_isolate_SZ11706Z_complete_genome____
↪2011,10957,291
Ref_gi|341819796|gb|DQ458789.2|_Nam_Dinh_virus_isolate_02VN178_complete_genome____
↪2002,7670,229
BaseRef_gi|499073354|gb|KC807170.1|_Ngewotan_virus_strain_JKT9982_complete_genome____
↪1981,0,0
```

Input File Requirements

The input must be fasta format. Both the query and ref files can have any number of sequences.

The year should be the last part of the ID, preceded by a quadruple underscore. e.g.:

```
>some|info|blah_blah____2001_09_2010
>some____1995
>some____09/09/2012
```

If the ID uses '/' rather than underscore, plot_muts currently accepts the year as the *fourth* field. e.g.:

```
>some/info/blah/1995
>some/info/blah/1995/more/info
```

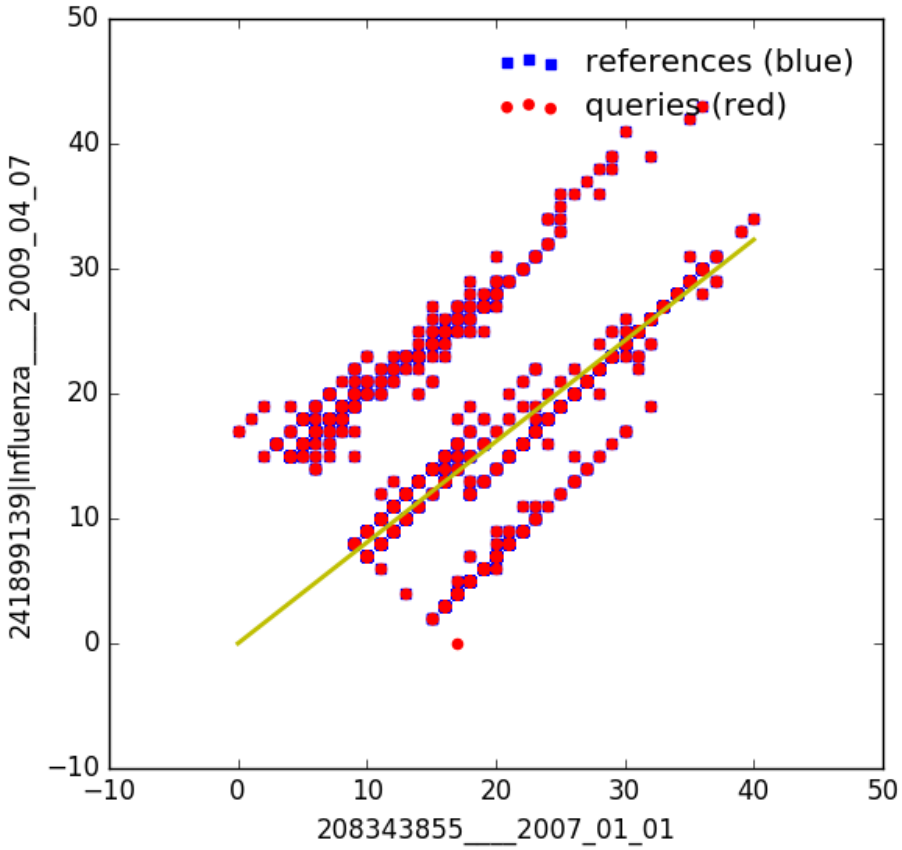
Example using cluster method to use two references as x and y axis

This is useful when you only have two references and no dates as all the sequences will be compared against these two sequences to give you a 'clustered' view

`--refs` is a fasta file that contains two references. The first being the x-axis and the second representing the y-axis
`--queries` is a fasta file that contains multiple sequences that will be plotted against the `--refs` sequences

```
plot_muts --query tests/testinput/ha/refall.ha.fasta --refs tests/testinput/ha/refall.
↪ha.fasta --cluster --out cluster.png
```

Example Output



Generating html graphics that are interactive

For both `--cluster` and non-cluster graphics you can optionally supply `--html` which will utilize the bokeh python project to build an interactive html output that you can open in your web browser.

fasta

fasta is a very simple script to help mangle fasta files.

- Supports converting multiline sequences into single line
- Supports splitting fasta file into separate files each named after the identifier
- Supports disambiguating ambiguous sequences

Usage

```
fasta --help
```

Examples

The following examples all use the test fasta file found under `tests/testinput/col.fasta`

```
>sequence1 some description !@#$%^&*()_+-=[]{}.,></?';:"
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
GGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG
CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
>sequence2!@#$%^&*()_+-=[]{}.,></?';:"
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
GGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG
CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
```

Convert column fasta into single lines

The following is a simple shell pipeline using fasta to ensure all sequences are on one line

```
$> cat tests/testinput/col.fasta | fasta -
```

Or if you want to you can read straight from a fasta file

```
$> fasta tests/testinput/col.fasta
```

Convert single line fasta into column fasta

The following would convert single line fasta sequences into column formatted fasta. It defaults to using 80 characters for each column

```
$> fasta tests/testinput/col.fasta
```

You can verify that it is wrapping correctly by simply piping the fasta command back into itself and then comparing to the original input file.

Here you can see we do that and then use diff to show there is no difference between the original file(col.fasta) and the new one(newline.fasta)

```
$> cat tests/testinput/col.fasta | fasta - | fasta --wrap - > newfile.fasta
$> diff tests/testinput/col.fasta newline.fasta
```

There will be no output as there is no difference between `newfile.fasta` and `tests/testinput/col.fasta`

Simple shell pipeline using fasta

The following is a simple shell pipeline to count how many A's there are in the sequence lines. There should be 160 since `col.fasta` is 80 characters per line and only the first line of each sequence has `A` and there are 2 sequences.

```
$> fasta tests/testinput/col.fasta | grep -v '>' | grep -Eo '[Aa]' | wc -l
160
```

Split fasta file into separate files named after identifiers

The following example shows how you can split a fasta file into multiple fasta files each named after an identifier in the original

```
$> fasta tests/testinput/col.fasta --split
$> ls -l *.fasta
sequence1.fasta
sequence2_____________________________.fasta
```

Note The reason sequence2 has such a long name is because it is replacing all punctionation characters with underscores. `col.fasta` is a test file that has a bunch of punctuation, hence all the underscores.

Similar to above, you can use input from standard input as the fasta input file

```
$> cat tests/testinput/col.fasta | fasta --split -
$> ls -l *.fasta
sequence1.fasta
sequence2_____________________________.fasta
```

Disambiguate ambiguous sequences

You can turn sequences that have ambiguous bases in them into all permutations of the same sequence with the ambiguous bases turned into non-ambiguous bases.

There is an upper limit of 100 for how many sequences can be generated to avoid creating thousands of sequences or consuming all of your computer's RAM.

If a sequence would generate more than 100 sequences, it will generate a message such as:

```
Sequence too_many has 7 ambiguous bases that would produce 128 permutations and was␣
↪skipped
```

and it will be skipped.

```
$> fasta --disambiguate tests/testinput/ambiguous.fasta > disambiguous.fasta
```

AMOS

AMOS is a file format that is similar to any assembly file format such as ACE or SAM. It contains information about each read that is used to assemble each contig.

The format is broken into different message blocks. For the Ray assembler, it produces an AMOS file that is broken into 3 types of message blocks

- RED

```
{RED
iid:\d+
eid:\d+
seq:
[ATGC]+
.
qlt:
```

```
[A-Z]+
}
```

iid Integer identifier

eid Same as iid?

seq Sequence data

qlt Should be quality, but is only a series of D's from Ray assembler

- TLE

```
{TLE
src:\d+
off:\d+
clr:\d+,\d+
}
```

src RED iid that was used

off One would think offset, but unsure what it actually means

clr Not sure what this is either

- CTG

```
{CTG
iid:\d+
eid:\w+
com:
.*$
.
seq:
[ATGC]+
.
qlt:
[A-Z]+
.
{TLE
...
}
}
```

iid integer id of contig

eid contig name

com Communication software that generated this contig

seq Contig sequence data

qlt Supposed to be contig quality data, but for Ray it only produces D's

TLE 0 or more TLE blocks that represent RED sequences that compose the contig

Parsing

bio_bits contains an interface to parse a given file handle that has been opened on an AMOS file.

To read in the AMOS file you simply do the following

```
from bio_bits import amos
a = None
with open('AMOS.afg') as fh:
    a = amos.AMOS(fh)
```

CTG

To get information about the contigs(CTG) you can access the `.ctgs` attribute. The contigs are indexed based on their iid so to get the sequence of contig iid 1 you would do the following:

```
ctg = a.ctgs[1]
seq = ctg.seq
```

To retrieve all the reads(RED) that belong to a specific contig:

```
reads = []
for tle in ctg.tlelist:
    reads.append(a.reds[tle.src])
```

RED

To get information about the reads(RED) you can access the `.reds` attribute. The reds are indexed based on their iid so to get the sequence of red iid 1 you would do the following:

```
red = a.reds[1]
seq = red.seq
```

If you want to convert a RED entry into anything you can use the `.format` method. The `.format` method allows you to utilize any of the properties of a RED object such as `.iid`, `.eid`, `.seq`, `.qlt`. You can see in the examples below how to do this.

Examples

Here is an example of how to convert all RED blocks into a single fastq file

```
from bio_bits import amos

# Fastq format string
fastq_fmt = '@{iid}\n{seq}\n+\n{qlt}'

with open('amos.fastq','w') as fh_out:
    with open('AMOS.afg') as fh_in:
        for iid, red in amos.AMOS(fh_in).reds.items():
            fq = red.format(fastq_fmt)
            fh_out.write(fq + '\n')
```

CHANGELOG

Version 1.4.0

- Switched to conda install

- Added continuous delivery

Version 1.3.2

- fasta added –disambiguate option to turn ambiguous sequences into all permutations possible

Version 1.3.1

- plot_muts added –cluster and –html options
- fasta added –split and –wrap options

Version 1.3.0

- Added fasta script that removes newlines from fasta sequences

Version 1.2.1

- Fixed some python3 and python2.6 incompatability issues
- Fixed some old bio_pieces references
- Added some simple tests for plot_muts

Version 1.2.0

- Renamed project to bio_bits to fix naming issue with other project
- GPL License added
- degen_regions script added
- parallel_blast added
- plot_muts script added

Version 1.1.0

- Renamed parse_contigs to group_references to better name functionality
- group_references now supports bam files

Version 1.0.0

- Version bump. Starting here we will employ semantic versioning
- Added version script to get version from project

Version 0.1.0

- Started project over to setup for Continuous Integration testing
- Added rename_fasta that can rename fasta sequence identifiers based on a input rename file
- Added travis, coveralls, readthedocs
- Added amos file parser that is specific to Ray assembler amos format
- Added format functionality for amos classes such that it is easy to convert to different formats
- Added amos2fastq to pull sequences out of AMOS files organized by their contigs.
- Added vcfcat.py, a commandline app for filtering and comparing vcf files.
- Completed documentation for vcfcat
- Added beast_checkpoint script and documentation
- Added beast_wrapper script that prints estimated time column in beast output
- Added beast_est_time script that allows you to easily get estimated time left from already running beast run

TODO

Todo

Could be possible to get beast_checkpoint to check for that scenario and use the last tree state that matches the last log state

(The original entry is located in /home/docs/checkouts/readthedocs.org/user_builds/bio-bits/checkouts/stable/docs/scripts/beast_checkpoint.rst, line 52.)

CHAPTER 2

Indices and tables

- genindex
- modindex
- search