align_contigs

March 14, 2018

1 Align contigs

In [3]:
```
%%bash
source activate secapr_env
secapr align_sequences -h
```

```
usage: secapr align_sequences [-h] --sequences SEQUENCES --output OUTPUT
                              [--aligner {muscle,mafft}]
                              [--output-format {fasta,nexus,phylip,clustal,emboss,s
                              [--no-trim] [--window WINDOW]
                              [--proportion PROPORTION]
                              [--threshold THRESHOLD]
                              [--max-divergence MAX_DIVERGENCE]
                              [--min-length MIN_LENGTH] [--ambiguous]
                              [--cores CORES]

Align sequences and produce separate alignment file for each locus, containing
the seqeunces of all taxa. Copyright (c) 2010-2012, Brant C. Faircloth All
rights reserved. Redistribution and use in source and binary forms, with or
without modification, are permitted provided that the following conditions are
met: * Redistributions of source code must retain the above copyright notice,
this list of conditions and the following disclaimer. * Redistributions in
binary form must reproduce the above copyright notice, this list of conditions
and the following disclaimer in the documentation and/or other materials
provided with the distribution. * Neither the name of the University of
California, Los Angeles nor the names of its contributors may be used to
endorse or promote products derived from this software without specific prior
written permission. THIS SOFTWARE IS PROVIDED BY THE COPYRIGHT HOLDERS AND
CONTRIBUTORS "AS IS" AND ANY EXPRESS OR IMPLIED WARRANTIES, INCLUDING, BUT NOT
LIMITED TO, THE IMPLIED WARRANTIES OF MERCHANTABILITY AND FITNESS FOR A
PARTICULAR PURPOSE ARE DISCLAIMED. IN NO EVENT SHALL THE COPYRIGHT OWNER OR
CONTRIBUTORS BE LIABLE FOR ANY DIRECT, INDIRECT, INCIDENTAL, SPECIAL,
EXEMPLARY, OR CONSEQUENTIAL DAMAGES (INCLUDING, BUT NOT LIMITED TO,
PROCUREMENT OF SUBSTITUTE GOODS OR SERVICES; LOSS OF USE, DATA, OR PROFITS; OR
BUSINESS INTERRUPTION) HOWEVER CAUSED AND ON ANY THEORY OF LIABILITY, WHETHER
IN CONTRACT, STRICT LIABILITY, OR TORT (INCLUDING NEGLIGENCE OR OTHERWISE)
ARISING IN ANY WAY OUT OF THE USE OF THIS SOFTWARE, EVEN IF ADVISED OF THE
```

```
POSSIBILITY OF SUCH DAMAGE. _____ Modified
by Tobias Hofmann (tobias.hofmann@bioenv.gu.se): Additions include: -
Standardizing script for incomplete data - More forgiving default options for
non-UCE datasets - Format the sequence headers of the output alignment files
to simply the sample name (no locus information in the header, only in the
filename) _____

optional arguments:
  -h, --help            show this help message and exit
  --sequences SEQUENCES
                        The fasta file containing the extracted contigs that
                        match the target loci
  --output OUTPUT       The directory in which to store the resulting
                        alignments.
  --aligner {muscle,mafft}
                        The alignment engine to use.
  --output-format {fasta,nexus,phylip,clustal,emboss,stockholm}
                        The output alignment format.
  --no-trim             Align, but DO NOT trim alignments.
  --window WINDOW       Sliding window size for trimming.
  --proportion PROPORTION
                        The proportion of taxa required to have sequence at
                        alignment ends.
  --threshold THRESHOLD
                        The proportion of residues required across the window
                        in proportion of taxa.
  --max-divergence MAX_DIVERGENCE
                        The max proportion of sequence divergence allowed
                        between any row of the alignment and the alignment
                        consensus.
  --min-length MIN_LENGTH
                        The minimum length of alignments to keep.
  --ambiguous           Allow reads in alignments containing N-bases.
  --cores CORES         Process alignments in parallel using --cores for
                        alignment. This is the number of PHYSICAL CPUs.
```

Let's run the alignment. In the example command below we added the flag `--no-trim`, which avoids the algorithm to cut the alignment at the ends (= full contig sequence length is being preserved) and the flag `--ambiguous`, which allows sequences with ambiguous bases ('N') to be included into the alignments. You can decide to not use the `--no-trim` flag if you want all seqeunces in the alignments to be of the same length. In that case there are a bunch of additional flags (see above) that you can use to adjust the trimming process.

```
secapr align_sequences --sequences ../../data/processed/target_contigs/extracted_ta
```

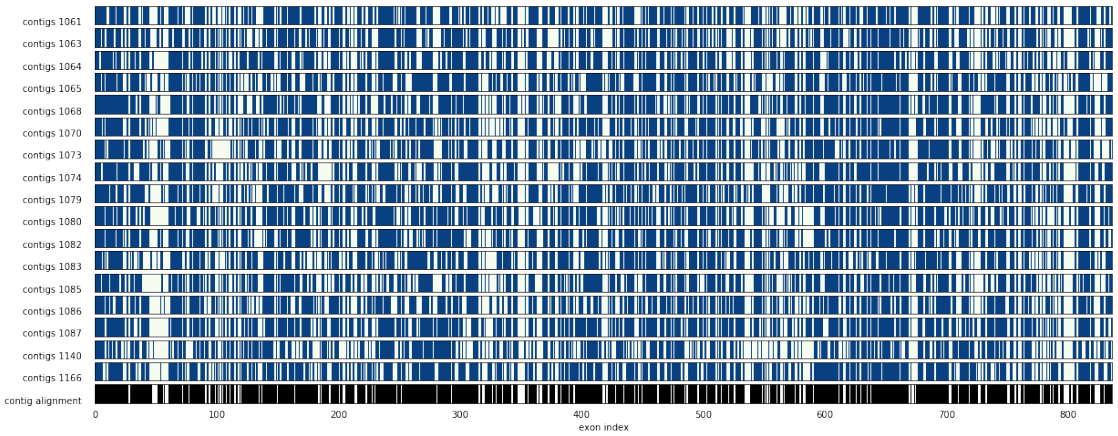
The align_sequences function by default creates multiple sequence alignments (MSAs) for all loci that are shared by at least 3 samples. This leads to alignments for most of the targeted exons. We can create an overview, that shows which loci we have MSAs for (bottom line on plot below).

```
In [3]: import sys
        sys.path.append("../../src")
        import plot_contig_data_function as secapr_plot

        match_table_path = '../../data/processed/target_contigs/match_table.txt'
        alignment_folder_path = '../../data/processed/alignments/contig_alignments'

        secapr_plot.plot_contigs_and_alignments_yield(match_table_path,alignment_fo
```

Out[3]:



1.1 Filling in missing sequences in alignments

Some applications (such as e.g. BEAST) require the same samples/taxa being present in every alignment. If you review your alignments you may find that many-most loci could not be assembled for all of your samples. Even though this is not the optimal turn-out it is quite normal and you can still proceed using your multilocus dataset. It is okay to have missing sequence data for some samples in the alignments, as long as it is correctly coded. Secapr has a function that adds dummy sequences consisting of ?'s for the missing taxa to your alignments, so that all alignments have the same set of taxa. All you have to do is to provide the path to the folder containign all alignments you want to sync and provide the link to the output folder.

```
secapr add_missing_sequences --input ../../data/processed/alignments/contig_alignme
```

Congratulations! You now hopefully have a whole folder full with alignments, which you can use for your downstream analyses. However, there is a lot more you can get out of your sequence capture data if you stick with us! Keep in mind that contig sequences (which your alignments consist of) constitute consensus sequences of the reads that were merged during assembly. Even though the algorithms behind assembly softwares (such as ABySS and Trinity) are well developed, they still may produce chimeric seqeunces. This means that the resulting sequence may well be a mixture between the sequences of the two possible alleles at the respective locus (for diploid organisms) or even worse a mixture between paralogous sequences from different sites. The rest

of this tutorial will take you through the steps of reference-based assembly, phasing and compiling of allele sequences. Further we provide different options for SNP extraction.

The next step is to generate a new reference library from our contig sequences and to remap the reads to this library (reference-based assembly).

Previous page | Next page

In [ ]: