RAPID CURATION OF GENOMIC ASSEMBLIES

The focus of rapid curation is to identify and correct large scale scaffolding errors of genomic assemblies. This is done by modifying an assembly via a Tile Path File (TPF) using evidence from Hi-C data.

Requirements

- Fasta file of genomic assembly
- Rapid curation scripts (https://gitlab.com/wtsi-grit/rapid-curation)
- Access to HiGlass (HiGlass)
- PretextMap (https://github.com/wtsi-hpag/PretextMap)
- PretextViewer (https://github.com/wtsi-hpag/PretextView)

Overview of workflow:

1. Convert your fasta file into a Tile Path File (TPF)

A Tile Path File (TPF) is a simple file that simply lists the order of sequences along a chromosome. These files are often used in genome assemblies in an effort to convey mapping information to the assembly program. Gap types and sizes can be specified, along with desired orientation of the component sequences.
The TPF format supports rapid manipulation of the underlying components thereby allowing the user to efficiently reshape a genome assembly.
For further information, please refer to Tiling Path File (TPF) Specification v1.4: 2/15/2011

rapid_split.pl will convert your fasta file into the tile path file format for you.

Eg:
rapid_split.pl -fa <yourfastafilename>

The created TPF file is sorted into 4 tab separated columns as in the following example:
1 2 3 4

```
?   scaffold_1:1-10627236   scaffold_1  PLUS
GAP TYPE-2  500
?   scaffold_1:10627737-26896573    scaffold_1  PLUS
?   scaffold_2:1-2001924    scaffold_2  PLUS
GAP TYPE-2  500
?   scaffold_2:2002425-4716520  scaffold_2  PLUS
GAP TYPE-2  500
?   scaffold_2:4717021-10487029 scaffold_2  PLUS
GAP TYPE-2  500
?   scaffold_2:10487530-12959303    scaffold_2  PLUS
GAP TYPE-2  500
?   scaffold_2:12959804-16728812    scaffold_2  PLUS
GAP TYPE-2  500
?   scaffold_2:16729313-19399053    scaffold_2  PLUS
GAP TYPE-2  500
?   scaffold_2:19399554-23803696    scaffold_2  PLUS
GAP TYPE-2  500
?   scaffold_2:23804197-26337532    scaffold_2  PLUS
?   scaffold_3:1-20769075   scaffold_3  PLUS
GAP TYPE-2  500
?   scaffold_3:20769576-24535592    scaffold_3  PLUS
GAP TYPE-2  500
?   scaffold_3:24536093-25417327    scaffold_3  PLUS
?   scaffold_4:1-5801213    scaffold_4  PLUS
GAP TYPE-2  500
?   scaffold_4:5801714-14085539 scaffold_4  PLUS
GAP TYPE-2  500
?   scaffold_4:14086040-16309388    scaffold_4  PLUS
GAP TYPE-2  500
?   scaffold_4:16309889-19868079    scaffold_4  PLUS
```

Content within the rows can take on one of two formats; Sequence line and non-sequence lines.

For **Sequence lines** Column 1 will be ?
When TPF formats were originally created, Column 1 was used for the Genbank sequence identifier. For our rapid curation purpose, this is not applicable, but an entry into column 1 is still required, hence we use the ?
Column 2 gives the scaffold Name followed by coordinates of the scaffold piece
Column 3 gives the scaffold ID
Column 4 gives the orientation of the scaffold

The scaffold name and ID will come from the name which is assigned during the assembly process.

E.g:
```
?   scaffold_1:1-10627236   scaffold_1  PLUS
```

rapid_split.pl will create the TPF file of your sequences using their scaffold identifier followed by its coordinates. Where the script sees a run of N's within the fasta sequence, it will add a **non-sequence line** to indicate a gap is present, with a default size of 500bp.
Column 1 states it is a gap
Column 2 states the gap type, in this process, we use only TYPE-2 gaps
Column 3 states the gap size
Column 4 is unused in this situation, but can be used to specify how the size of the gap was determined.

E.g:
```
GAP TYPE-2  500
```

2. Use PretextView to manipulate your assembly, and generate an AGP file

See PretextView - Tutorial for detailed instructions on use of pretext.
Arrange scaffolds into updated order.

For an explanation of AGP files please refer to AGP Specification v2.1

Where gaps exist in the assembly, you do not need to change anything in your TPF file in order for the script to incorporate breaks and joins correctly.
If there is no gap in the TPF where you wish to make an update, you would need to manually insert a gap into your TPF, editing the coordinates of the scaffold accordingly.

Wherever you wish to make a break within your TPF, you need to add **>** to the corresponding gap line in the TPF.

For example, if you wished to insert a scaffold into scaffold_1 at position 10982492 where there are no N's within the assembly, your TPF would originally look like this.

```
?       scaffold_1:1-14982492       scaffold_1      PLUS
```

You would have to edit your TPF file to make it look like this:

```
?       scaffold_1:1-10982492       scaffold_1      PLUS
>GAP    TYPE-2          200
?       scaffold_1:10982493-14982492        scaffold_1      PLUS
```

You can then paint the scaffolds into chromosomal units, ordered and oriented, label where appropriate (sex chromosomes, unlocalised, haplotigs) and then generate an AGP as described in the pretextview instructions.

3. Convert your AGP file to an updated TPF

In order to make an updated fasta for submission, we first need to have an updated TPF. To do this, we use the TPF file generated from your original assembly.fasta file with the ">" added into gaps where breaks and joins are to be made, and the .agp file extracted from Pretext.

```
rapid_pretext2tpf_XL.py <your_tpf> <Your_trimmed.agp>
```

Eg:

rapid_pretext2tpf_XL.py iyLasLatv2.tpf iyLasLatv2_1.agp

This will produce the following files:

- rapid_prtxt_XL.tpf
- haps_rapid_prtxt_XL.tpf
- chrs.csv

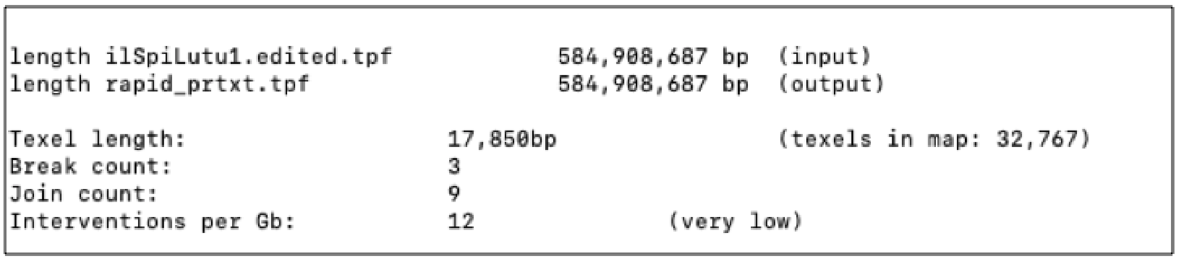
rapid_prtxt_XL.tpf will have renamed the scaffolds which you painted in pretext, in size order, using the prefix RL_ and also contains the remaining scaffolds of the assembly with their original names.

haps_rapid_prtxt_XL.tpf will contain any haplotigs you specified in pretext

chrs.csv will contain a simple .csv file which is used for making the files for submission

Anywhere a join has been made between scaffolds which you have specified using the scaffold painting in PretextView will be assigned a default gap size of 200bp. We use this so that we can easily see which changes we have made during the curation process.

When the script runs successfully you should get a printed message within the terminal window. This contains some information and statistics about your assembly and will look like this:



```
length ilSpiLutu1.edited.tpf          584,908,687 bp  (input)
length rapid_prtxt.tpf                584,908,687 bp  (output)

Texel length:               17,850bp              (texels in map: 32,767)
Break count:                3
Join count:                 9
Interventions per Gb:       12              (very low)
```

4. Create files for submission

In order to create the files needed for submission, the following files are required:

- Original fasta file
- Your updated tpf (rapid_pretext_XL.tpf)
- Your .csv file (chrs.csv)

The script takes the original assembly fasta file, a one-line per chromosome csv file and a TPF and creates the updated assembly from the TPF.

This is done by using rapid_join.pl

rapid_join.pl -fa <original.fasta> -tpf <your updated tpf file> -csv <your.csv> -out <outfile.fasta>

Eg:

rapid_join.pl -fa -fa iyLasLatv2.original.fa -tpf rapid_prtxt.tpf -csv iyLasLatv.csv -out iyLasLatv2

You do not need to use the -out option. If you don't then files will be written to <original.fasta**.new**>

You will now have an updated, curated version of your assembly ready for submission, and a related .csv file indicating the chromosome names.

If you have identified any haplotigs, you will also needs to run rapid_join.pl on the haplotig TPF produced earlier (**haps_rapid_prtxt_XL.tpf)** in order to also create the haplotigs fasta file for submission:

rapid_join.pl -fa <input.fa> -tpf <haplotype.tpf> -out <outfile> -hap

Assembly statistics

If you wish to know how many manual interventions you have made to your assembly, there are scripts to do this for you:

To count rapid breaks:

```
python rapid_count_breaks.py original.tpf rapid_prtxt.tpf haps.tpf
```

To count rapid joins:

```
grep 'GAP'$'\t''TYPE-2'$'\t''200' rapid_prtxt.tpf | wc -l
```