

Introduction

There are some useful scripts.

vcf2snpbinner.R

A R script for transforming vcf file to [SNPBinner](#) input file. The heterozygosity and missing rate will be calculated for output SNP marker.

Usage

```
Rscript ./vcf2snpbinner.R -h
```

```
usage: reform.R [--] [--help] [--opts OPTS] [--input INPUT] [--out OUT]
      [--parent1 PARENT1] [--parent2 PARENT2] [--minDP_p1 MINDP_P1]
      [--minDP_p2 MINDP_P2]
```

a program for converting vcf to table of snpbinner. genotype same as parent_1 is designated 'a', genotype same as parent_2 is designated 'b', heterozygous genotype is designated 'h', missing genotype is designated '-'

flags:

-h, --help show this help message and exit

optional arguments:

-x, --opts RDS file containing argument values
-i, --input vcf or vcf.gz file containing two parents and progeny
 lines
-o, --out output file prefix
-p, --parent1 name of parent_1
--parent2 name of parent_2
-m, --minDP_p1 Minimum depth of parent_1 [default: 5]
--minDP_p2 Minimum depth of parent_2 [default: 5]

Haplotype.R (GeneStructure with Variation)

A R script for drawing gene structure and the variation of this gene in a population. A gtf file containing target gene, a vcf file containing variation of this gene and phenotype data is needed. Hierarchical clustering algorithm was adopted to distinguish different haplotype, the number of haplotype can be designated according clustering result. Some polymorphism may exist within samples belonging to the same haplotype, you can divided them into different haplotypes by setting more haplotypes.

 A figure containing gene structure and variation information

ePCR.pl

A perl script for ePCR. Input is tsv (tab-separated values) file containing three columns (PrimerID, forwardPrimer, Reverse Primer).

Requiemment

- [ePCR](#)

Preparation

```
# lower letter to UPPER letter
seqkit seq -u reference.fa > genome.fa
# prepare sequence database for re-PCR searches
famap -t N -b genome.famap genome.fa
fahash -b genome.hash -w 12 -f 3 genome.famap
```

Usage

```
perl ePCR.pl -h
```

```
#####
#
# Usage:  ePCR.pl --input primer.txt --output output.txt
#
# Required:
#
#   --input <string>          input filename, one pair primer per line, tab
seperated, e.g.:
#                               primerID    Left_primer_seq Right_primer_seq
#
#   --output <string>         output filename.
#
#####
```

slidingWindow.R

A sliding window function in R. The R package [tidyverse](#) should be installed. [values](#) is a vector containing column names which need be calculated.

```
source("./slidingWindow.R")
# An example
sldwid <- slidingWindow(df = df, winSize = 1000000, winStep = 200000, groups =
"CHROM", position = "POS", values = c("R.R3.depth", "R.qY.depth"), fun = "mean")
```

addUp.R

A R function for calculating accumulation value for a column of a table. For example, a data.frame contain two columns, "chromosome" and "position", this function will calculate the accumulation position of different chromosome, then a list containing a table with a new column "position_add_Up", a vector containing breaks position, a vector containing labels, a vector containing gaps position, will be returned.

```
source("./addUp.R")
# An example
addUp(df = df, len = len, group = "chromosome", pos = "position", band = 0.01)
addUp(df = df, len = len, group = "chromosome", pos = c("start", "end"), band =
0.01)
```

run_DESeq2.R

A R script for differential expression analysis using DESeq2 (with biological replication). You need to prepare three files:

Requiemment

- **read count matrix file.**
- **samples file**, tab-delimited text file indicating biological replicate relationships. e.g.

```
cond_A  cond_A_rep1
cond_A  cond_A_rep2
cond_B  cond_B_rep1
cond_B  cond_B_rep2
```

- **contrasts file**, tab-delimited text file containing the pairs of sample comparisons to perform. e.g.

```
cond_A  cond_B
cond_Y  cond_Z
```

Usage

```
Rscript run_DESeq2.R -h
```

```
usage: run_DESeq2.R [--] [--help] [--opts OPTS] [--matrix MATRIX]
      [--samples_file SAMPLES_FILE] [--min_reps MIN_REPS] [--min_cpm
      MIN_CPM] [--contrasts CONTRASTS]
```

Run differential expression analysis using DESeq2.

flags:

```
-h, --help          show this help message and exit
```

optional arguments:

```
-x, --opts          RDS file containing argument values
-m, --matrix        matrix of raw read counts (not normalized!)
-s, --samples_file  tab-delimited text file indicating biological
                    replicate relationships.
--min_reps          At least min count of replicates must have cpm
                    values > min cpm value. [default: 2]
--min_cpm           At least min count of replicates must have cpm
                    values > min cpm value. [default: 1]
-c, --contrasts     file (tab-delimited) containing the pairs of
                    sample comparisons to perform.
```

extractSeq.sh

If there is a gene/transcript id you are interested and the corresponding genomics data, then you want to obtain genomic, CDS, pep sequence and gene structure information of this gene/transcript, you can use this shell script.

Usage

```
#chmod u+x extractSeq.sh
./extractSeq.sh --help
```

```
Usage: /home/wangpf/bin/extractSeq.sh [--genome genome_file] [--gff3 gff3_file] [-
-cds cds_file] [--pep pep_file] [--id gene/mRNA_id] [--up up] [--down down] [--gz]
Options:
```

```
--genome  Specify the genome fasta file
--gff3    Specify the gff3 file
--cds     Specify the cds fasta file
--pep     Specify the pep fasta file
--id      Specify the gene/mRNA id
--up      Specify how many bp upstream for gene/mRNA
--down    Specify how many bp downstream for gene/mRNA
```

```
--gz      Compress all the result if this option is present
--help    Display this help message
```

run_edgeR.R

A R script for differential expression analysis using edgeR (without biological replication). You need to prepare three files:

Requiemment

- **read count matrix file**.
- **samples file**, tab-delimited text file indicating biological replicate relationships. e.g.

```
cond_A  sample_A
cond_B  sample_B
```

- **contrasts file**, tab-delimited text file containing the pairs of sample comparisons to perform. e.g.

```
cond_A  cond_B
cond_Y  cond_Z
```

Usage

```
Rscript run_edgeR.R -h
```

```
usage: run_edgeR.R [--] [--help] [--opts OPTS] [--matrix MATRIX]
      [--samples_file SAMPLES_FILE] [--min_reps MIN_REPS] [--min_cpm
      MIN_CPM] [--contrasts CONTRASTS] [--dispersion DISPERSION]
```

Run differential expression analysis using DESeq2.

flags:

-h, --help show this help message and exit

optional arguments:

-x, --opts RDS file containing argument values
-m, --matrix matrix of raw read counts (not normalized!)
-s, --samples_file tab-delimited text file indicating biological
 replicate relationships.
--min_reps At least min count of replicates must have cpm
 values > min cpm value. [default: 1]
--min_cpm At least min count of replicates must have cpm
 values > min cpm value. [default: 1]

```
-c, --contrasts      file (tab-delimited) containing the pairs of
                     sample comparisons to perform.
-d, --dispersion     edgeR dispersion value. [default: 0.1]
```

get_longest_seq.pl

In some genomics data, there are multiple isoforms for one gene because of alternative splicing. This perl script can get the longest CDS or pep sequence of genes.

Requiemment

- perl module
 - Bio::SeqIO
- data file
 - CDS or pep sequence file
 - gff file

Usage

```
perl get_longest_seq.pl -h
```

```
#####
#
# Usage:  /public/home/wangpf/bin/get_longest_seq.pl --fasta <cds_or_pep.fa> --gff
<genes.gff> --out <outprefix>
#
# Required:
#
#      --fasta <string>                CDS or pep fasta file.
#
#      --gff    <string>                gff file.
#
#      --out    <string>                output prefix.
#
#####
```

Output

- <outprefix>.longest.fa, the longest CDS or pep sequence in fasta format with gene id as sequence identifier
- <outprefix>.longest.list, a list contain gene id, longest mRNA id and the length of longest mRNA

find_gaps.py

There are gaps in majority genome fasta files except T2T genome. This `find_gaps.py` script will find gaps position.

Requiemment

- python3 module
 - argparse
 - Biopython
- input data
 - genome fasta file

Usage

```
chmod u+x find_gaps.py
find_gaps.py -h
```

```
usage: find_gaps.py [-h] -i INPUT -o OUTPUT [-s] [-c CONTIG]
```

Find gaps (N regions) in a genome FASTA file and optionally split at gaps.

optional arguments:

-h, --help	show this help message and exit
-i INPUT, --input INPUT	Input genome FASTA file
-o OUTPUT, --output OUTPUT	Output file for gap positions
-s, --split	Split the FASTA file at gaps
-c CONTIG, --contig CONTIG	Output file for split contigs (default: split.fa)

Output

A tab-separated text file containing three columns.

```
<chromosome_id> <gap_start> <gap_end>
```

A fasta file with split contigs.

mummerCoordsPlot.R

This script can draw a alignment plot according to mummer's show-coords program. In fact, this script is modeled after `mummerCoordsDotPlotly.R` in `dotPlotly`. `mummerCoordsDotPlotly.R` is a excellent program, but there are a few aspects that I am not entirely satisfied with:

- It will sort query ID by length, but I want keep query ID order in the query genome;
- `Yellow` is not very noticeable on a white background, so I use `red` and `blue`;
- Add the ability to color the alignment results based on whether they are forward or reverse complementary;
- IDs was not on the middle of chromosomes or contigs in figure;
- I typically work with the `tidyverse` ecosystem, so I have rewritten this script using `tidyverse` syntax for clarity and consistency;
- Some other modifications.

Requiemment

- R package
 - `argparser`
 - `tidyverse`
- input data
 - Alignment result from mummer's show-coords program;

```
show-coords example.filter.delta > example.coords
```

- Tab-separated text files containing reference and query IDs separately, first column is IDs in genome, the second is what you want to show in figure, only chromosomes or contigs in these files will be shown (optional). If not assigned, all IDs of chromomsomes or contigs longer than `--min-query-length` will be shown in figure;

```
GWHERGL00000001 A01
GWHERGL00000002 A02
GWHERGL00000003 A03
GWHERGL00000004 A04
GWHERGL00000005 A05
... ..
```

- Tab-separated text files containing chromosome or contig length, first column is IDs in genome, the second is the corresponding length (optional);

```
GWHERGL00000001 59205763
GWHERGL00000002 39005548
GWHERGL00000003 44769757
GWHERGL00000004 28346059
GWHERGL00000005 46671336
... ..
```


Usage

```
Rscript mummerCoordsPlot.R -h
```

```
usage: mummerCoordsPlot.R [--] [--help] [--opts OPTS] [--input INPUT]
      [--out OUT] [--ref REF] [--query QUERY] [--refIDs REFIDS]
      [--queryIDs QUERYIDS] [--refLen REFLen] [--queryLen QUERYLEN]
      [--min-query-length MIN-QUERY-LENGTH] [--min-alignment-length
      MIN-ALIGNMENT-LENGTH] [--min-identity MIN-IDENTITY] [--color-by
      COLOR-BY] [--width WIDTH] [--height HEIGHT]
```

Generates plots of alignment data produced by show-coords.

flags:

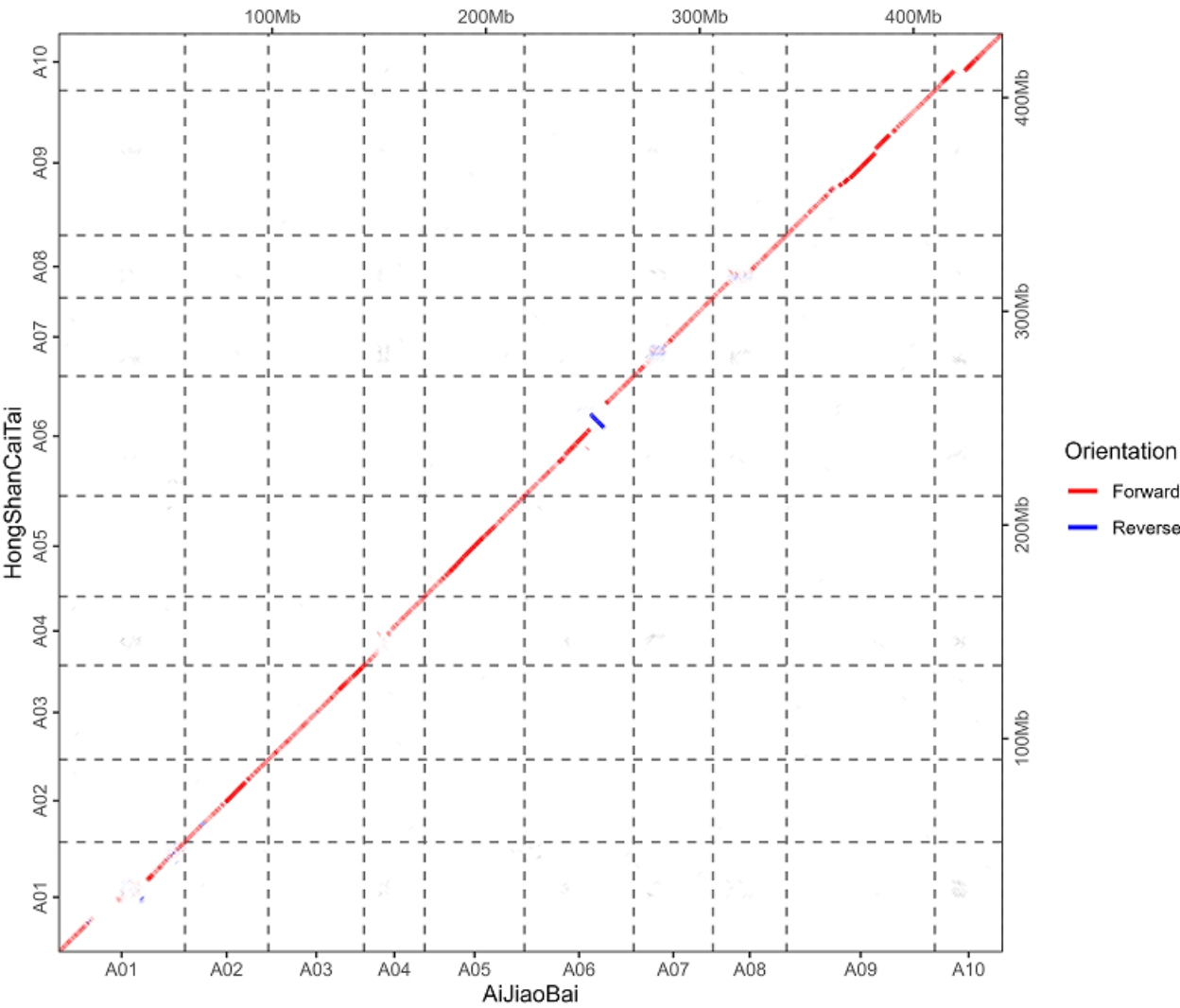
-h, --help show this help message and exit

optional arguments:

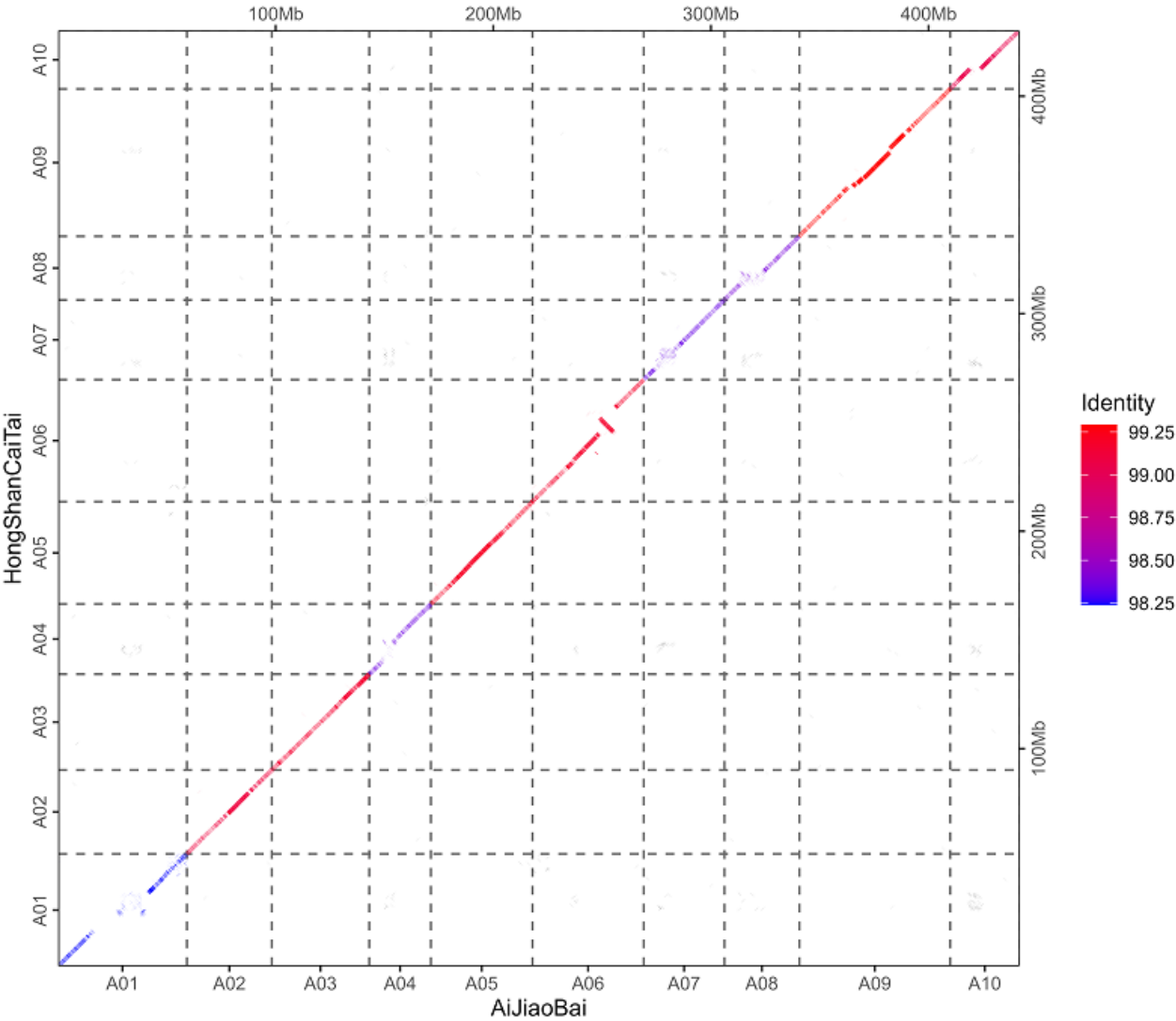
-x, --opts	RDS file containing argument values
-i, --input	coords file from mummer program 'show-coords'
-o, --out	outfile prefix
-r, --ref	reference name shown in plot [default: Reference]
-q, --query	query name shown in plot [default: Query]
-R, --refIDs	a tab-separated file containing two columns, first column is IDs in reference genome, second column is names to shown in plot
-Q, --queryIDs	a tab-separated file containing two columns, first column is IDs in query genome, second column is names to shown in plot
-l, --refLen	a tab-separated file containing two columns, first column is IDs in reference genome, second column is chr length
-L, --queryLen	a tab-separated file containing two columns, first column is IDs in query genome, second column is chr length
-M, --min-query-length	filter queries with total alignments less than cutoff X bp [default: 4e+05]
-m, --min-alignment-length	filter alignments less than cutoff X bp [default: 10000]
-s, --min-identity	filter alignments with identity less than X % [default: 90]
-c, --color-by	turn on color alignments by 'direction' or 'identity', no color if not assign
-W, --width	plot width (inches) [default: 10]
-H, --height	plot height (inches) [default: 10]

Output

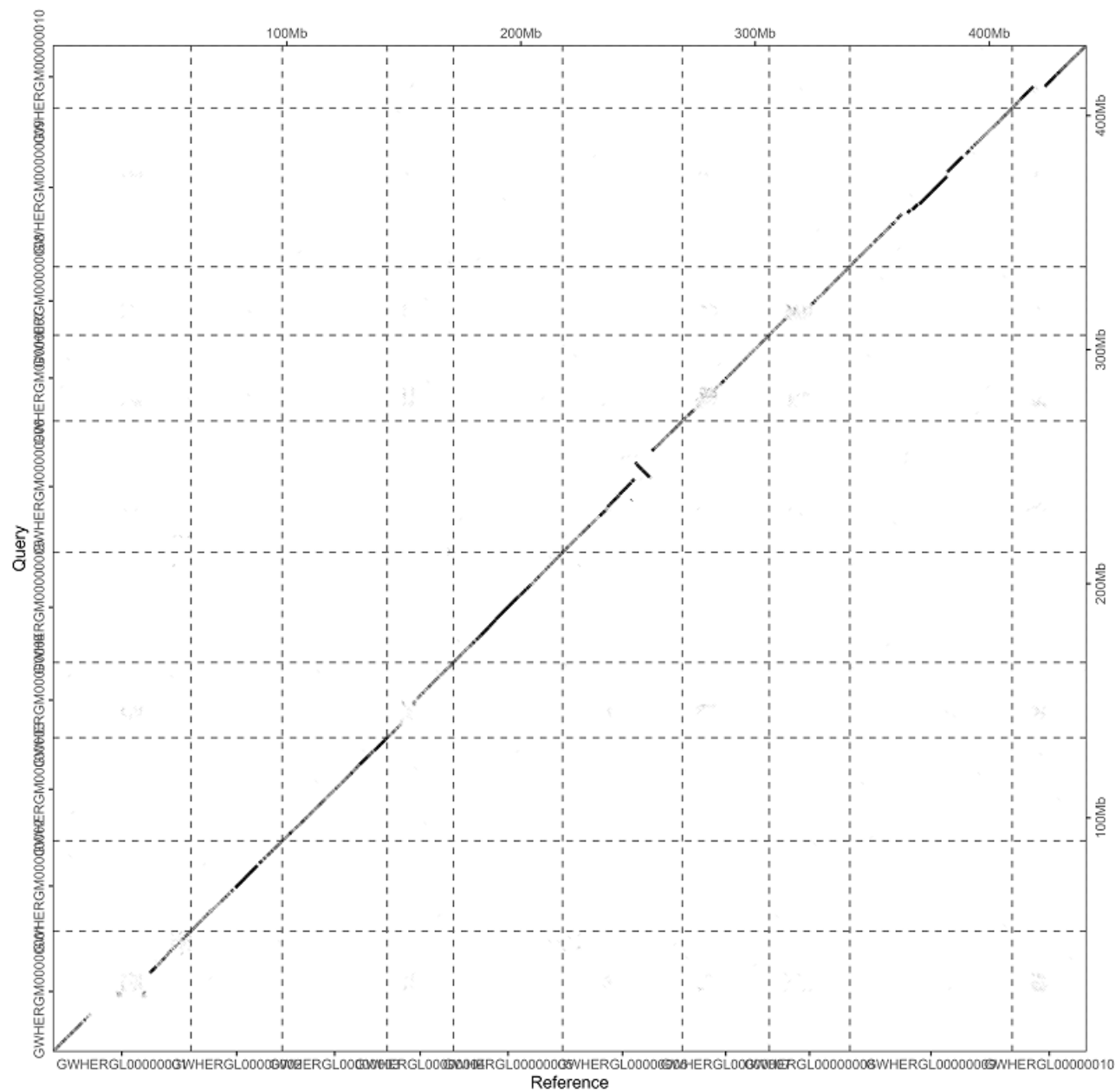
- Color the alignment results based on direction.



- Color the alignment results based on identity.



- default.



Data Source

These two genome is from this paper:

Zhou, Yifan et al. "The complexity of structural variations in Brassica rapa revealed by assembly of two complete T2T genomes." Science bulletin vol. 69,15 (2024): 2346-2351. doi:10.1016/j.scib.2024.03.030