

# HiCHap

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## Introduction

**HiCHap** is a Python package designed to process and analyze Hi-C data, primarily for diploid Hi-C by using phased SNPs. First, the Hi-C reads are split in ligation junction sites, and then all split parts are used in mapping to maximumly utilize SNPs in allele assignment, thus improving the ratios of allele-assigned reads. The noisy reads are further eliminated. Second, except for traditional data bias caused by Hi-C experiments, the unevenly distributed genetic variants lead to additional bias in reconstructed Hi-C haplotype because it is potentially easier to assign allelic contacts in the chromatin regions with denser genetic variants. HiCHap utilizes a two-step strategy to reduce these two types of data biases by using the mapped and allele-assigned contacts only. Third, with the improved quality of reconstructed Hi-C haplotype, HiCHap can identify compartments, topological domains/boundaries and chromatin loops at haplotype level, and also provide testing on the allelic specificity for these structures. Finally, HiCHap also supports data processing, bias correction and structural analysis for traditional Hi-C without separating homologous chromosomes.

## Download :

PyPI : (<https://pypi.org/project/HiCHap/>)

Github : ([https://github.com/Prayforhanluo/HiCHap\\_master](https://github.com/Prayforhanluo/HiCHap_master))

## Requirement

1. Python 2.7+
2. Multiprocess
3. Numpy
4. Scipy
5. statsmodels
6. Scikit-Learn
7. xml
8. mirnylib
9. pysam
10. ghmm
11. Bio
12. bowtie2 (we used 2.2.9)
13. samtools (we used 1.5)
14. cooler

Please use **conda** , **pip** or the source code to install them if necessary

## Install HiCHap

Download the HiCHap source code from PyPI or Github, unzip the files and run the setup.py script::

```
$ python setup.py install
```

Or use the pip install

```
$ pip install HiCHap
```

By the way, When I developed HiCHap, I have found a strange bug. when HiCHap has been installed, I use the `hichap -h` to get help information. But it bugs with "**pkgresources.ResolutionError::**". I just figure out how to fix it but i can't figure out why it happens. The fixing code is in the `setup.py` at the last few lines. If anyone can tell me how to avoid this bug. Please connect with me ([hluolc@outlook.com](mailto:hluolc@outlook.com)) and thanks so much :)

**HiCHap** will be installed successfully if no exception occurs in the above process.

## Input data formats

### Hi-C data

Hi-C data format is fastq.gz(or unzipped fastq), and canonical name will be nice, for example::

```
$ ls -lh
-rw-r--r-- 1 hluo CPeng 42G Sep 14 00:30 GM12878_R1_1.fastq.gz
-rw-r--r-- 1 hluo CPeng 42G Sep 14 00:30 GM12878_R1_2.fastq.gz
```

Both compressed and uncompressed fastq data are available for Hi-C data

### genome data

The genome data format is .fa. Please use the same reference genome version when processing different data, such as phased genetic variants and allelic read mapping..

```
$ ls -lh
-rwxr-xr-- 1 hluo CPeng 2780868912 Sep  4  2017 mm10.fa
-rwxr-xr-- 1 hluo CPeng 3187102198 Sep  4  2017 hg19.fa
```

### SNPs data

The phased SNPs can be given in a TXT-like file. The file has the following five columns :

1. chromosome ID
2. genome position
3. reference base
4. Maternal base
5. Paternal base

For example::

```
$ ls -lh
-rw-r--r-- 1 hluo CPeng 56524229 Mar  9 11:07 GM12878_F1_maternal_paternal_SNP.txt

$ head -5 GM12878_F1_maternal_paternal_SNP.txt

1  10492  C  T  C
1  10927  A  A  G
1  10938  G  G  A
1  13302  C  C  T
1  13813  T  G  T
```

## How to use HiCHap ?

First, try to get the help message! The executable code in the command line of HiCHap is **hichap**.

HiCHap has the general parameters : -w (--workspace), -log (--logfile) and -N (--NonAllelic).

Specifically, **hichap** will output the results to the current workspace (-w) and search the input there. Users can also set the workspace by themselves. If -N (--NonAllelic) is set on the command, this sub-module will run the traditional Hi-C pipeline.

If -N (--NonAllelic) is set on the sub-command. the sub-module will running traditional Hi-C pipeline.

Try to get help information:

```
$ hichap --help

usage: hichap [-h] [-v] {rebuildG,rebuildF,GlobalMapping,Rescue,ReMapping,bamProcess,filtering,matrix}
...
...
```

There are several major functions available in HiCHap sub-commands.

| Subcommand | Description | |-----|-----| | rebuildG | Build Genome index, genome size and enzyme fragment locations | | rebuildF | Chunk fastq files. | GlobalMapping | Mapping the raw chunked reads to genome Rescue | Rescue the unmapped reads by cutting the Ligation site.

ReMapping | Re-mapping the rescued reads to genome bamProcess | Integrate all the mapping information filtering | Hi-C filtering and Allelic assignment (if necessary). matrix | Interaction Matrix Construction

Please use 'hichap SUB-COMMAND -h' to see the detail description for each option of each module.

## rebuildG

For traditional Hi-C pipeline, build the genome index and enzyme-fragment locations for the reference genome.

The command line is ::

```
$ hichap rebuildG -w ./GM12878 -log ./GM12878.log -N -g ./hg19.fa -e MboI -t 4
```

For Hi-C haplotype pipeline, build the maternal and/or paternal genomes and corresponding indexes, enzyme-fragment locations.

The command line is ::

```
$ hichap rebuildG -w ./GM12878 -log ./GM12878.log -g ./hg19.fa -S ./GM12878_F1_maternal_paternal_SNP.txt -e MboI -t 4
```

After subcommand rebuildG, a folder (./GM12878/genome) containing the genome indexes and the txt files containing fragment locations will be created under the workspace(./GM12878)

## rebuildF

Chunk the fastq by a given step. The command line eg ::

```
$ hichap rebuildF -w ./GM12878 -log ./GM12878.log -1 GM12878_R1_1.fastq.gz -2 GM12878_R1_2.fastq.gz -c 4000000 -t 2
```

After rebuildF, a folder(./Genome/fastqchunks) contains the chunked files will be created under the workspace(./Genome) eg:

```
$ ls -lh ./Genome/fastqchunks

-rw-r--r-- 1 hluo CPeng 410M Nov 13 10:44 GM12878_R1_chunk0_1.fastq.gz
-rw-r--r-- 1 hluo CPeng 410M Nov 13 10:43 GM12878_R1_chunk0_2.fastq.gz
...
...
-rw-r--r-- 1 hluo CPeng 407M Nov 13 10:49 GM12878_R1_chunk9_1.fastq.gz
-rw-r--r-- 1 hluo CPeng 406M Nov 13 10:48 GM12878_R22_chunk9_2.fastq.gz
```

## GlobalMapping

After genome rebuilding and fastq chunking, you need to start mapping tasks. Each chunk represents a single mapping task. The parallel mode is used to reduce the time cost. And two sets of mapping APIs are designed for different computer environments.

### 1. PBS-Mode

If you use the clusters (**based on PBS for jobs management**), you can choose the PBS API. You can submit N tasks to the computing nodes and M threads for each task.

For traditional Hi-C pipeline, there is only one index parameter. For example:

```
$ nohup hichap GlobalMapping -w ./GM12878 -log GM12878.log -b ~/tools/bowtie2/bowtie2 -i ./GM12878/genome/hg19/hg19
-m PBS -pt 10 4 &
```

For Hi-C haplotype pipeline, there are two index parameters, maternal and paternal indexes. For example:

```
$ nohup hichap GlobalMapping -w ./GM12878 -log GM12878.log -b ~/tools/bowtie2/bowtie2 -i ./GM12878/genome/Maternal/Maternal
./GM12878/genome/Paternal/Paternal -m PBS -pt 10 4 &
```

**Make sure that this command is run on the login node or the node where jobs are submitted..**

The key parameter of this command -m(--mode) must be PBS. The parameter -pt (--PBSthreads) 10 4 means that 10 chunks will run mapping tasks parallelly, and each task uses 4 threads. Using the **qstat** to check the tasks.

```
$ qstat
```

Job ID	Name	User	Time	Use	S	Queue
2266086.admin	GM12878_R1	hluo	0	R	batch	
2266087.admin	GM12878_R1	hluo	0	R	batch	
2266088.admin	GM12878_R1	hluo	0	R	batch	
2266089.admin	GM12878_R1	hluo	0	R	batch	
2266090.admin	GM12878_R1	hluo	0	R	batch	
2266091.admin	GM12878_R1	hluo	0	R	batch	
2266092.admin	GM12878_R1	hluo	0	R	batch	
2266093.admin	GM12878_R1	hluo	0	R	batch	
2266094.admin	GM12878_R1	hluo	0	R	batch	
2266095.admin	GM12878_R1	hluo	0	R	batch	

## 2. WS-Mode

If you are not using the cluster, please use WS API to start mapping tasks.

For traditional Hi-C pipeline, there is only one index parameter. For example:

```
$ hichap GlobalMapping -w ./GM12878 -log GM12878.log -b ~/tools/bowtie2/bowtie2 -i ./GM12878/genome/hg19/hg19 -m WS -wt 16
```

For Hi-C haplotype pipeline, there are two index parameters, maternal and paternal indexes. For example:

```
$ hichap GlobalMapping -w ./GM12878 -log GM12878.log -b ~/tools/bowtie2/bowtie2 -i ./GM12878/genome/Maternal/Maternal  
./GM12878/genome/Paternal/Paternal -m WS -wt 16
```

The key parameter of this command `-m(--mode)` must be `WS`. The parameter `-wt(--PBStreads)` 16 means that 16 threads will be shared by 4 mapping tasks. That is, 4 chunk mapping tasks are running parallelly and each task occupies 4 threads.

## Rescue

Rescue the unmapped reads. For unmapped reads in `GlobalMapping`, `hichap` will search the ligation-junction sites and use the rescuing mode to make full use of sequence information on reads.

For traditional Hi-C pipeline:

```
$ hichap Rescue -w ./GM12878 -log GM12878.log -e MboI -t 8 -N
```

For Hi-C haplotype pipeline:

```
$ hichap Rescue -w ./GM12878 -log GM12878.log -e MboI -t 8
```

## ReMapping

Except for the inputs, the other parameters are same as `GlobalMapping ..`

## bamProcess

Integrate all the mapping information.

For traditional Hi-C pipeline, the fragment parameter(`-f`) only have one fragment-location file and the SNP parameter should be default (None). For example:

```
$ hichap bamProcess -w ./GM12878 -log ./GM12878.log -N -f ./GM12878/genome/GATC_hg19_fragments.txt -t 16 --rfo
```

For Hi-C haplotype pipeline, the fragment parameter(`-f`) should have two fragment-location files, maternal and paternal files. The SNP parameter should be set. For example:

```
$ hichap bamProcess -w ./GM12878 -log ./GM12878.log -f ./GM12878/genome/GATC_Maternal_fragments.txt
```

```
./GM12878/genome/GATC_Paternal_fragments.txt -s ./GM12878/genome/SNPs/Snps.pickle -t 16 --rfo
```

The parameter `--rfo` means filtering the unique reads softly. If your data have high sequencing depths, you can remove this parameter by sacrificing the data utilization.

## filtering

The **filtering** sub-command is designed to perform some basic filtering on the mapped Hi-C read pairs:

### Hi-C filtering

1. Remove redundant PCR duplicates
2. Remove the read pair that maps to the same restriction fragment

### Allelic assignment

1. assignment maternal interaction pairs
2. assignment paternal interaction pairs
3. assignment regroup interaction pairs

Here's the command you should type in the terminal:

For traditional Hi-C pipeline

```
$ hichap filtering -w ./GM12878 -log ./GM12878.log -N -t 16
```

For Hi-C haplotype pipeline

```
$ hichap filtering -w ./GM12878 -log ./GM12878.log -t 16
```

After this sub-command, some bed files will be created under the workspace, such as "FilteredBed" folder for traditional Hi-C pipeline, "AllelicBed" folder for Hi-C haplotype pipeline. The main file is "\*\*\*Validsorted.bed", which is the valid Hi-C interactions. This file has 23 columns and you can do some custom processing by using it. The description of each column is :

```
----- | Hi-C interaction -----| ----- column | description 1 | Pair Name 2 | R1 mate Reference 3 | R1 mate Strand 4 | R1 mate Position 5 | R1 mate Length 6 | R1 mate AS score 7 | R1 mate Fragment Middle point 8 | R1 mate SNP Matching num (traditional Hi-C results in 0) 9 | R1 mate Reference 10 | R2 mate Strand 11 | R2 mate Position 12 | R2 mate Length 13 | R2 mate AS score 14 | R2 mate Fragment Middle point 15 | R2 mate SNP Matching num (traditional Hi-C results in 0) ----- | candidate mate if it is possible 16 | Candidate mate Reference 17 | Candidate mate Strand 18 | Candidate mate Position 19 | Candidate mate Length 20 | Candidate mate AS score 21 | Candidate mate Fragment Middle point 22 | Candidate mate SNP Matching num (traditional Hi-C results in 0) 23 | Candidate Index for which mate.
```

For Hi-C haplotype pipeline, the output files of haplotype interactions have the targets like "MM", "PP", "MP", "PM", "BiAllelic". The "MM" represents the maternal-maternal interactions. The "MP" represents the maternal-paternal interactions. "BiAllelic" represents no allelic assignment. The files have 5 columns. The description of each column is:

```
----- | Haplotype-resolved Hi-C interactions -----| ----- column|description 1 | chromosome ID for interaction loci 1 2 | fragment ID for interaction loci 1 3 | chromosome ID for interaction loci 2 4 | fragment ID for interaction loci 2 5 | assignment target (R1 means assigned by R1, R2 means assigned by R2, Both means both mate can be assigned)
```

## matrix

Interaction Matrix Construction. The cooler format file will be generated. For this sub-command, You need to set the output-folder path. The matrix will be saved in cooler files at different resolutions

For traditional Hi-C pipeline

```
$ hichap matrix -b GM12878_R1_workspace/Filtered_Bed GM12878_R2_worspace/Filtered_Bed -N -o ./GM12878_Matrix -gs ./genome/genomeSize -wR 2000000 1000000 -lR 200000 40000 20000
```

For Hi-C haplotype pipeline

```
$ hichap matrix -b GM12878_R1_workspace/Allelic_Bed GM12878_R2_workspace/Allelic_Bed -o ./GM12878_Matrix
```

```
-gs ./genome/genomeSize -wR 5000000 2000000 -lR 500000 40000
```

The Imputation parameters can be changed. try --help for more information.

### !!! Notice !!!

The traditional matrix in cool are balanced by ICE.

The haplotype-resolved matrices are not stored as balance data in cool way. The raw count values in haplotype-resolved matrices are already corrected by HiCHap and the type is float.

The Gap file are saved into NPZ file. U can use numpy to load it.

## How to get matrix with python code.

Loading the Matrix in cooler. Open a python interpreter and follow the code below:

```
>>> import cooler
>>> GM12878_T = cooler.Cooler('Merged_Traditional_Multi.cool::40000')
>>> GM12878_T.matrix(balance = False).fetch('1')      #Get the raw chromosome 1 Matrix
>>> GM12878_T.matrix(balance = True).fetch('1')      #Get the balanced chromosome 1 Matrix
>>> # Haplotype-resolved Matrix
>>> GM12878_Haplotype = cooler.Cooler('Merged_Imputed_Haplotype_Multi.cool::40000')
>>> GM12878_Haplotype.matrix(balance = False).fetch('M1') #Get chromosome 1 Maternal Matrix
>>> GM12878_Haplotype.matrix(balance = False).fetch('P1') #Get chromosome 1 Paternal Matrix
```

More information about cooler format [here](#)

## Structure Analysis

Structure analysis is integrated in the module **StructureFind**. The source code can be found in the lib/StructureFind.py.

Use the API like :

```
>>> from HiCHap.StructureFind import StructureFind

>>> #===== Compartment=====
>>> ## For traditional Hi-C
>>> GM_T_PC = StructureFind(cooler_fil = 'Merged_Traditional_Multi.cool', Res = 500000, Allelic = False)
>>> GM_T_PC.run_Compartment(OutPath = 'Traditonal_PC', plot = True, MS = 'IF', SA = False)

>>> ## For haplotype-resolved Hi-C
>>> GM_M_PC = StructureFind(cooler_fil = 'Merged_Imputed_Haplotype_Multi.cool', Res = 500000, Allelic = 'Maternal')
>>> GM_M_PC.run_Compartment(OutPath = 'Maternal_PC', plot = True, MS = 'IF', SA = False,
                           Traditional_PC_file = 'Traditional_PC/Traditional_PC_Compartment_500K.txt')

>>> GM_P_PC = StructureFind(cooler_fil = 'Merged_Imputed_Haplotype_Multi.cool', Res = 500000, Allelic = 'Paternal')
>>> GM_P_PC.run_Compartment(OutPath = 'Paternal_PC', plot = True, MS = 'IF', SA = False,
                           Traditional_PC_file = 'Traditional_PC/Traditional_PC_Compartment_500K.txt')

>>> #===== TADs calling=====
>>> ## For traditional Hi-C
>>> GM_tads_T = StructureFind(cooler_fil = 'Merged_Traditional_Multi.cool', Res = 40000, Allelic = False)
>>> GM_tads_T.run_TADs(OutPath = 'Traditional_TADs', plot = True)
>>>
>>> ## For haplotype-resolved Hi-C
>>> GM_tads_M = StructureFind(cooler_fil = 'Merged_Imputed_Haplotype_Multi.cool', Res = 40000, Allelic = 'Maternal')
>>> GM_tads_M.run_TADs(OutPath = 'Maternal_TADs', plot = True)

>>> GM_tads_P = StructureFind(cooler_fil = 'Merged_Imputed_Haplotype_Multi.cool', Res = 40000, Allelic = 'Paternal')
>>> GM_tads_P.run_TADs(OutPath = 'Paternal_TADs', plot = True)

>>> #===== Loops calling=====
>>> ## For traditonal Hi-C
>>> GM_Loop_T = StructureFind(cooler_fil = 'Merged_Traditional_Multi.cool', Res = 40000, Allelic = False)
>>> GM_Loop_T.run_Loops(OutPath = 'Traditional_Loops', plot = True)
```

```

>>> ## For haplotype-resolved Hi-C
>>> GM_Loop_M = StructureFind(cooler_fil = 'Merged_Imputed_Haplotype_Multi.cool', Res = 40000, Allelic = 'Maternal')
>>> GM_Loop_M.run_Loops(OutPath = 'Maternal_Loops', plot = True)

>>> GM_Loop_P = StructureFind(cooler_fil = 'Merged_Imputed_Haplotype_Multi.cool', Res = 40000, Allelic = 'Paternal')
>>> GM_Loop_P.run_Loops(OutPath = 'Paternal_Loops', plot = True)

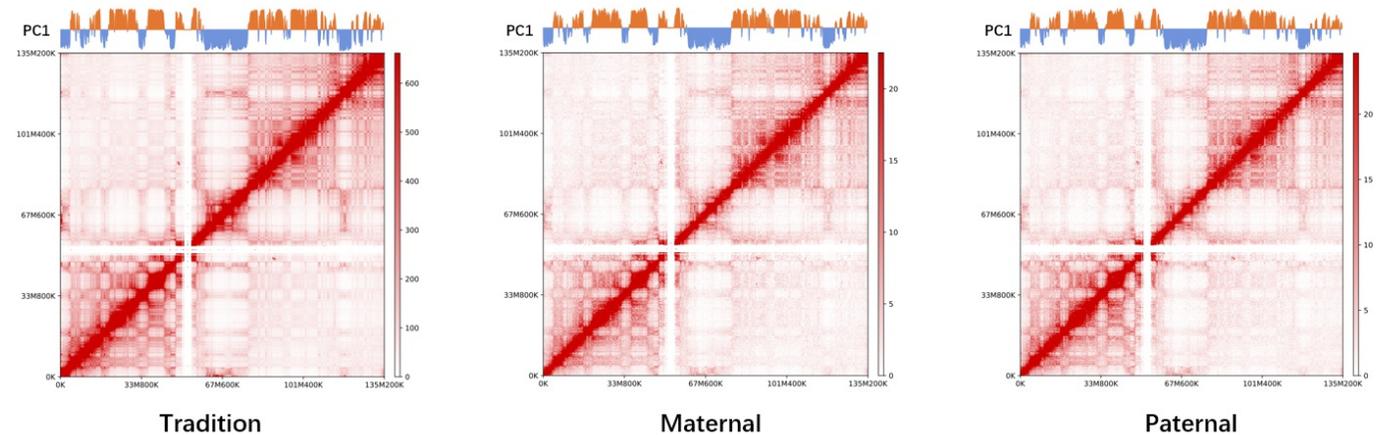
```

Notice that the most important parameter is **Allelic**, **False** for traditional Hi-C and **Maternal/Paternal** for Maternal/paternal..

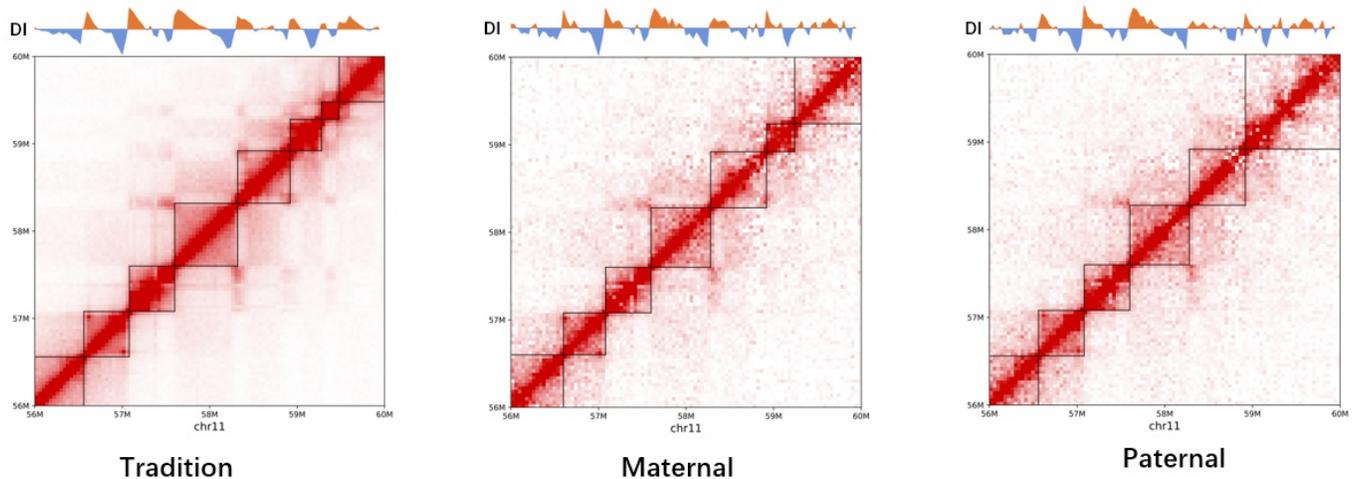
You can read the source code in `StructureFind.py` for more details.

## The Structure examples:

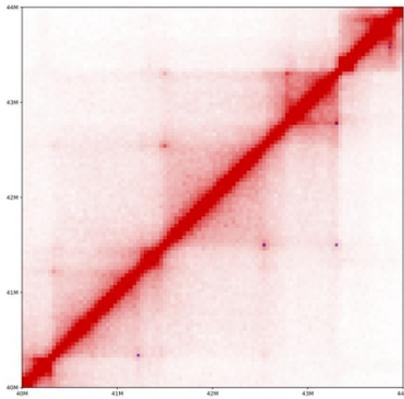
### Compartment:



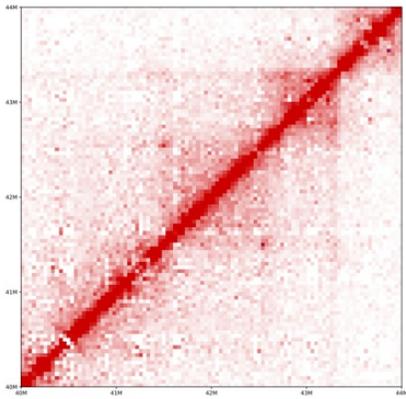
### TADs:



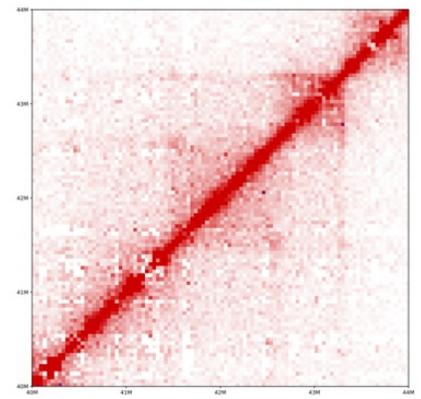
### Loop:



Tradition



Maternal



Paternal

## Allele-Specificity of Chromatin Structure

The calculation method of Allelic Specificity is integrated in the **AllelicSpecificity** module.

### Compartment Allele-Specificity calculation

Using the maternal and paternal PC1 values as input. with the format as:

1) chromosome ID. 2) PC1 values

Each row indicates a chromatin bin and its pc values. Bins are ordered from 5' to 3'.

```
$ less GM12878_Maternal_PC.txt

1 0.007763622511004936
1 0.03661900228230602
1 0.031072969611405883
1 0.03303907288771536
1 0.03185222709883546
...
```

Use the API like:

```
>>> from HiChap.AlelicSpecificity import CompartmentAllelicSpecificity

>>> Allel_PC = CompartmentAllelicSpecificity(Maternal_PC = 'GM12878_Maternal_PC.txt',
                                             Paternal_PC = 'GM12878_Paternal_PC.txt',
                                             Res = 500000)

>>> Allel_PC.Running('Output.txt')
```

The output file is similar to the input file. with the format as:

1. chromosome ID
2. position
3. Maternal PC value
4. Paternal PC value
5. difference
6. p-value
7. q-value

### Boundary Allele-Specificity calculation

Using the Candidate boundaries as input. TXT file contains 3 columns format as

1. chromosome ID.
2. Maternal Boundary

### 3. Paternal Boundary

Each row indicates a pair of candidate boundary to calculate the allelic-specificity. Maternal and Paternal boundary can be different (Same boundary with a little translation.) We suggest the distance of translation should be less than 3 bins.

```
$ less Candidate_Boundary.txt

1      800000  800000
1      1240000 1240000
1      1680000 1680000
1      1840000 1840000
1      2080000 2040000
1      2320000 2320000
...
```

Use the API like:

```
>>> from HiChap.AllelicSpecificity import BoundaryAllelicSpecificity

>>> Allel_Boundary = BoundaryAllelicSpecificity(cooler_fil = 'Merged_Imputed_Haplotype_Multi.cool',
        Boundary_fil = 'Candidate_Boundary.txt',
        Res = 40000, offset = 10)

>>> Allel_Boundary.Running('OutPut.txt')
```

The output file is similar to the input file. with the format as:

1. chromosome ID
2. Maternal boundary position
3. Paternal boundary position
4. Mean value of Maternal boundary
5. Mean value of Paternal boundary
6. stat
7. p-value
8. q-value

### Loop Allele-Specificity calculation

Using the Candidate loops as input. TXT file contains 5 columns format as

1. chromosome ID.
2. Maternal loop loci 1.
3. Maternal loop loci 2.
4. Paternal loop loci 1.
5. Paternal loop loci 2

like:

```
$ less Candidate_Loop.txt

1      2320000      2560000      2320000      2560000
1      13840000     14160000     13840000     14160000
1      35320000     35640000     35320000     35640000
1      47640000     48160000     47640000     48160000
...
```

Use the API like:

```
>>> from HiChap.AllelicSpecificity import LoopAllelicSpecificity

>>> Allel_Loop = LoopAllelicSpecificity(cooler_uri = 'Merged_Imputed_Haplotype_Multi.cool',
        Loop_file = 'Candidate_Loop.txt',
        Res = 40000)
```

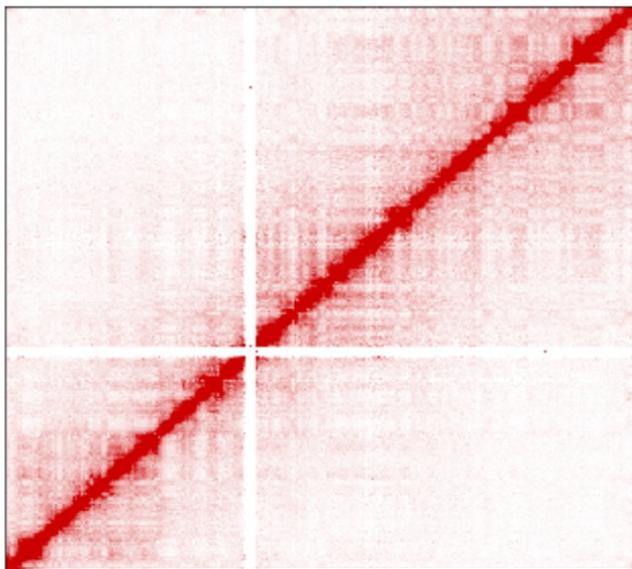
```
>>> Allel_Loop.Running('OutPut.txt')
```

The output file is similar to the input file. with the format as:

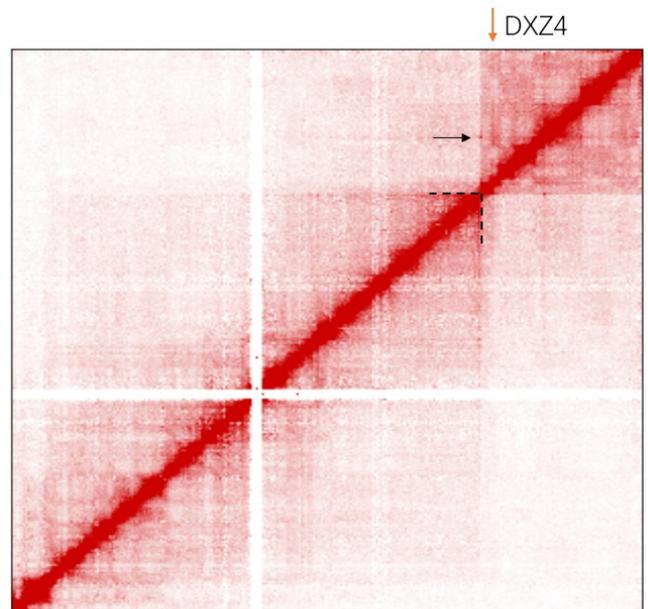
1. chromosome ID
2. Maternal loop loci 1
3. Maternal loop loci 2
4. Paternal loop loci 1
5. Paternal loop loci 2
6. Maternal loop Contact strength
7. Paternal loop Contact strength
8. quantile ratio
9.  $\log_2(\text{fold-change})$
10. stat
11. p-value

## Examples of Allel-Specific Structure

### X-Chromosome-Inactivation

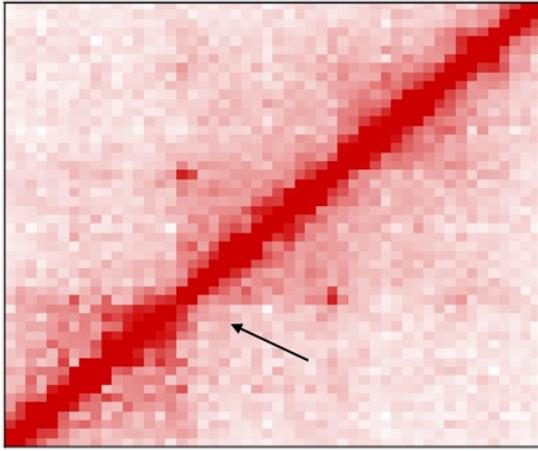


Maternal

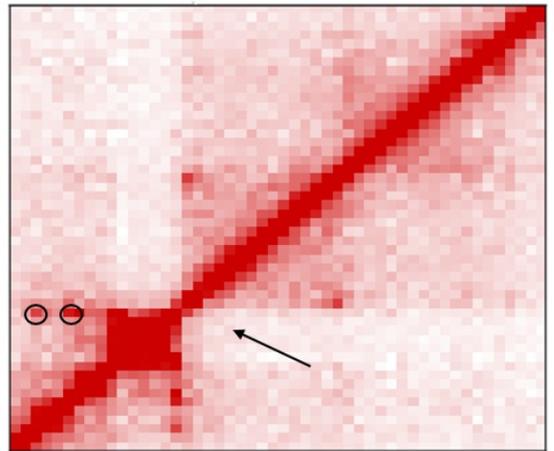


Paternal

### Allel-Specific Boundary and Loop



Maternal



Paternal