

Group 3

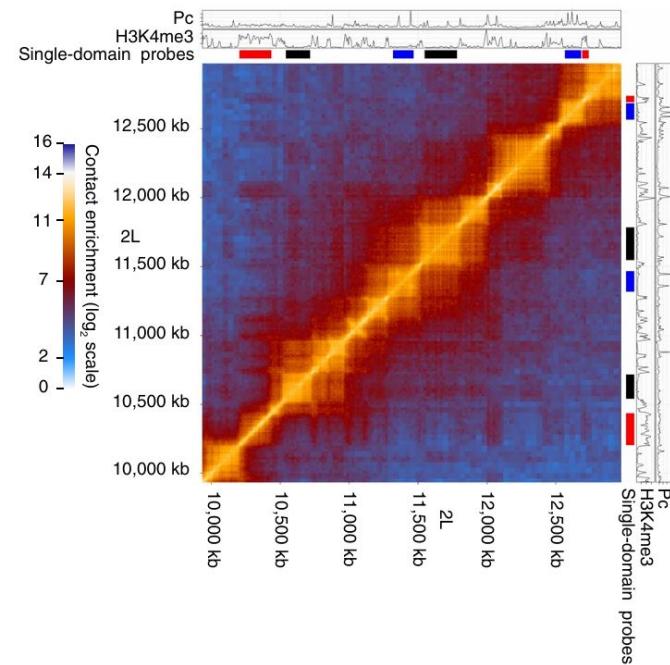
paper 2 presentation

Szabo, Q. *et al.* TADs are 3D structural units of higher-order chromosome organization in Drosophila.
Science Advances 4, eaar8082 (2018).

Motivation: Why Study TADs?

Are TADs Real Physical Units?

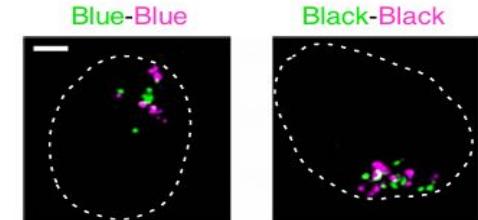
- Hi-C: Captures contact frequencies between DNA regions in 3D space
- TAD (Topologically Associating Domain):
 - Visible as small triangular domains in Hi-C maps
 - DNA within a TAD interacts frequently
 - Different TADs interact less frequently
 - *Serves as a basic 3D structural unit in this study*



TADs are real 3D structures, not Hi-C artifacts

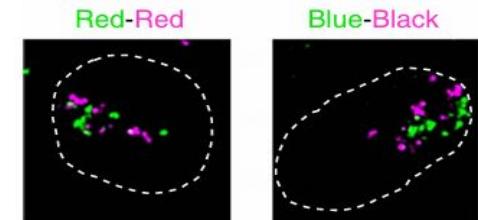
Polycomb-repressed TADs (Blue)

- Very strong Hi-C signal (high contact frequency)
- Highly compact in 3D → forms *nanocompartment* spheres



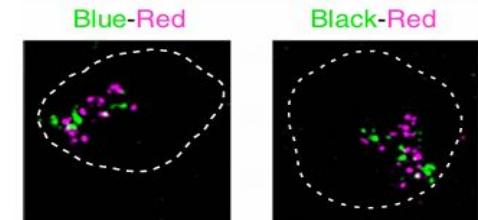
Inactive TADs (Black)

- Intermediate Hi-C intensity
- Moderately compact



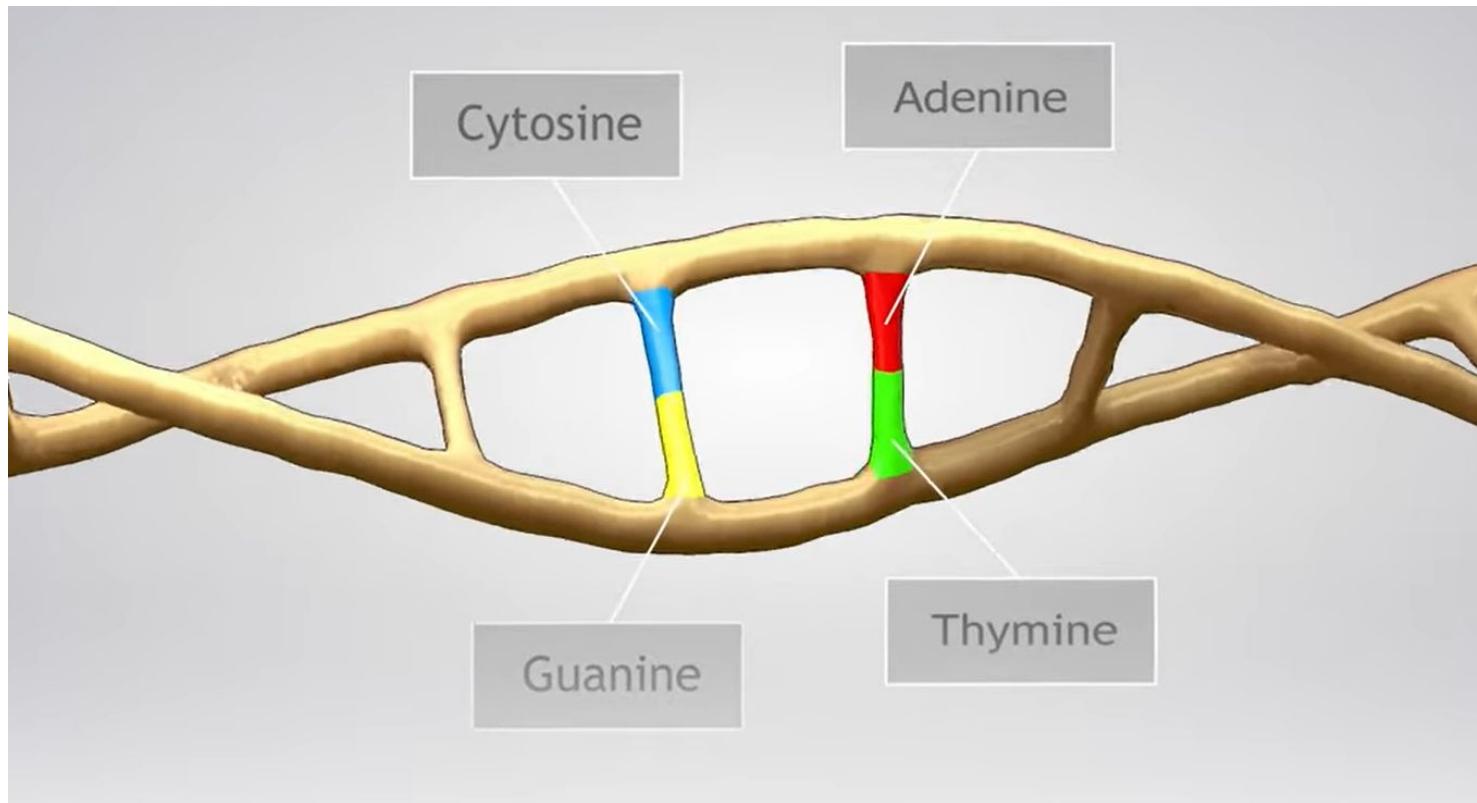
Active TADs (Red)

- Weak Hi-C signal → low density
- In 3D: remain loose and dispersed, never forming spheres

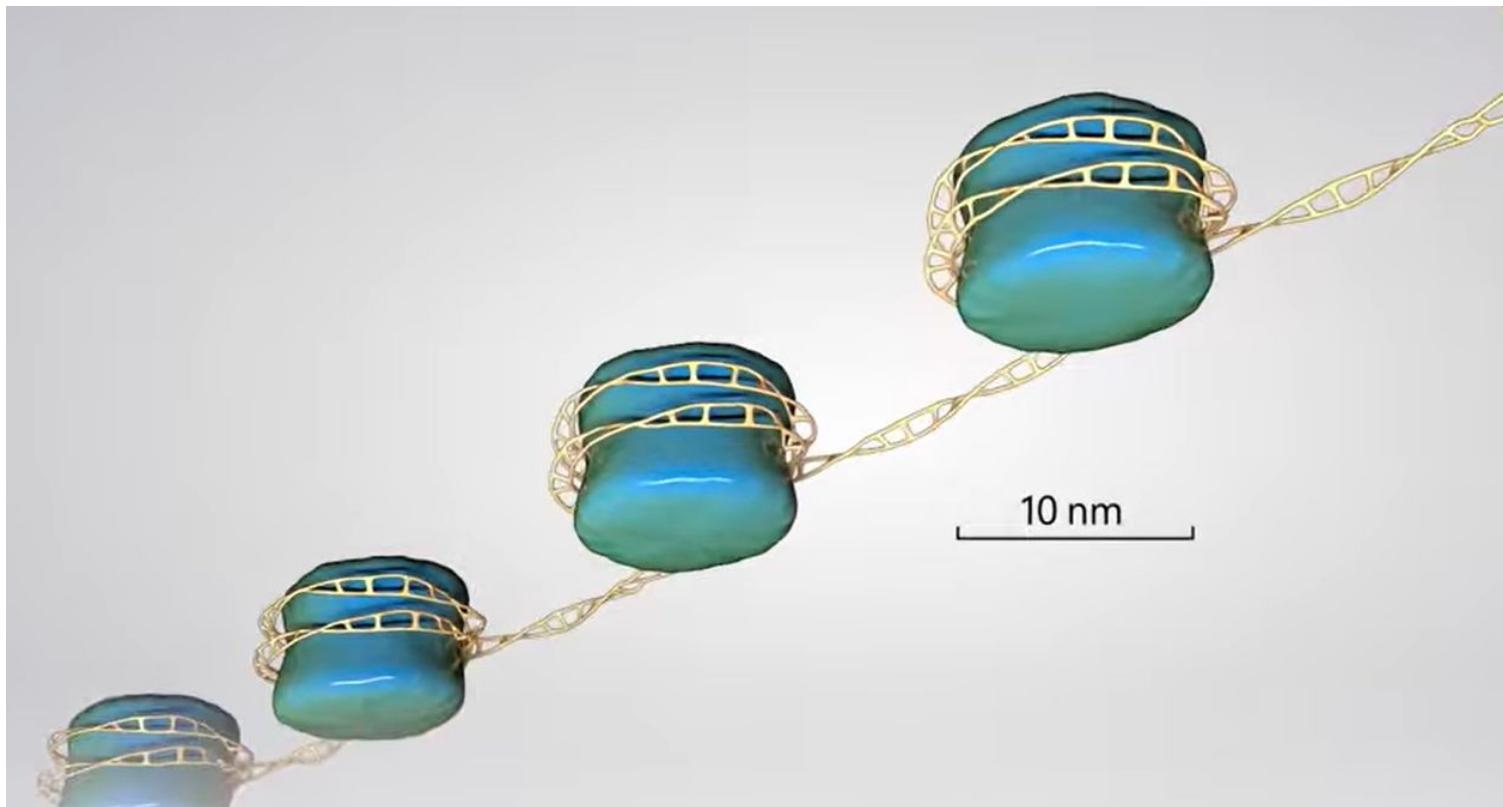


Biological Definition of TADs

DNA Strand



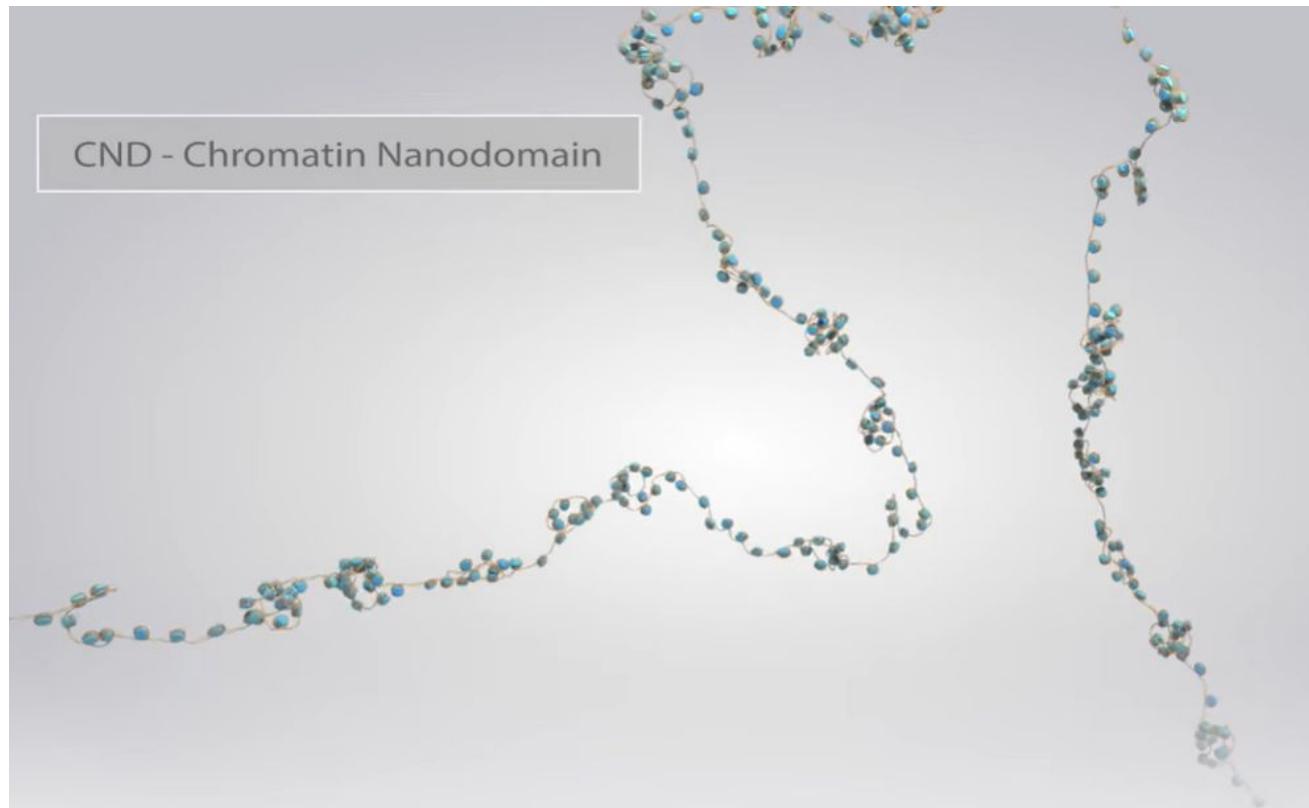
Histone Proteins & Nucleosomes



Clutches



CND



Cohesin



Loop Extrusion



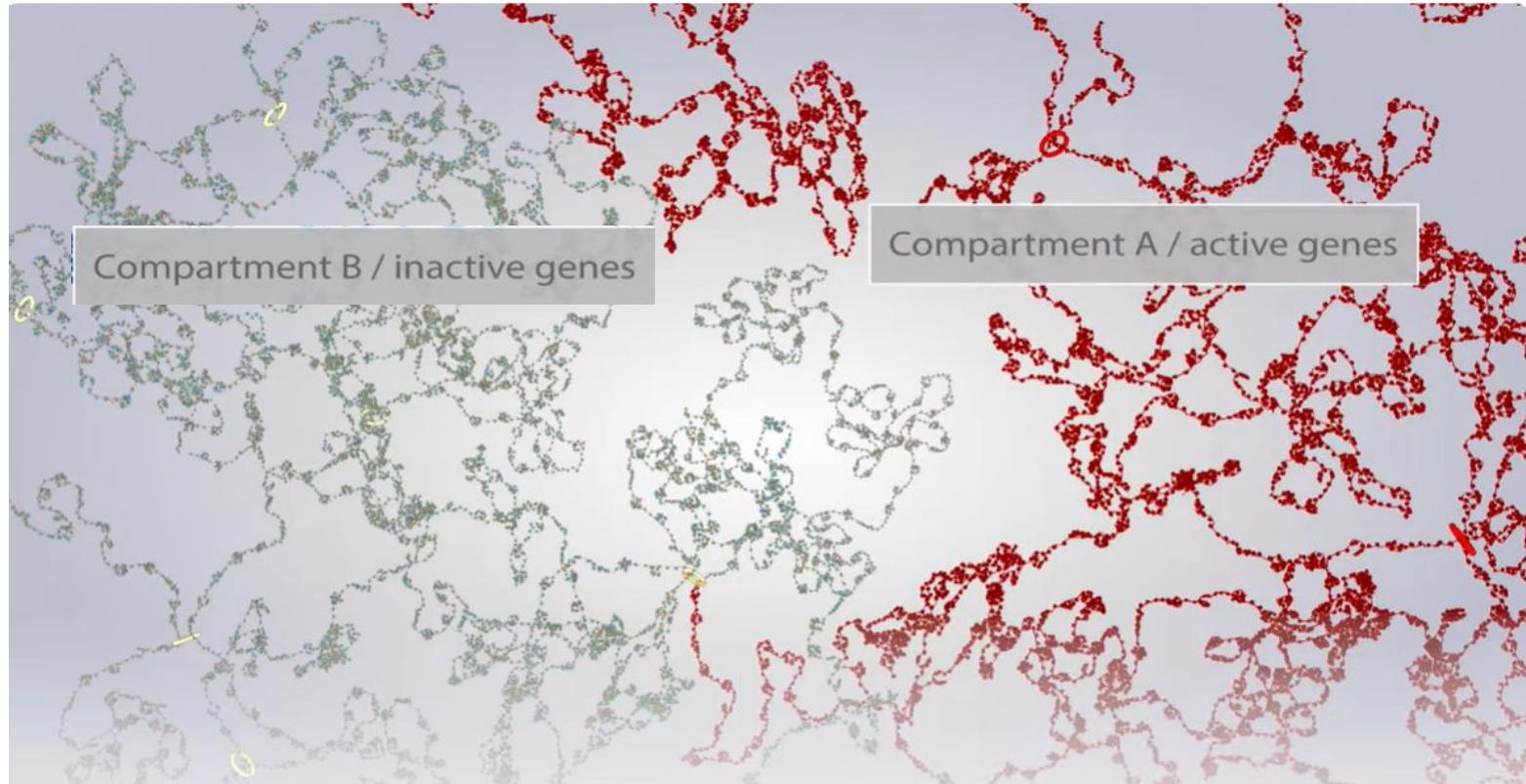
CTCF



TAD



Different Compartment



Reconstructing Hi-C Maps from Contact Matrix

Goal: Reconstruct Figures 1A, 5A, and 5G

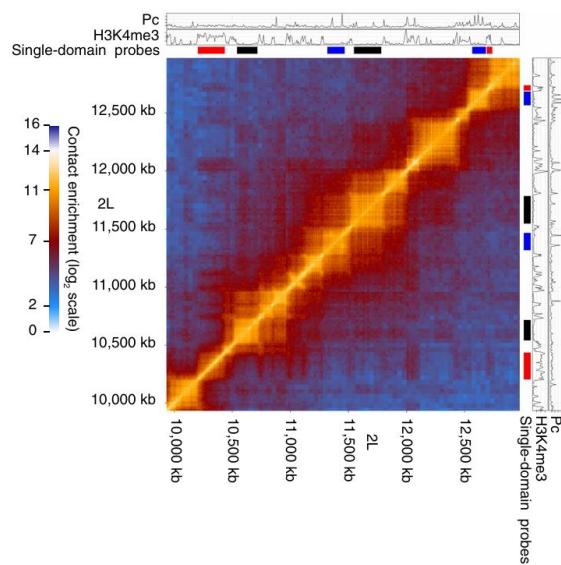


figure 1A

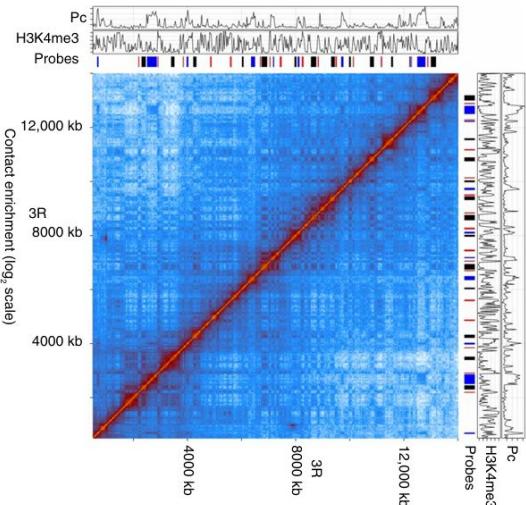


figure 5A

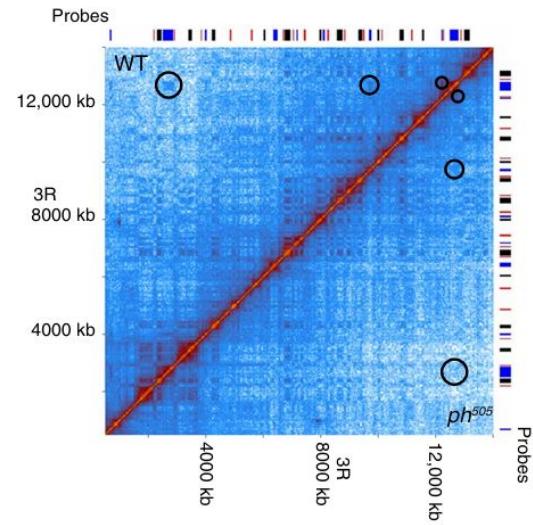


figure 5G

Contact Matrix Dataset Source

The screenshot shows the NCBI Gene Expression Omnibus (GEO) Accession Display page for dataset GSE99107. The top navigation bar includes links for HOME, SEARCH, SITE MAP, GEO Publications, FAQ, MIAME, Email GEO, and a login link. The main content area displays the dataset details for GSE99107, including its status as public, title, organism, experiment type, summary, overall design, and citation information.

Scope: Self **Format:** HTML **Amount:** Quick **GEO accession:** GSE99107 **GO**

Series GSE99107 **Query DataSets for GSE99107**

Status	Public on Jan 03, 2018
Title	TADs are 3D structural units of higher-order chromosome organization in Drosophila
Organism	Drosophila melanogaster
Experiment type	Other
Summary	This SuperSeries is composed of the SubSeries listed below.
Overall design	Refer to individual Series
Citation(s)	Szabo Q, Jost D, Chang JM, Cattoni DI et al. TADs are 3D structural units of higher-order chromosome organization in Drosophila. <i>Sci Adv</i> 2018 Feb;4(2):eaar8082. PMID: 29503869

Contact Matrix Dataset Source

Samples (6)	GSM2633507 S2R+ rep 1
≡ Less...	
	GSM2633508 S2R+ rep 2
	GSM2633509 male rep 1
	GSM2633510 male rep 2
	GSM2633511 ph rep 1
	GSM2633512 ph rep 2

This SuperSeries is composed of the following SubSeries:

- [GSE99104](#) TADs are 3D structural units of higher-order chromosome organization in Drosophila [S2R+]
- [GSE99105](#) TADs are 3D structural units of higher-order chromosome organization in Drosophila [male]
- [GSE99106](#) TADs are 3D structural units of higher-order chromosome organization in Drosophila [ph]

Contact Matrix Dataset Source

Supplementary file	Size	Download	File type/resource
GSE99104_nm_none_10000.bins.txt.gz	92.7 Kb	(ftp)(http)	TXT
GSE99104_nm_none_10000.n_contact.txt.gz	114.7 Mb	(ftp)(http)	TXT
GSE99104_nm_none_160000.bins.txt.gz	6.0 Kb	(ftp)(http)	TXT
GSE99104_nm_none_160000.n_contact.txt.gz	4.7 Mb	(ftp)(http)	TXT
GSE99104_nm_none_20000.bins.txt.gz	46.6 Kb	(ftp)(http)	TXT
GSE99104_nm_none_20000.n_contact.txt.gz	45.8 Mb	(ftp)(http)	TXT
GSE99104_nm_none_40000.bins.txt.gz	23.3 Kb	(ftp)(http)	TXT
GSE99104_nm_none_40000.n_contact.txt.gz	62.6 Mb	(ftp)(http)	TXT
GSE99104_nm_none_5000.bins.txt.gz	174.7 Kb	(ftp)(http)	TXT
GSE99104_nm_none_5000.n_contact.txt.gz	232.9 Mb	(ftp)(http)	TXT
GSE99104_nm_none_80000.bins.txt.gz	12.0 Kb	(ftp)(http)	TXT
GSE99104_nm_none_80000.n_contact.txt.gz	17.1 Mb	(ftp)(http)	TXT

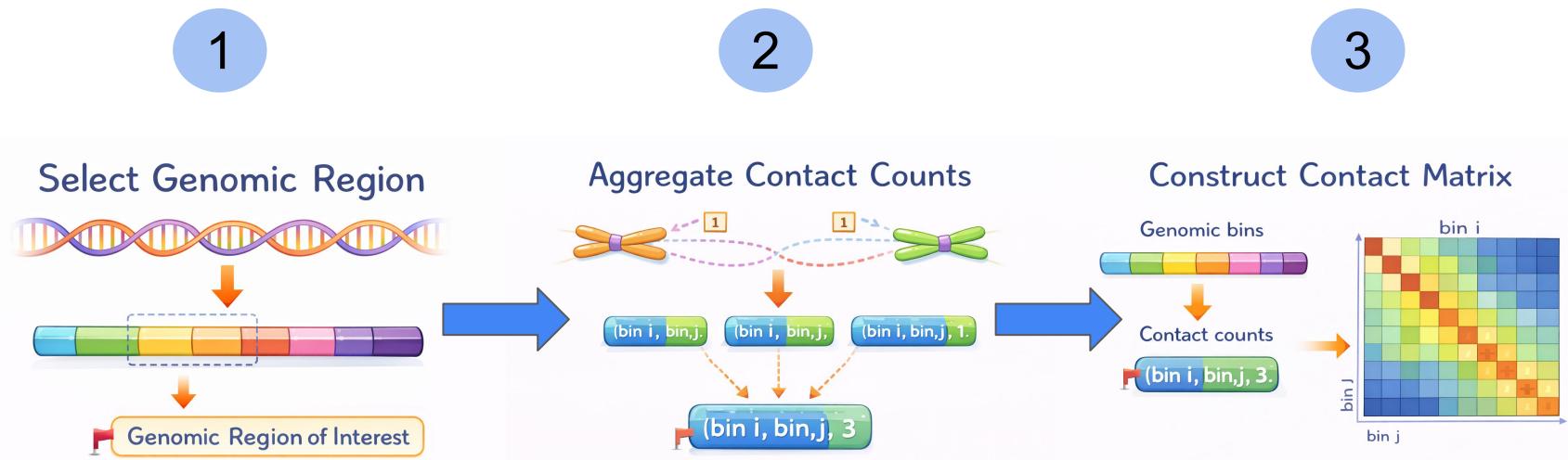
Dataset 1: Genomic Bins for Hi-C Analysis

cbin	chr	from	to
1	2L	0	40,000
2	2L	40,000	80,000
3	2L	80,000	120,000
4	2L	120,000	160,000
5	2L	160,000	200,000

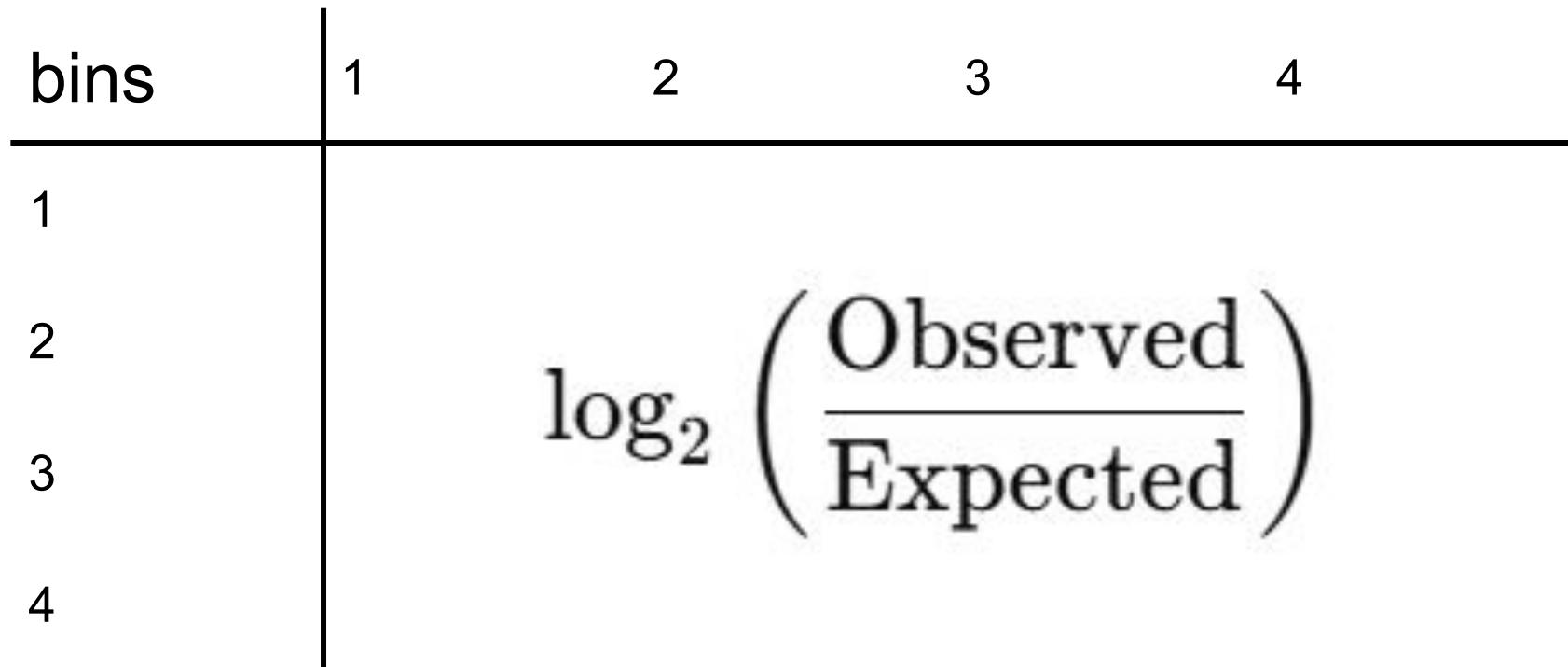
Dataset 2: Hi-C Contact Counts

cbin1	cbin2	expected count	observed count
1	1	1.731	1,803
1	2	3.831	1,698
1	3	5.677	457
1	4	5.445	183
1	5	4.283	88

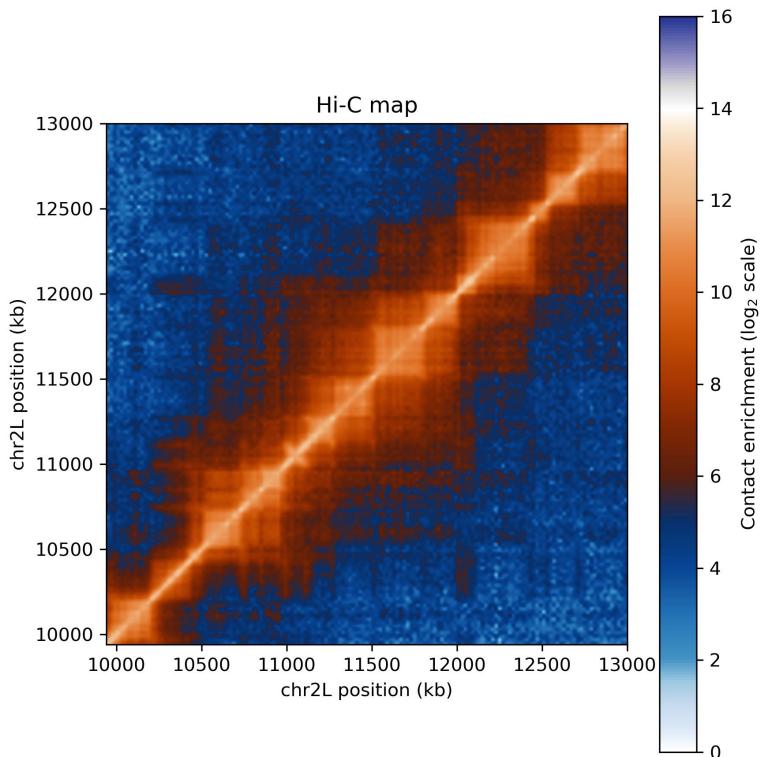
Hi-C Contact Matrix Construction Pipeline



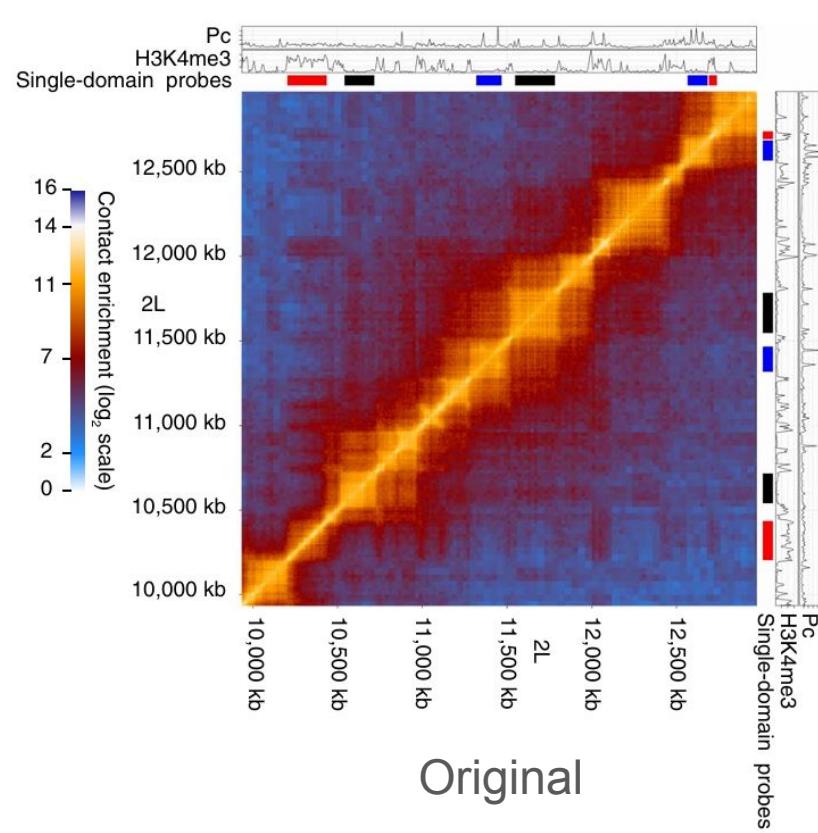
Hi-C Contact Matrix Construction Pipeline



Reconstruction of Figure 1A from S2R+ Hi-C Map

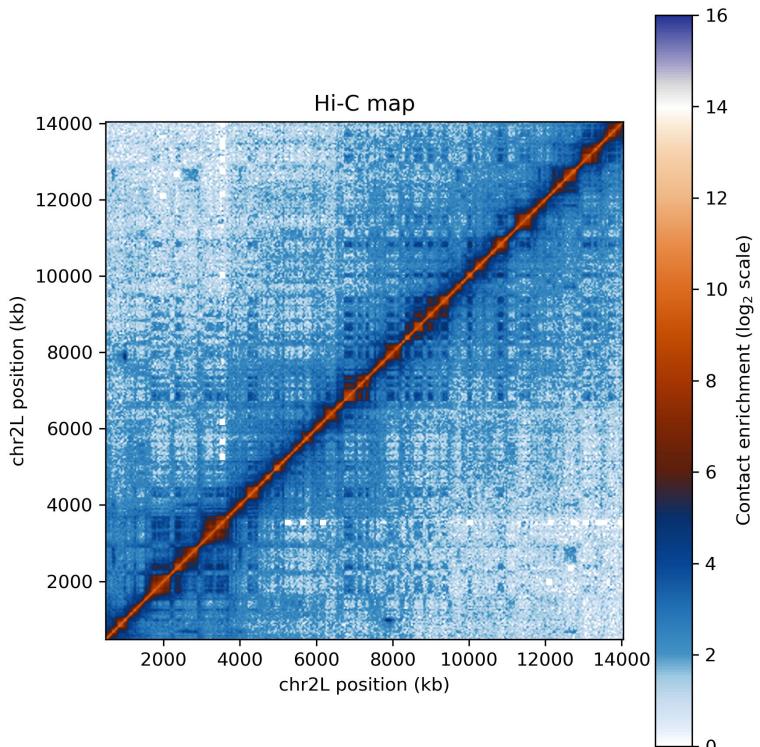


Reconstructed

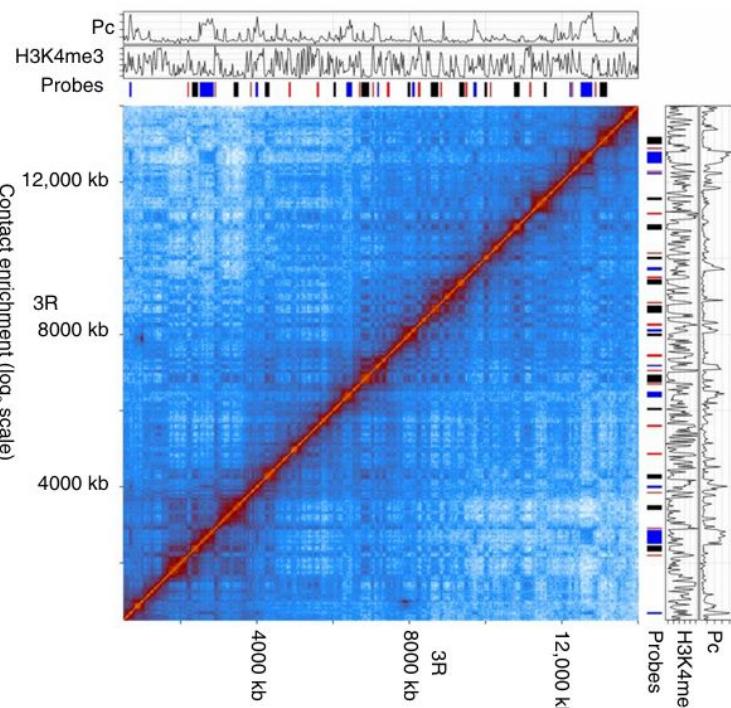


Original

Reconstruction of Figure 5A from Embryonic Hi-C Map



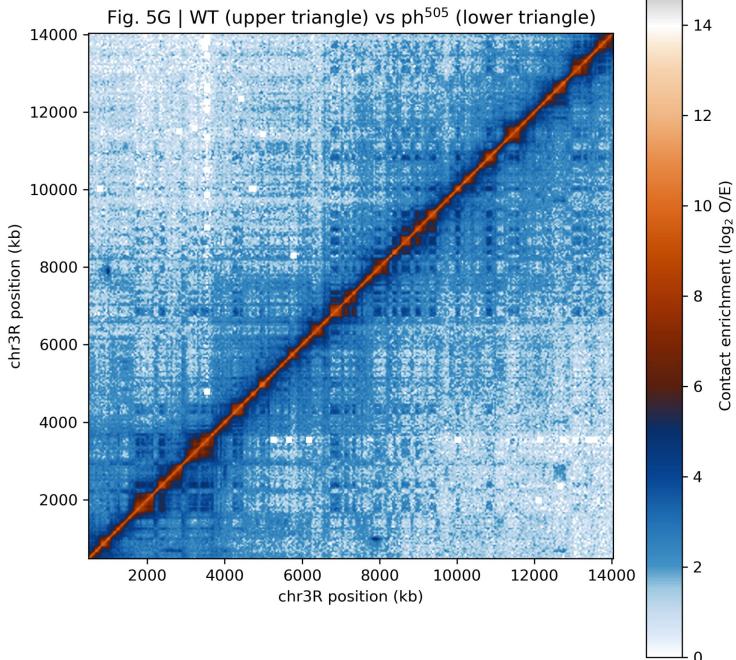
Reconstructed



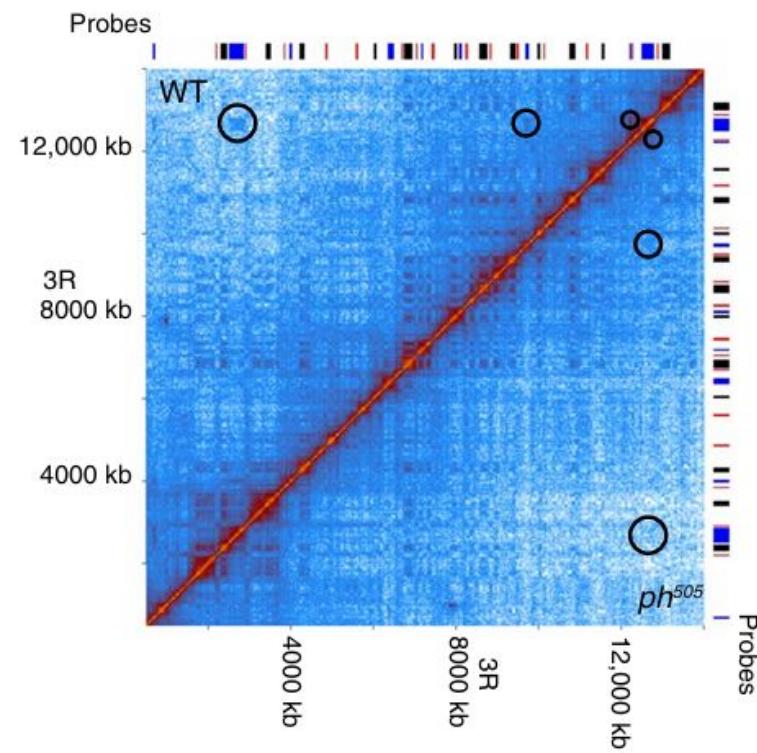
Original

Reconstruction of Figure 5G: Loss of PcG-Mediated

(



Reconstructed



Original

Step-by-step construction of the HI-C diagram

Contact Matrix Dataset Source

Found 19 Items

<input checked="" type="checkbox"/>	<input type="checkbox"/>	Run	1	BioProject	2	BioSample	3	AvgSpotLen	4	Bases	5	Bytes	6	Developmental_Stage	7	Experiment	8	genotype	9	GEO_Accession	10	Ins
<input type="checkbox"/>	1	SRR5579160	PRJNA387323	SAMN07146998				65	9.05 G	7.81 Gb	Embryo			SRX2837376		ph[505]/KrGFP-FM7c			GSM2633511	Illumir		
<input type="checkbox"/>	2	SRR5579161	PRJNA387323	SAMN07146998				65	8.76 G	7.57 Gb	Embryo			SRX2837376		ph[505]/KrGFP-FM7c			GSM2633511	Illumir		
<input type="checkbox"/>	3	SRR5579162	PRJNA387323	SAMN07146998				65	7.94 G	6.85 Gb	Embryo			SRX2837376		ph[505]/KrGFP-FM7c			GSM2633511	Illumir		
<input type="checkbox"/>	4	SRR5579163	PRJNA387323	SAMN07146998				65	7.83 G	6.79 Gb	Embryo			SRX2837376		ph[505]/KrGFP-FM7c			GSM2633511	Illumir		
<input type="checkbox"/>	5	SRR5579164	PRJNA387323	SAMN07146998				65	9.11 G	7.83 Gb	Embryo			SRX2837376		ph[505]/KrGFP-FM7c			GSM2633511	Illumir		
<input type="checkbox"/>	6	SRR5579165	PRJNA387323	SAMN07146998				65	8.79 G	7.61 Gb	Embryo			SRX2837376		ph[505]/KrGFP-FM7c			GSM2633511	Illumir		
<input type="checkbox"/>	7	SRR5579166	PRJNA387323	SAMN07146998				65	8.43 G	7.29 Gb	Embryo			SRX2837376		ph[505]/KrGFP-FM7c			GSM2633511	Illumir		
<input type="checkbox"/>	8	SRR5579167	PRJNA387323	SAMN07146997				98	19.36 G	11.73 Gb	Embryo			SRX2837377		ph[505]/KrGFP-FM7c			GSM2633512	Illumir		
<input type="checkbox"/>	9	SRR5579168	PRJNA387323	SAMN07146997				98	17.35 G	10.54 Gb	Embryo			SRX2837377		ph[505]/KrGFP-FM7c			GSM2633512	Illumir		
<input type="checkbox"/>	10	SRR5579169	PRJNA387323	SAMN07146997				98	17.63 G	10.84 Gb	Embryo			SRX2837377		ph[505]/KrGFP-FM7c			GSM2633512	Illumir		
<input type="checkbox"/>	11	SRR5579170	PRJNA387324	SAMN07147000				98	15.74 G	9.56 Gb	Embryo			SRX2837378	y[1],w[67c23];Dp(1;Y),y[+]P{ry+11}P{w[+mC]=ActGFP}JMR1			GSM2633509	Illumir			
<input type="checkbox"/>	12	SRR5579171	PRJNA387324	SAMN07147000				98	15.50 G	9.40 Gb	Embryo			SRX2837378	y[1],w[67c23];Dp(1;Y),y[+]P{ry+11}P{w[+mC]=ActGFP}JMR1			GSM2633509	Illumir			
<input type="checkbox"/>	13	SRR5579172	PRJNA387324	SAMN07147000				98	15.73 G	9.57 Gb	Embryo			SRX2837378	y[1],w[67c23];Dp(1;Y),y[+]P{ry+11}P{w[+mC]=ActGFP}JMR1			GSM2633509	Illumir			
<input type="checkbox"/>	14	SRR5579173	PRJNA387324	SAMN07147000				98	15.70 G	9.53 Gb	Embryo			SRX2837378	y[1],w[67c23];Dp(1;Y),y[+]P{ry+11}P{w[+mC]=ActGFP}JMR1			GSM2633509	Illumir			
<input type="checkbox"/>	15	SRR5579174	PRJNA387324	SAMN07146999				98	17.43 G	10.51 Gb	Embryo			SRX2837379	y[1],w[67c23];Dp(1;Y),y[+]P{ry+11}P{w[+mC]=ActGFP}JMR1			GSM2633510	Illumir			
<input type="checkbox"/>	16	SRR5579175	PRJNA387324	SAMN07146999				98	17.34 G	10.43 Gb	Embryo			SRX2837379	y[1],w[67c23];Dp(1;Y),y[+]P{ry+11}P{w[+mC]=ActGFP}JMR1			GSM2633510	Illumir			
<input type="checkbox"/>	17	SRR5579176	PRJNA387324	SAMN07146999				98	17.70 G	10.83 Gb	Embryo			SRX2837379	y[1],w[67c23];Dp(1;Y),y[+]P{ry+11}P{w[+mC]=ActGFP}JMR1			GSM2633510	Illumir			
<input type="checkbox"/>	18	SRR5579177	PRJNA387300	SAMN07147001				100	30.13 G	15.40 Gb	Late embryonic stage			SRX2837380	wild type			GSM2633507	Illumir			
<input type="checkbox"/>	19	SRR5579178	PRJNA387300	SAMN07147002				100	31.13 G	16.21 Gb	Late embryonic stage			SRX2837381	wild type			GSM2633508	Illumir			

SRA Toolkit

1. 下載壓縮檔(prefetch)

2. 解封包(fastQdump)=>forward reverse

.sra → .fastq

01. Downloading SRA Toolkit

Andrew Klymenko edited this page 2 weeks ago · 41 revisions

NCBI SRA Toolkit

Below are the latest releases of various tools and release checksum file.

SRA Toolkit

Compiled binaries/install scripts of December 2, 2025, version 3.3.0:

- [AlmaLinux 64 bit architecture](#) - non-sudo tar archive
- [Ubuntu Linux 64 bit architecture](#) - non-sudo tar archive
- [Cloud - apt-get install script](#) - for Debian and Ubuntu - requires sudo permissions
- [Cloud - yum install script](#) - for AlmaLinux - requires sudo permissions
- [MacOS x86 64 bit architecture](#)
- [MacOS Arm64 bit architecture](#)
- [MS Windows 64 bit architecture](#)
- [Docker image repository](#)
- [md5 checksums](#)

Concatenate the data

一共4個file = { SRR5579177_1.fastq (front), SRR5579177_2.fastq(reverse)

,SRR5579178_1.fastq (front) , SRR5579178_2.fastq(reverse) }

concatenate 成一條 => { front front reverse reverse }

之後壓縮檔案 (gzip + pigz(加速)) 後得到 .gz 檔

生成Hi-C: jucier(pipeline)

pipeline有什麼？

pipeline :

Split : 將巨大的 FASTQ 切成小塊 (batch) (~30mins)。

Align : 將 reads 比對到 dm3 基因組(~12hrs)。(得到每一條 read 在基因組上的位置)

Merge & Sort: 合併比對結果並排序。

Chimeric Handling: 處理 Hi-C 特有的嵌合 reads (找Ligation junctions)。

Deduplicate: 移除 PCR 重複 (Duplicates)。

Final: 生成 .hic 檔案 (用於 Juicebox) 和 .hic 統計數據。

Practice

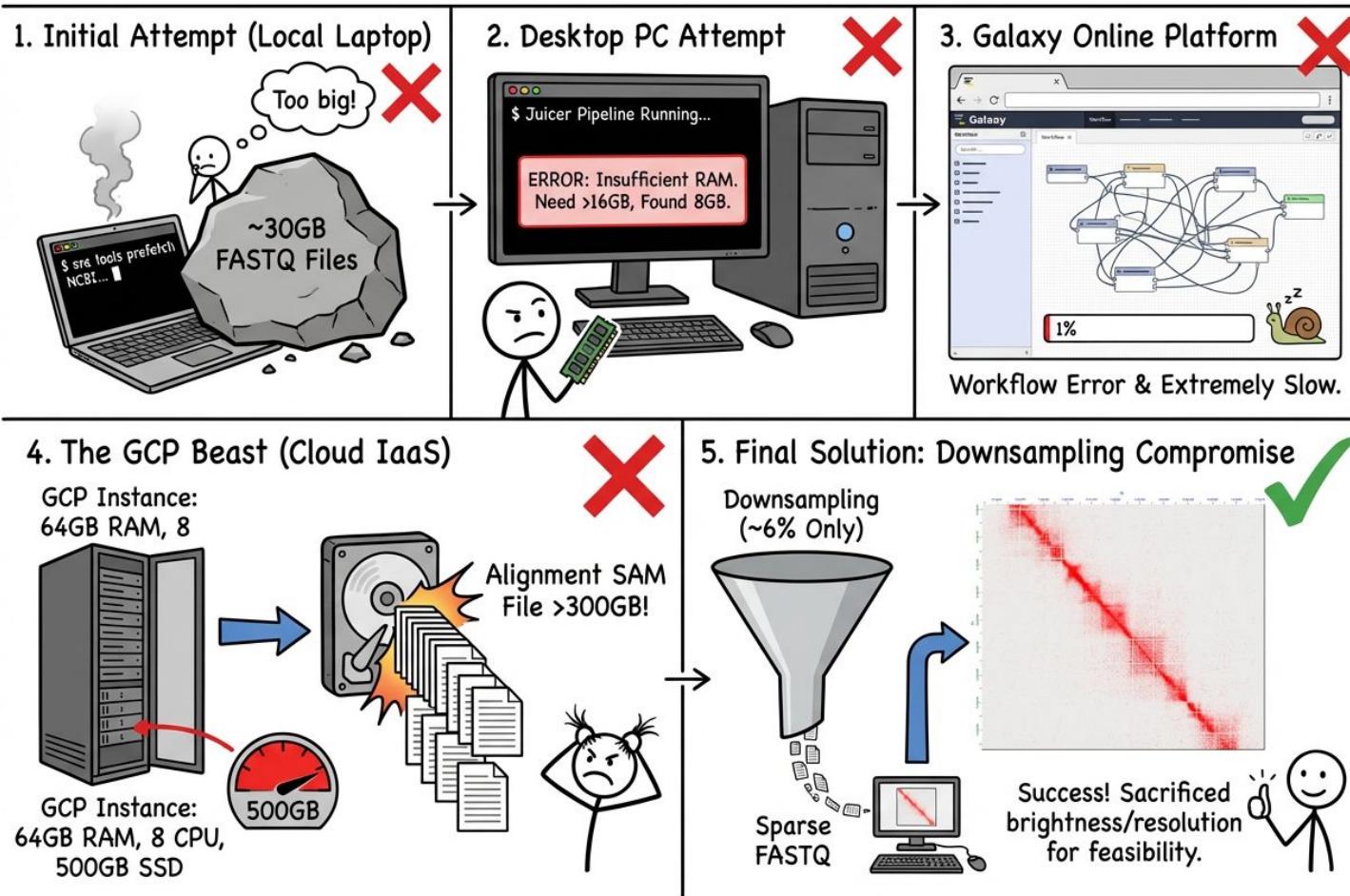
裡用jucier套件 要做的就是引入pipeline所需的額外套件

Align (BWA) => BW transform => .sam => 2L 2R

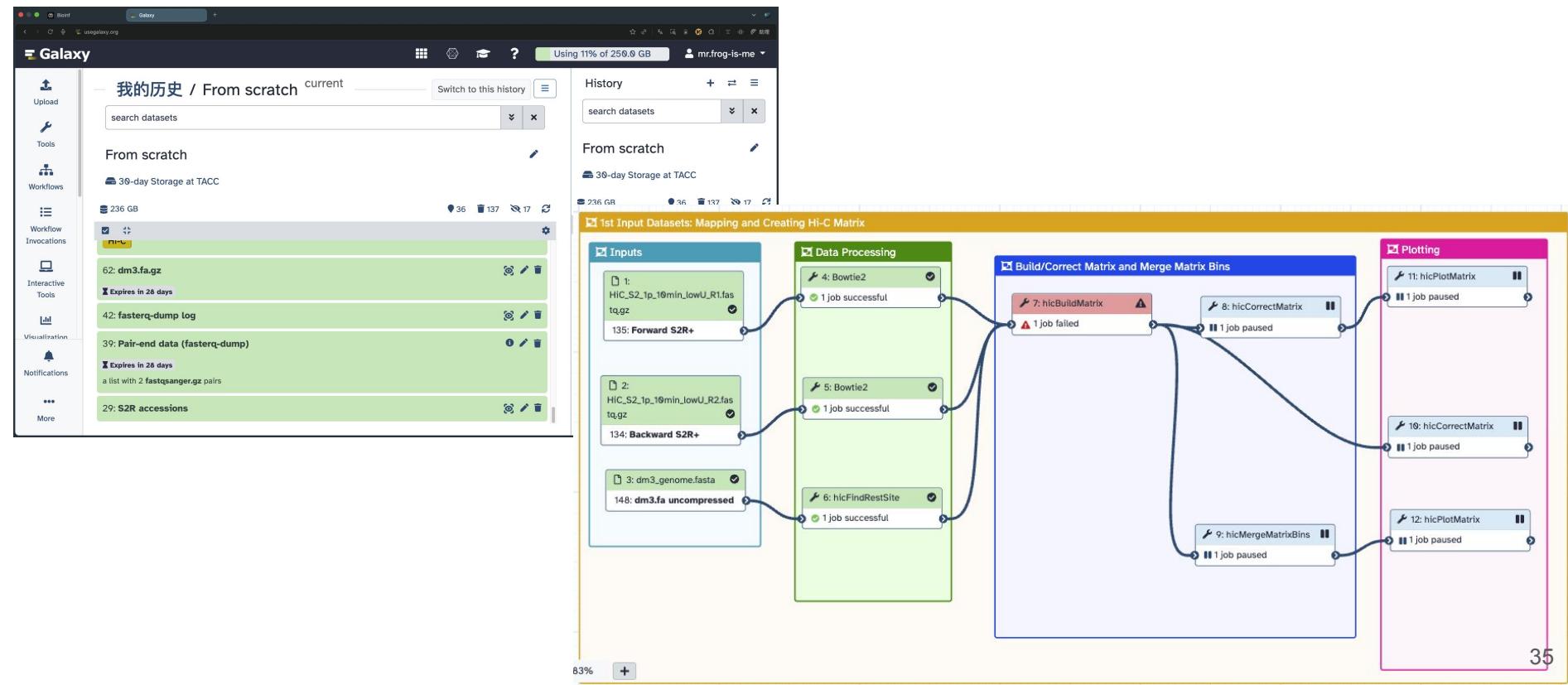
Chimeric Handling : samtools

Reconstruction Roadmap

My Bioinformatics Final Project Journey: Reproducing Hi-C Contacts from Raw FASTQ.



Galaxy (<https://usegalaxy.org/>)



GCP 雲端伺服器

機器設定

機型

必須先停止 VM 執行個體才能編輯機型

e2-highmem-8



vCPU
8

Memory
64 GB

CPU 平台

AMD Rome

名稱	↑	類型	大小
bioinf-hic		已平衡的永久磁碟	500 GB

總費用 (2025/12/1至2025/12/18) ②

費用

\$613

省下的費用

\$613

總費用

\$0.00

[查看詳細資料](#)

Getting Data From NCBI (RSA tools)

Prefetch (2~3hr) & Dump (~3hr)

```
#!/bin/bash
prefetch --option-file srr/SRR_Acc_List.txt -O srr/
mkdir -p fastq
cat srr/SRR_Acc_List.txt | while read SRR; do
    echo "Processing $SRR"
    fasterq-dump "srr/$SRR" \
        --outdir fastq \
        --split-files \
        --threads 8
done
```

~2.4B Lines, ~0.6B Reads

```
~/bioinf/old_files
> zcat S2R_plus_R1.fastq.gz | wc -l
2450250500
```

Original Fastq size (after concatenate)

```
~/bioinf/old_files
> du -sch $(ls -A .) | sort -h
140G    S2R_plus_R1.fastq
140G    S2R_plus_R2.fastq
279G    total
> du -sch $(ls -A .) | sort -h
28G      S2R_plus_R1.fastq.gz
29G      S2R_plus_R2.fastq.gz
57G      total
```

Pipeline - Juicer

Run juicer.sh

```
#!/bin/bash

rm -rf aligned/
# rm -rf splits/

./scripts/juicer.sh \
-D "$(pwd)" \
-z "$(pwd)/references/dm3.fa" \
-p "$(pwd)/references/dm3.chrom.sizes" \
-y "$(pwd)/restriction_sites/dm3_DpnII.txt" \
-s DpnII \
-g dm3 \
-t 12
# -S chimeric
```

JucierFile Structure

```
~/bioinf/juicer
> tree
.
├── aligned
│   ├── header
│   ├── inter.hic
│   ├── inter.txt
│   ├── inter_30.hic
│   ├── inter_30.txt
│   └── inter_30_contact_domains
│       └── 10000_blocks.bedpe
├── merged1.txt
├── merged30.txt
└── merged_dedup.bam

fastq
├── S2R_subset_R1.fastq.gz
├── S2R_subset_R2.fastq.gz
└── downsample.sh

references
├── dm3.chrom.sizes
├── dm3.fa
├── dm3.fa.amb
├── dm3.fa.ann
├── dm3.fa.bwt
├── dm3.fa.pac
└── dm3.fa.sa

restriction_sites
└── dm3_DpnII.txt

run.sh

scripts -> /home/g112703043/juicer/CPU
splits
├── S2R_subset.fastq.gz.bam
├── S2R_subset.fastq.gz_linecount.txt
├── S2R_subset.fastq.gz_norm.txt.res.txt
└── S2R_subset_R1.fastq.gz -> /home/g112703043/bioinf/juicer/fastq/S2R_subset_R1.fastq.gz
    S2R_subset_R2.fastq.gz -> /home/g112703043/bioinf/juicer/fastq/S2R_subset_R2.fastq.gz

8 directories, 26 files
```

Bottleneck

1. 做 Align (bwa mem) 非常吃記憶體 (~24GB), 也很花時間 (~12hr)
 - a. 前前後後嘗試了 7~8 次
 - b. 花了半週的時間等待輸出
2. 做 Align 輸出的 sam 檔案超！極！大！ (~300GB)
 - a. 內容包括：染色體、位置、CIGAR、插入片段長度、mapping quality、比對分數等。
 - b. 把硬碟塞爆，使得後面的 Pipeline 失敗，又要重新來過
 - c. 可惜沒有截到圖 QQ



~2.4B Lines, ~0.6B Reads

```
~/bioinf/old_files
> zcat S2R_plus_R1.fastq.gz | wc -l
2450250500
```

Size before downsampling

```
> du -sch $(ls -A .) | sort -h
28G    S2R_plus_R1.fastq.gz
29G    S2R_plus_R2.fastq.gz
57G    total
```

Size after downsampling

→	1.8G	S2R_subset_R1.fastq.gz
	1.9G	S2R_subset_R2.fastq.gz
	3.7G	total

Downsampling ~6.5% (40M Reads)

```
zcat S2R_plus_R1.fastq.gz | head -n 1600000000 | pigz -p 12 > S2R_subset_R1.fastq.gz
zcat S2R_plus_R2.fastq.gz | head -n 1600000000 | pigz -p 12 > S2R_subset_R2.fastq.gz
```



Fig 1A. Result (HiC Explorer)

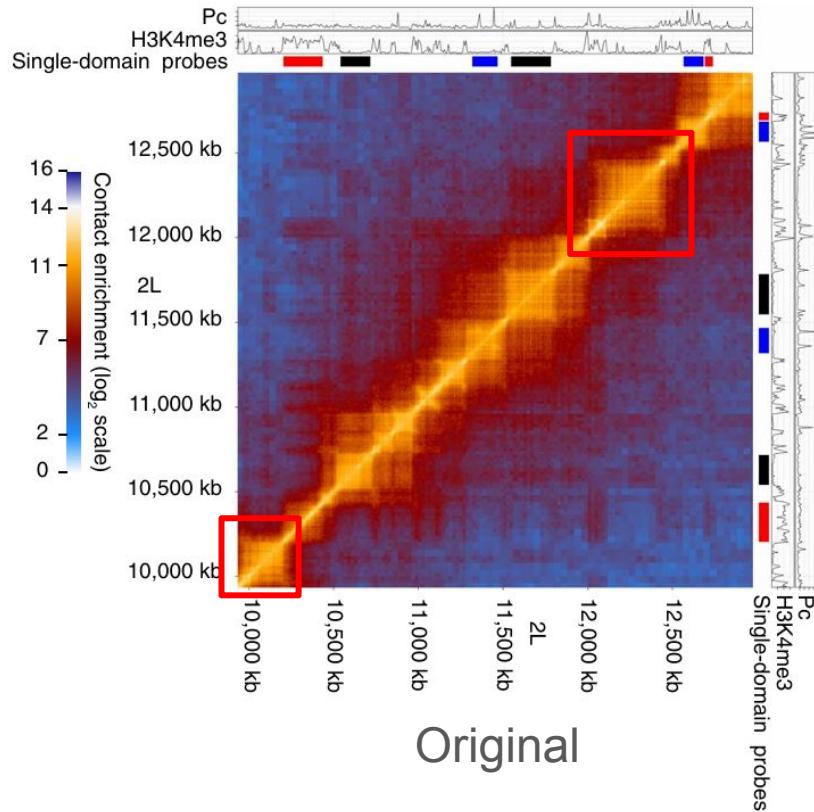
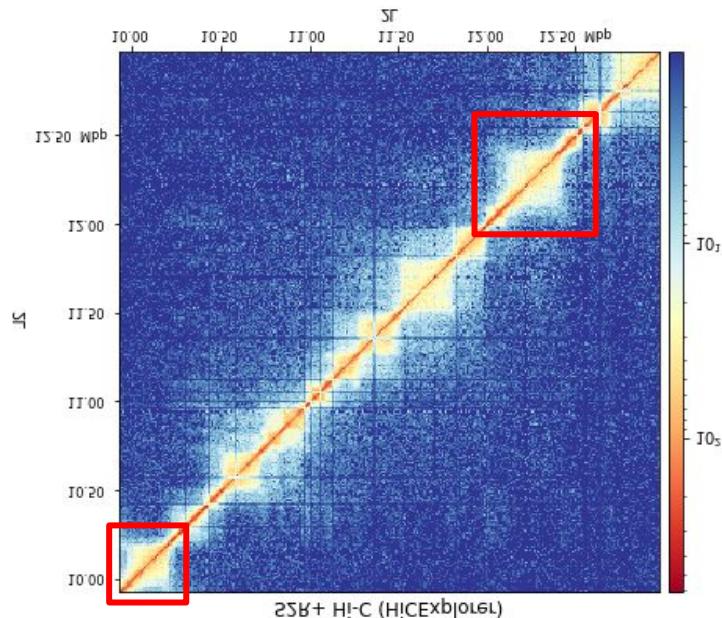
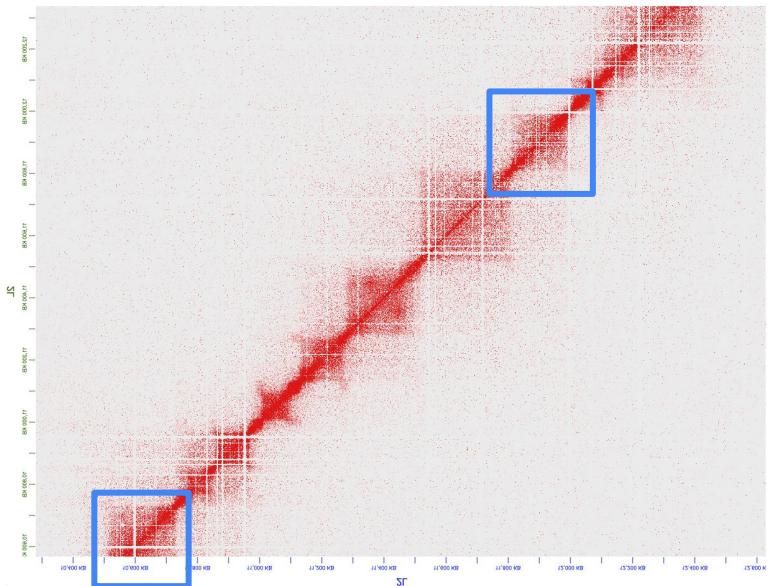
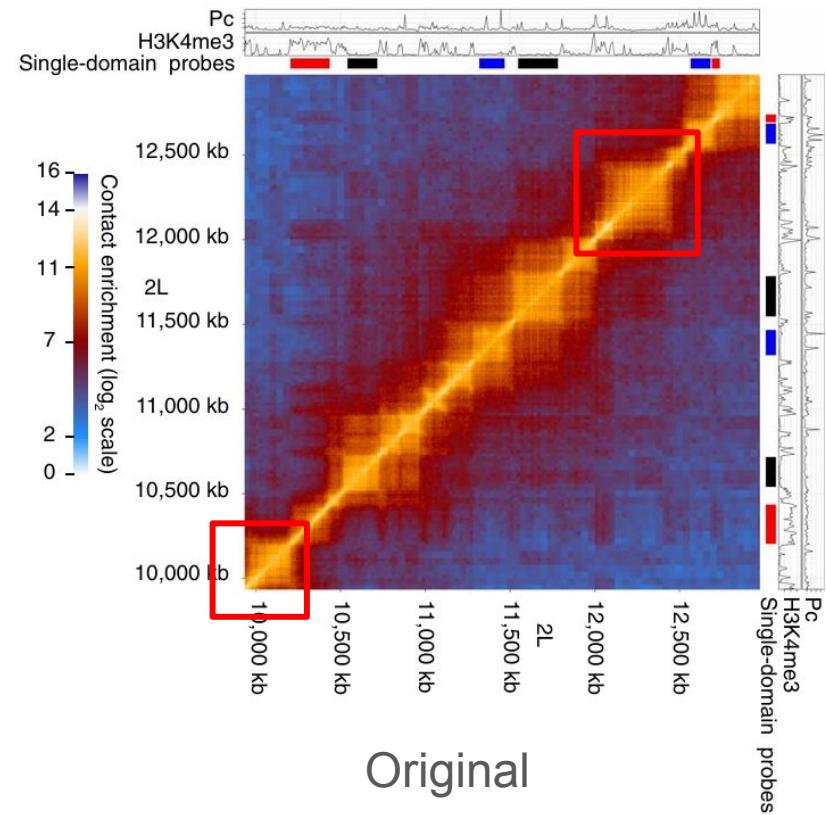


Fig 1A. Result (JuiceBox GUI)



Reconstructed
(JuiceBox)



Original

Future Work

1. 如果有足夠的運算資源、硬碟空間和時間，會想要用完整的數據重跑Fig 1A.
2. 花太多時間在Fig 1A上，沒有處理其他圖的序列
 - a. Fig 3A: Male Embryos
 - b. Fig 5A: WT & Mutant
3. 網站上抓得到 H3K4me3、Pc等等histone mark的資料，會希望也做出論文中圖表旁邊的附加資訊。
 - a. <https://flybase.org/reports/FBIC0002296>