期末專題報告

De novo assembly of the Aedes aegypti genome using Hi-C yields chromosome-length scaffolds

《基於Hi-C輔助從頭組裝-埃及斑蚊基因體組裝生成染色體尺度之長片段》

/ Paper Reproduction

/ 論 文 覆 現 實 驗

NCCUCS 楊明翰 108753203
NCCUCS 曾偉綱 108753122
NCCUCS 王神鐸 107753048

■ https://github.com/1081-Bioinformatics/finalproject-3ddna_108

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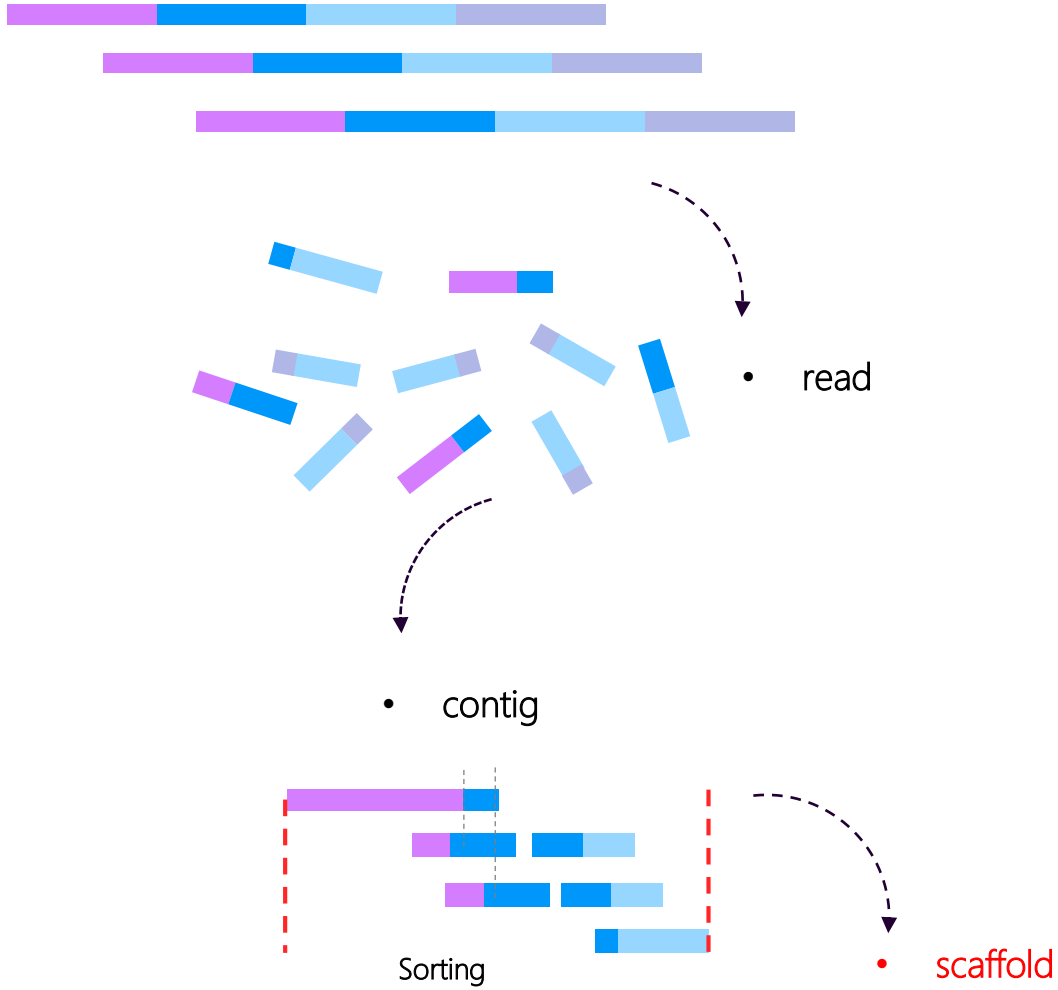
Part 1 / Introduction

基本概念介紹

NGS 次世代定序

把原本的DNA打成小段的碎片之後利用重複的頭尾關係，從較短的片段組合回長序列。而所謂的從頭組裝，是指不依靠既有的參考基因組(Reference Genome) 直接從短片段組合出定序結果。

因為快速和便宜，雖然單一樣本的準確率不如第一代測序Sanger Method，但依靠大量的數據可以更方便的投入疫區有更多醫療和商業價值。

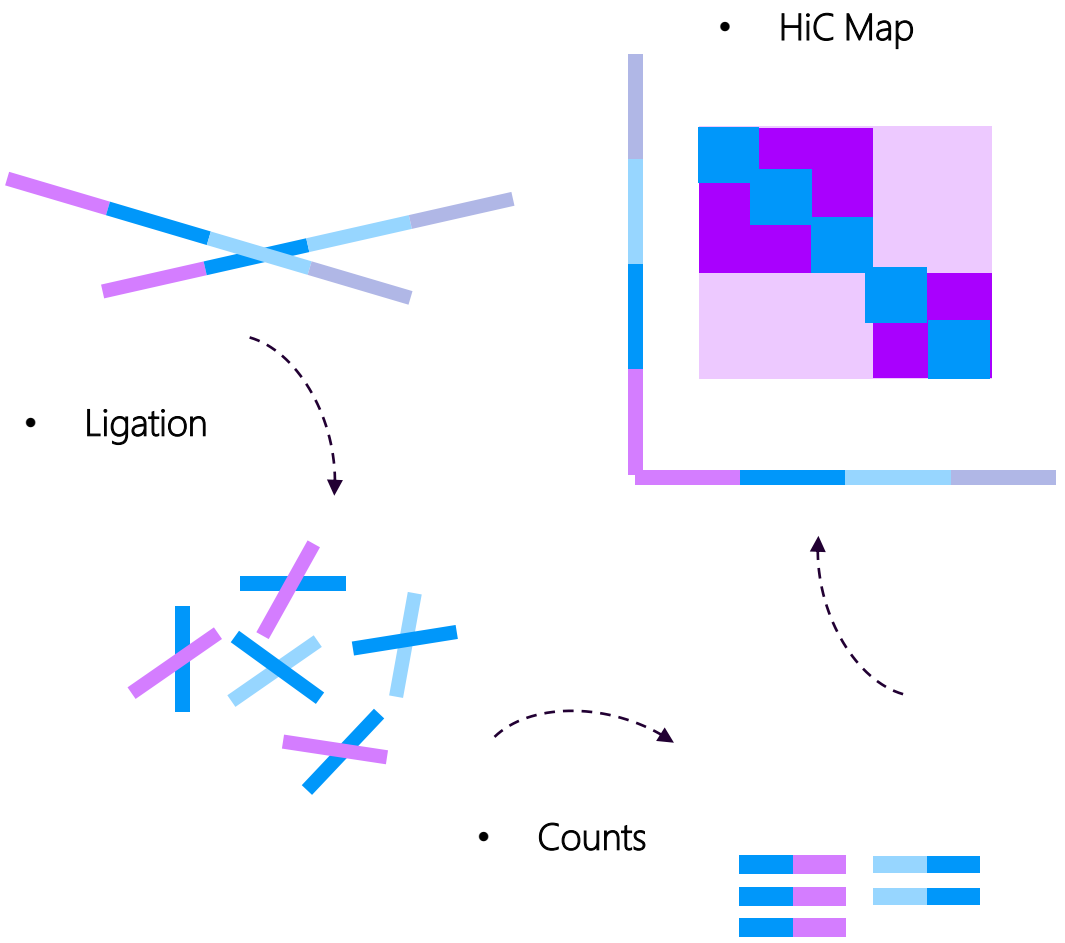


基本概念介紹

Hi-C 染色體結構捕獲

主要是利用染色體之間存在實際上的折疊三維空間關係，利用交聯反應捕捉後，計算次數，最後得到能夠反應三維遠近關係的二維矩陣

額外多了3維的空間關係資訊除了去了解染色體的各種性質之外也可以利用較近的片段容易被交聯而數量較多，越遠則越少的關係，去輔助染色體定序的組裝排序。

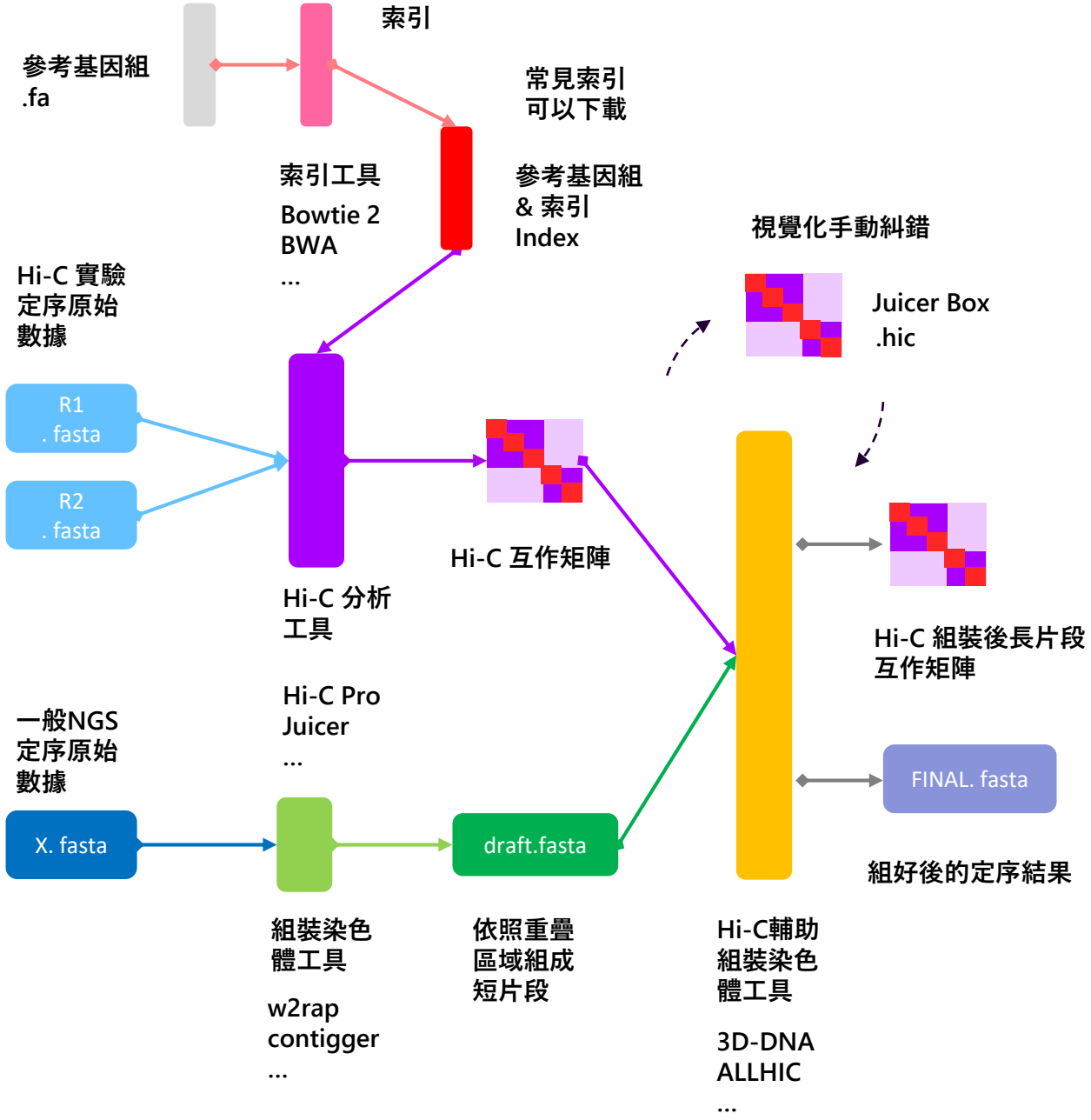


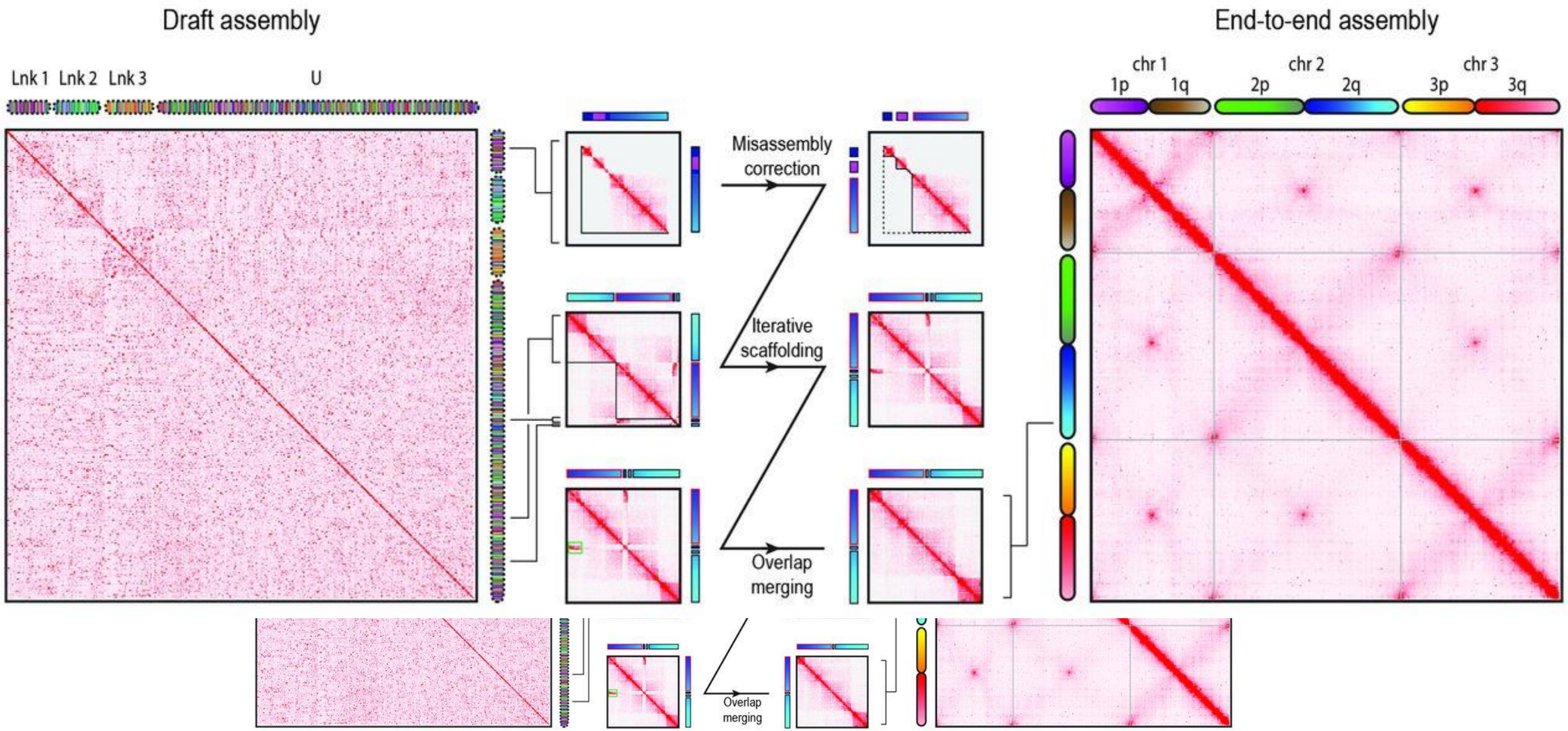
基本概念介紹

基於Hi-C輔助從頭組裝

本論文的主軸是在介紹他們新設計的從頭組裝軟體3D-DNA，具有使用Hi-C數據輔助組裝的能力

整體分析流程如右圖，本次專案覆現實驗會從進入3D-DNA之後開始，直接使用原論文提供的輸入前處理數據





本論文利用組裝前，中，後的 Hi-C圖表，去展現出輔助組裝後長片段在互作圖的表現，會明顯清晰很多。利用這部分的現象，想要簡易的覆現這篇論文，可以把組裝前中後的圖表進行比較。

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Part 2 /
Environment Setup

系統安裝方法

3D-DNA 環境需求與安裝

3D-DNA 需要的使用的程式包含了, Java Python , Shell 這三種語言。本專案安裝的部分是事先寫好每一步驟的安裝腳本，再逐一執行。

如果想要使用原始的3D-DNA進行實驗覆現，可以利用版控跳到第 745779b 版本

< /Source Code >

- https://github.com/1081-Bioinformatics/finalproject-3ddna_108/tree/master/code/install_3ddna_dependence

Install Oracle Java 8

Using JDK version jdk-8u231-linux-x64 (change if you need for other hardware.)

```
sudo sh install_jdk8.sh
```

Install GNU Tools (gawk , coreutils , parallel)

```
sudo sh install_gnuutil.sh
```

Install Lastz

```
sudo source install_lastz.sh
```

Install Python & lib

Install 3D-DNA Package

```
git clone https://github.com/theaidenlab/3d-dna
cd 3d-dna
```

For the old stable version (This vsersion using run-pipeline.sh to run.):

```
git checkout 745779b
```

系統安裝方法

AaegL2 &Human 實驗數據準備

	組裝草稿 (待組序列)	Hi-C 合併的片段數量統計 (輔助組裝 上游分析為 Juicer)
AaegL2 埃及斑蚊	GSE95797_AaegL2.fasta.gz	GSE95797_AaegL2.mnd.txt.gz
Human 人類	GSE95797_Hs1.fasta	GSE95797_Hs1.mnd.txt

< /Source Code >

- https://github.com/1081-Bioinformatics/finalproject-3ddna_108/tree/master/code/download_data

< /Tips to Speed up>

- axel -n 30 ftp://...

AaegL

Download AaegL2.mnd.txt (After unzip will be 76GB)

```
sudo sh download_GSE95797_AaegL2.mnd.txt.gz.sh
```

Download AaegL2.fasta

This code was provide by 3ddna project the same file in project ./supp/get-AaegL2.sh

```
sudo sh download_GSE95797_AaegL2.fasta.gz.sh
```

Human

Download GSE95797_Hs1.mnd.txt

```
sudo sh download_GSE95797_Hs1.mnd.txt.gz.sh
```

Download GSE95797_Hs1.fasta

```
sudo sh download_GSE95797_Hs1.fasta.gz.sh
```

3D-DNA 程式除錯

系統安裝方法

$$\frac{y}{x} \longrightarrow \frac{(y+1)}{(x+1)}$$

執行舊版的時候有時候會遇到
fatal: division by zero attempted
為了順利進行分析，以拉普拉斯平滑修正了出錯的地方。

```
if (($1!=prev1 || $2!=prev2 || $3!=prev3) && FNR!=1)
{
        print prev1, prev2, prev3, (count+1)/(slength[prev1]*slength[prev2]+1), (count+1)
        count = 0
}
```

< /Source Code >

- https://github.com/1081-Bioinformatics/finalproject-3ddna_108/tree/master/code/fixbug_code

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Part 3 /
Run Analysis

3D-DNA 執行

執行輸出結果

$ chmod +x run-asm-pipeline.sh

$ nohup ./run-asm-pipeline.sh GSE95797_Hs1.fasta GSE95797_Hs1.mnd.txt > run_GSE95797_Hs1.log 2>&1 &

(新版)

< /Source Code >

- **https://github.com/1081-Bioinformatics/finalproject-3ddna_108/tree/master/code/run_analysis**

3D-DNA
運行情況

執行輸出結果

- Google Cloud Platform
  n1-standard-16 (16 vCPUs, 60 GB memory, 1TB HDD)
- OS Version
  Ubuntu 16.04.6 LTS (Xenial Xerus)
- Run both AaegL2&Human

- CPU Usage Plot (The analysis process run 5 Days)

Part 4 /
Output Result

- Hi-C Figure
https://github.com/1081-Bioinformatics/finalproject-3ddna_108/tree/master/results

- .hic File
https://github.com/1081-Bioinformatics/finalproject-3ddna_108/tree/master/data

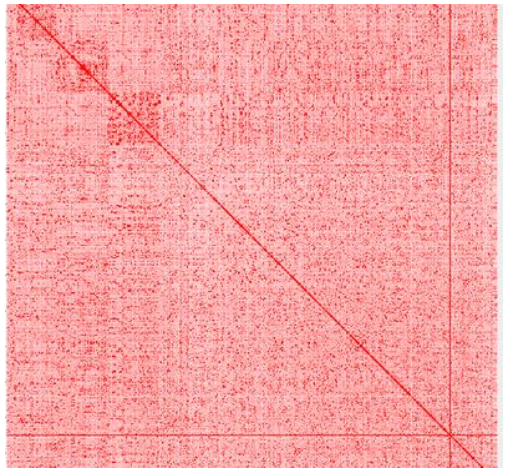
實驗結果整理

埃及斑蚊Hi-C圖變化

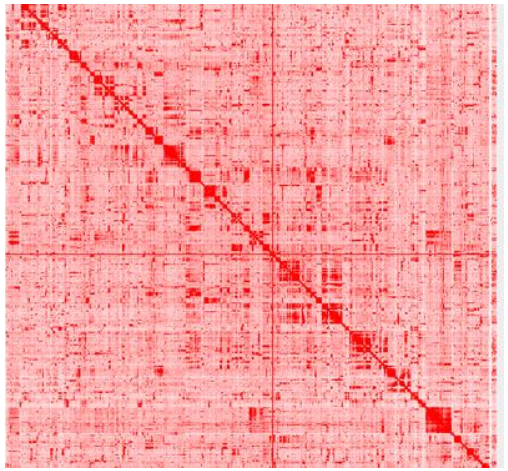
從左上，右上，左下，右下依序是從草稿Hi-C是視覺化圖，一直到最後一輪迭代的Hi-C圖。視覺圖有由本次專案輸出的.hic檔經Juicer Box 視覺化。

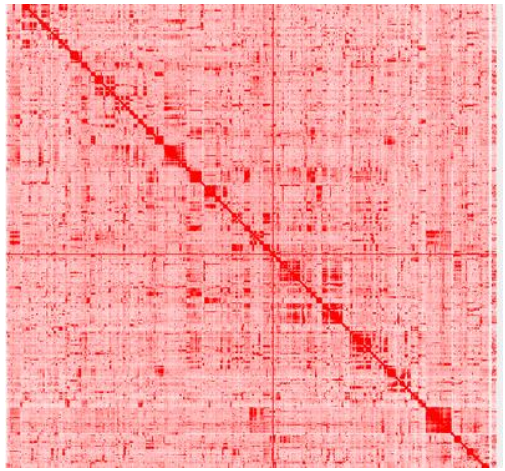
可以明顯的比較出來，隨著3D-DNA正確的參照Hi-C的資訊進行長片段組裝迭代，Hi-C圖有越來越清晰的傾向，表示隨著組裝序列越長，越有回復到原本的序列。

AaegL2.2.draft.hic

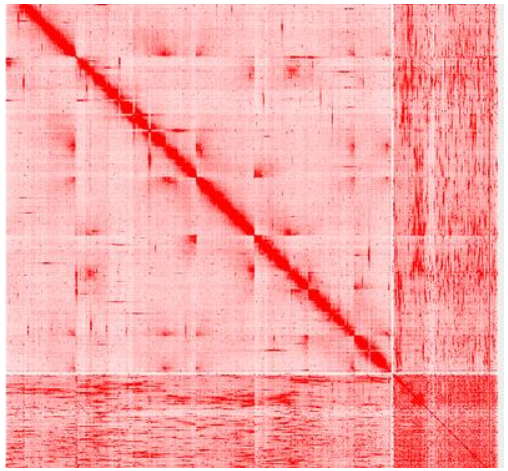


AaegL2.2.0.hic





AaegL2.2.1.hic



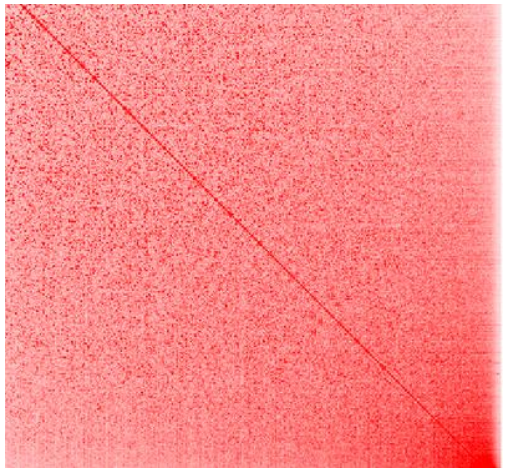
AaegL2.2.2.hic

實驗結果整理

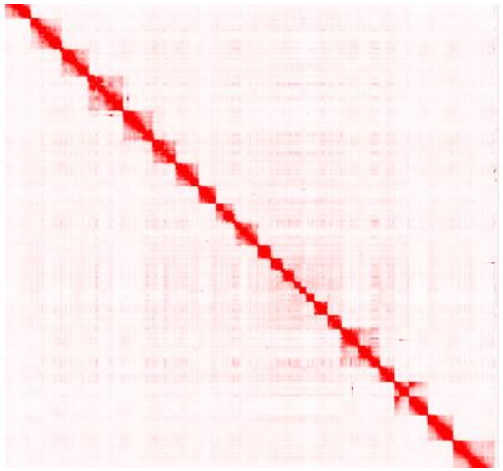
人類Hi-C圖變化

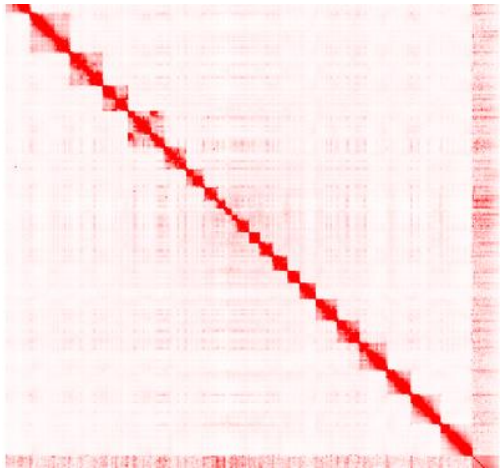
與前面一樣，迭代次數增加，Hi-C圖有越來越清晰的傾向，恢復的資訊越多。從以上兩組Hi-C圖的視覺化，就能夠清楚知道其作用。

GSE95797_Hs1.draft.hic

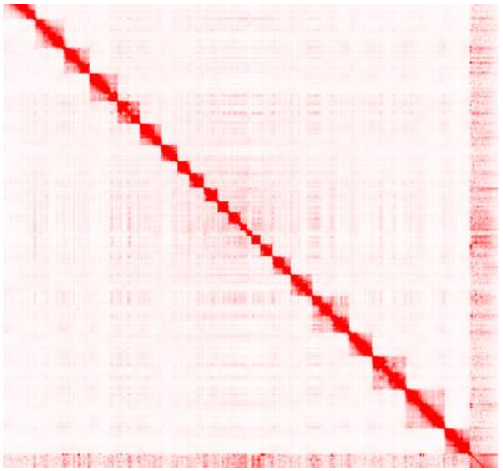


GSE95797_Hs1.0.hic





GSE95797_Hs1.1.hic

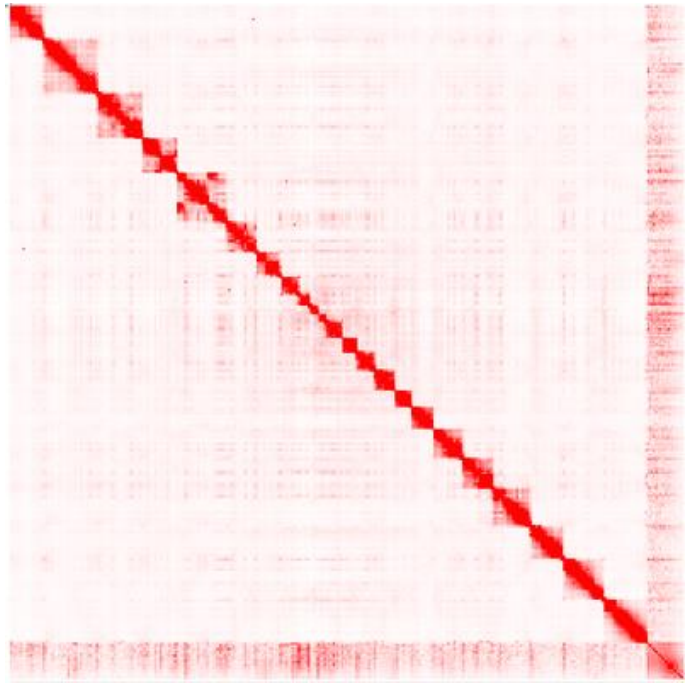


GSE95797_Hs1.2.hic

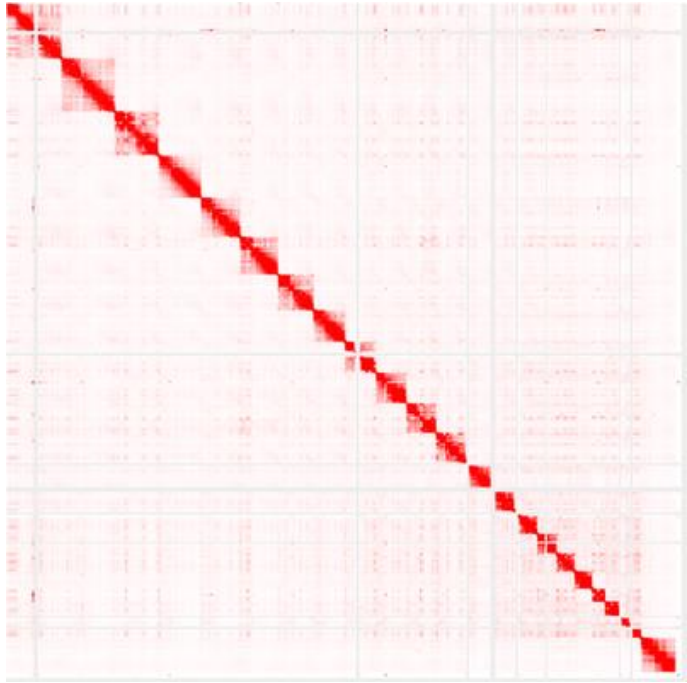
實驗結果整理

人類Hi-C圖比較

左邊是本論文的資料經過輔助組裝後的結果，右邊是2014年其他人實驗所繪製的Hi-C圖，兩者具有類似結構



- GSE95797_Hs1.2.hic



- GM12878_Cell_2014

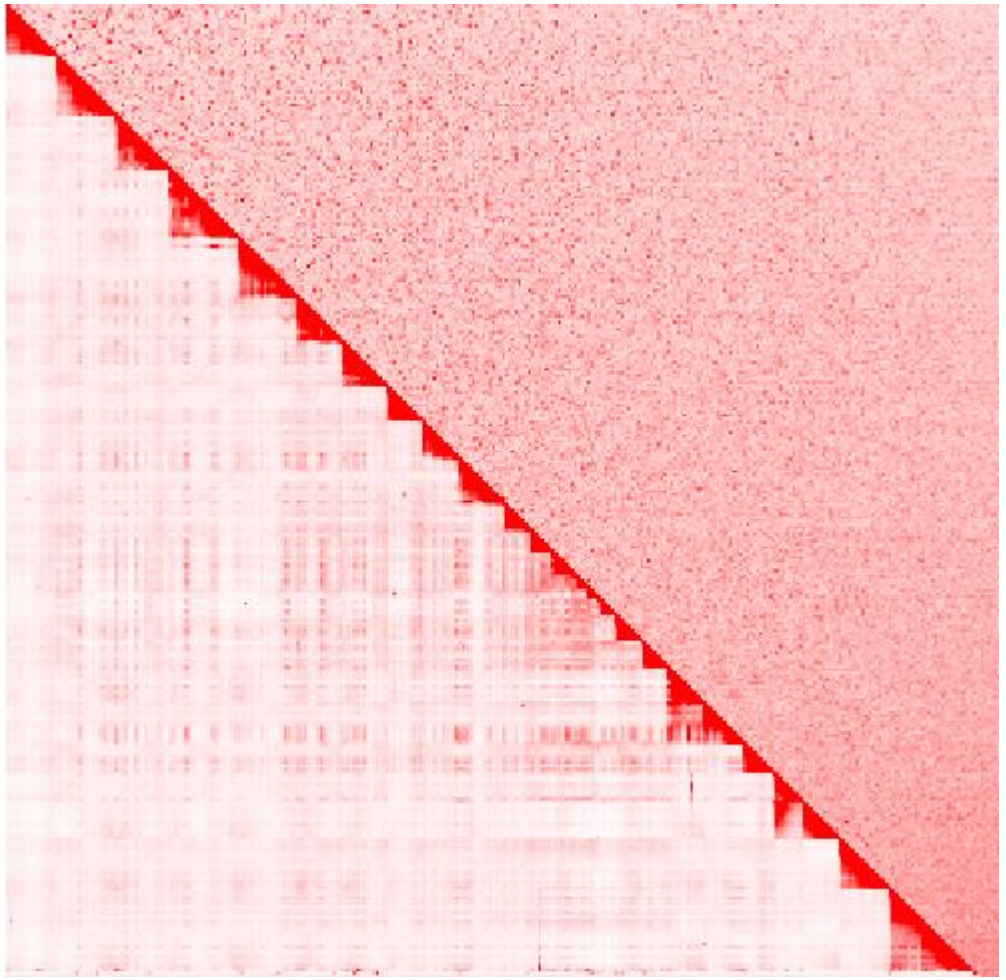
(HIC001(148M) Cell. 2014 Dec 18;159(7):1665-80. doi: 10.1016/j.cell.2014.11.021. Epub 2014 Dec 11.)

實驗結果整理

人類 Hi-C圖 疊圖比較

左下半較清晰的是組裝後的圖，右上半是尚未組裝的圖。

GSE95797_Hs1.draft.hic



GSE95797_Hs1.2.hic

結論

實驗結果整理

本專案是部分
<De novo assembly of the Aedes aegypti genome using Hi-C yields chromosome-length scaffolds>論文再現實驗。

並且得到了由Hi-C輔助組裝的序列。其中，3D-DNA透過修正與排序疊代調整組裝得長片段，右邊列出最後三點總結。

01 Hi-C能夠有效協助序列組裝，3D-DNA組裝效果佳。

02 3D-DNA 計算效能消耗大。如果需要在有限時間內完成，需要預留更多時間。

03 3D-DNA 部分程式碼應該可以更加完善

Supp

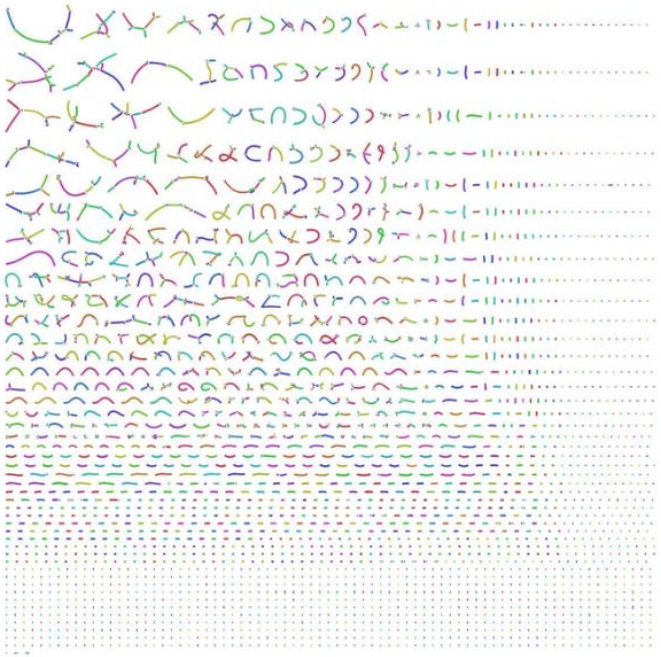
實驗結果整理

1. With the Zika virus spreading largely unchecked in Latin America and the Caribbean by way of a now-notorious insect, some of the nation's leading mosquito researchers are striving to assemble a state-of-the-art DNA map that they say will help them fight the disease with the mosquito's own genetic code.

2. Some want to hunt for genes that, if altered in mosquitoes released into the wild, could drive the species to extinction. Others are trying to identify genes that control how mosquitoes sense human prey so as to devise better repellents. Still others favor the idea of selectively breeding populations of mosquitoes, like corn or cattle, for desirable — or, at least, less undesirable — traits, such as a preference for biting animals other than humans.

Supp

實驗結果整理



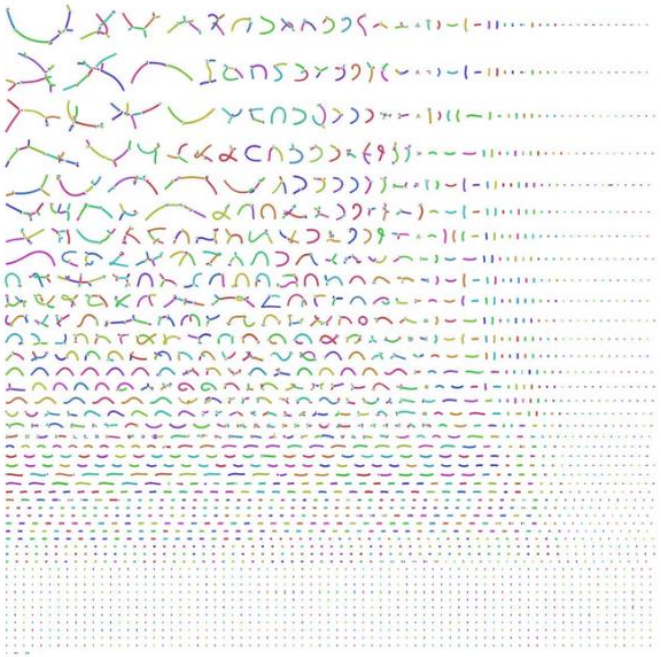
A visualization of the recently sequenced Aedes aegypti genome. Each of the 3,752 colored lines is a fragment of its three chromosomes that could not be fit together without the additional information that the Aedes Genome Working Group hopes to produce. A 2007 genome map for Aedes aegypti is fragmented into about 10 times as many pieces. Mark Kunitomi

1. That a genome map for Aedes aegypti, however imperfect, was published in 2007 is a testament to the species' longtime status as a potent human foe. (Its genus name, bestowed by 18th-century naturalists with a penchant for accuracy, means "unpleasant" in ancient Greek; "aegypti" refers to Egypt, where it was first believed to have been collected.)

2. Besides Zika and yellow fever, the insect carries dengue, which causes a severe and sometimes fatal flulike illness, and chikungunya, which can cause intense joint pain that lasts for years.

Supp

實驗結果整理



A visualization of the recently sequenced Aedes aegypti genome. Each of the 3,752 colored lines is a fragment of its three chromosomes that could not be fit together without the additional information that the Aedes Genome Working Group hopes to produce. A 2007 genome map for Aedes aegypti is fragmented into about 10 times as many pieces. Mark Kunitomi

1. But the technology used for the 2007 Aedes map, and many others, could only read relatively short stretches of DNA at a time, which were then pieced together by matching up the sections where they overlapped in a process that often left some areas garbled and riddled with gaps. And since more than half of the Aedes genome consists of sequences that repeat again and again, it has proved more difficult than many genomes to make sense of.

Supp

實驗結果整理

“The Zika outbreak, spread by the Aedes aegypti mosquito, highlights the need to create high-quality assemblies of large genomes in a rapid and cost-effective way. Here we combine Hi-C data with existing draft assemblies to generate chromosome-length scaffolds. We validate this method by assembling a human genome, de novo, from short reads alone (67× coverage). We then combine our method with draft sequences to create genome assemblies of the mosquito disease vectors Ae. aegypti and Culex quinquefasciatus, each consisting of three scaffolds corresponding to the three chromosomes in each species. These assemblies indicate that almost all genomic rearrangements among these species occur within, rather than between, chromosome arms. The genome assembly procedure we describe is fast, inexpensive, and accurate, and can be applied to many species.”

Supp2

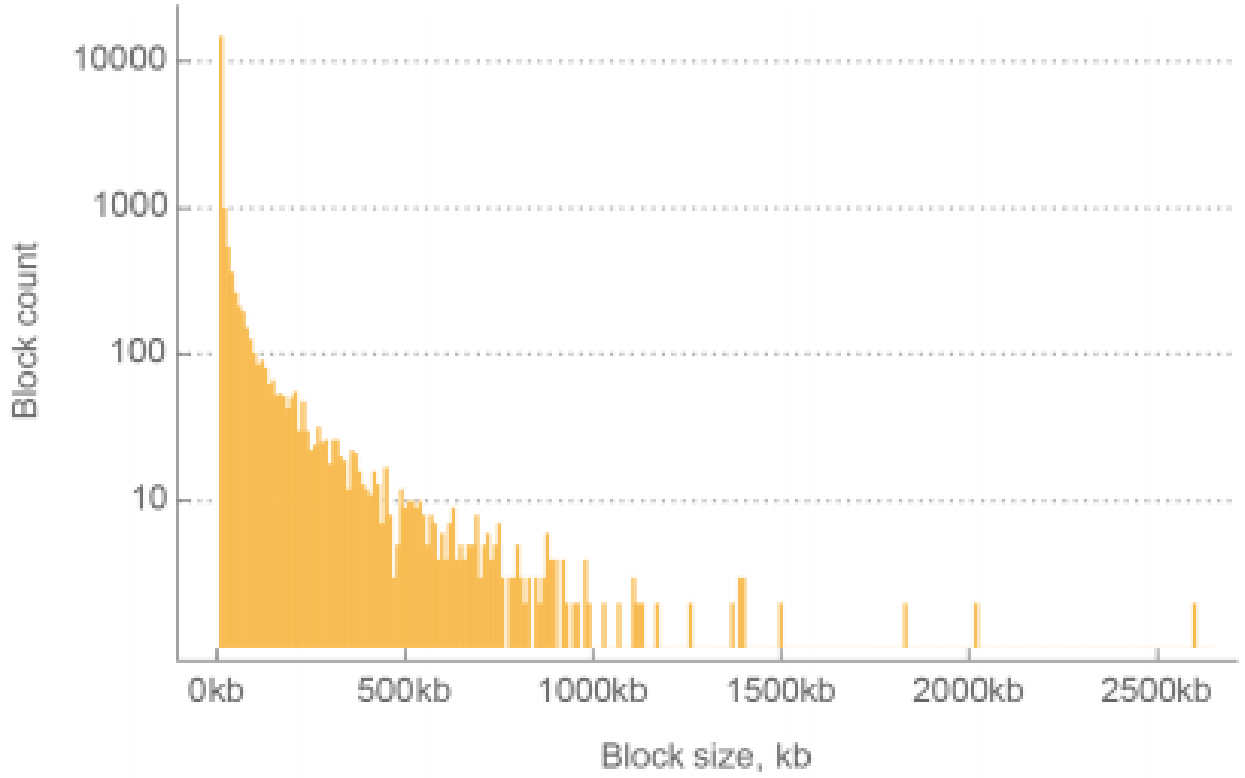
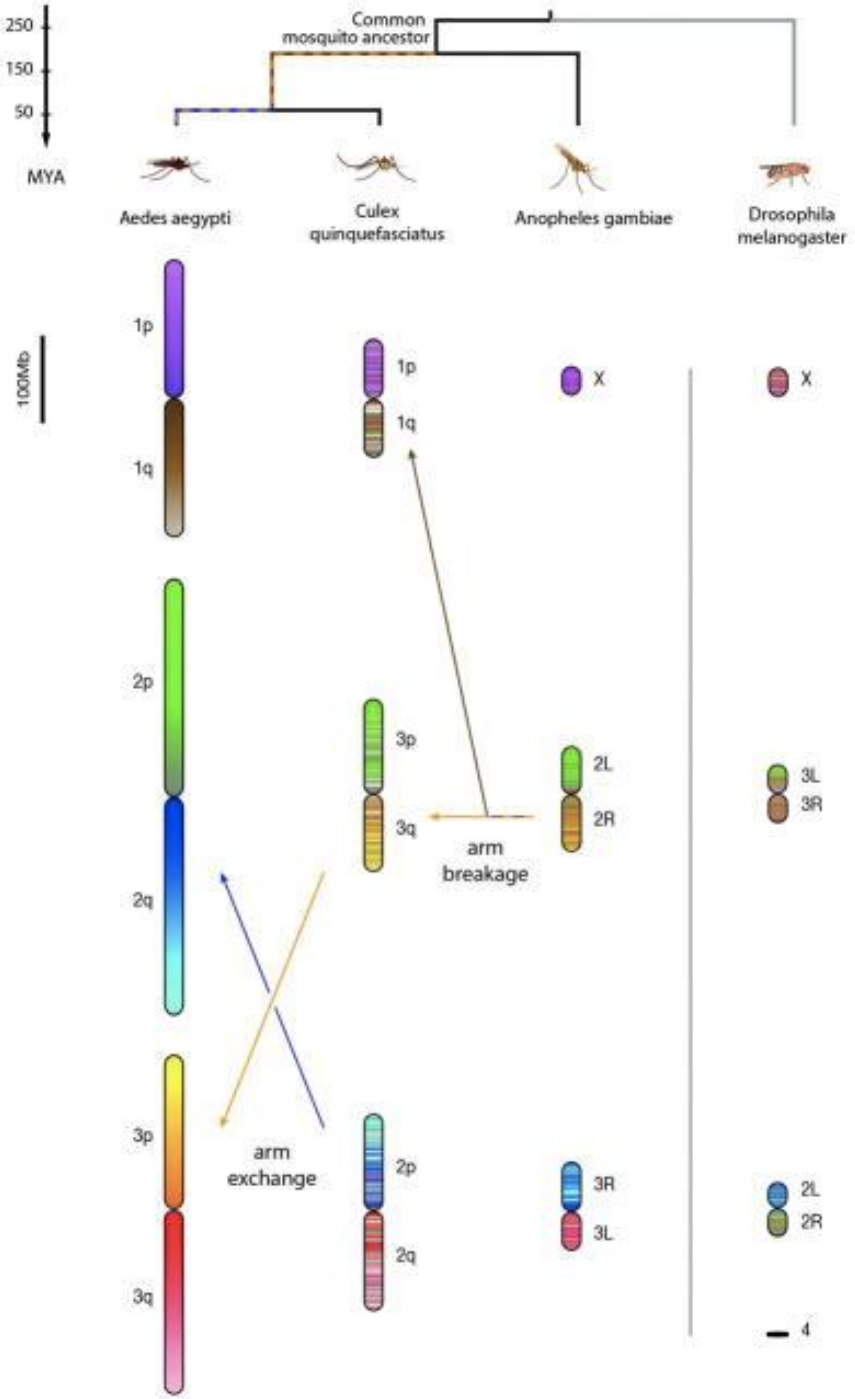


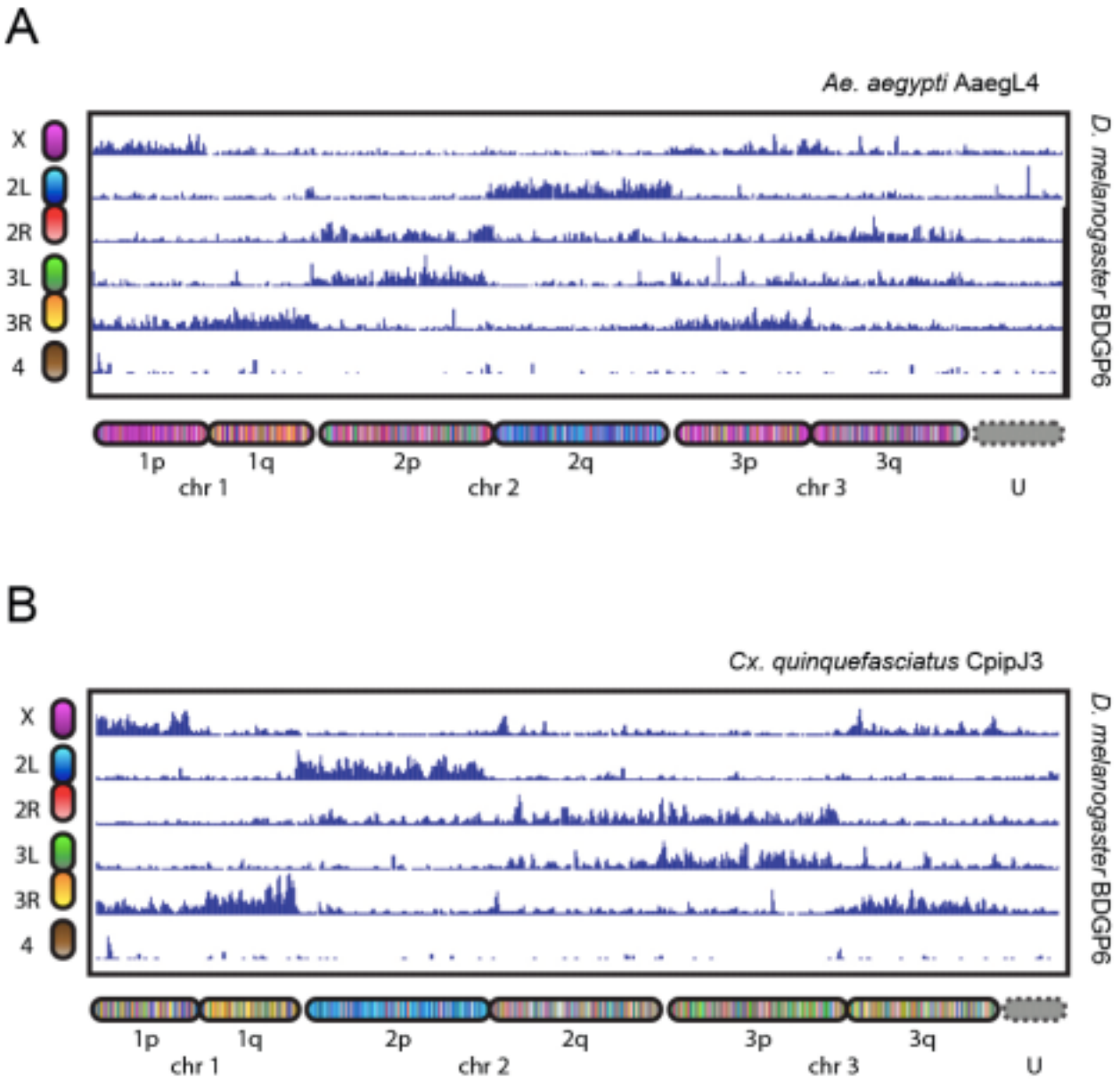
Fig. S16.

Size distribution for synteny blocks between *Ae. aegypti* and *An. gambiae*. The block sizes are measured with respect to the *Ae. aegypti* genome. The blocks are defined as chains of conserved sequence markers that are both consecutive and collinear in both genomes. The chain ends when two consecutive markers disagree with the rest of the chain; however, one marker in the wrong order and/or the wrong orientation does not break the chain.

Supp2


250
150
50
MYA
Common mosquito ancestor
Aedes aegypti
Culex quinquefasciatus
Anopheles gambiae
Drosophila melanogaster
100Mb
1p
1q
2p
2q
3p
3q
arm breakage
arm exchange
X
2L
2R
3L
3R
4

Supp2


A
Ae. aegypti AaegL4
X
2L
2R
3L
3R
4
D. melanogaster BDGP6
1p
1q
2p
2q
3p
3q
chr 1
chr 2
chr 3
U
B
Cx. quinquefasciatus CpipJ3
X
2L
2R
3L
3R
4
D. melanogaster BDGP6
1p
1q
2p
2q
3p
3q
chr 1
chr 2
chr 3
U

Supp2

- provides a rapid, inexpensive methodology for generating highly accurate de novo assemblies with chromosome-length scaffolds.
- the current approach is not perfect for local ordering of small adjacent contigs.
- This might be circumvented by more sophisticated analysis of Hi-C data. Additional data (such as long or paired-end reads) could also improve the results.

參考資料

實驗結果整理

1. THE CENTER FOR GENOME ARCHITECTURE Baylor College of Medicine & Rice University http://aidenlab.org/assembly/manual_180322.pdf

2. De novo assembly of the Aedes aegypti genome using Hi-C yields chromosome-length scaffolds. https://www.ncbi.nlm.nih.gov/pubmed/28336562

3. Hi-C辅助基因组安装 https://www.jianshu.com/p/95792fbde9c3

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THANKS