Developments on genome assembly

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Why genome assembly?

Keywords to get your assembly founding accepted

- For SARS-CoV-2: Find putative drugs, understand its mode of action.
- For human: cure/prevent genetic diseases.
- For cattle: improve production, informed selection.
- For plant: hydric stress resistance, pathogen resistance.

In general, crucial for:

- genetics studies,
- molecular studies.

Genome sequencing

Definition

- Extracting DNA.

→ GATTACA
GATTACA
GATTACA

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ATT
ACA
GAT
TAC
TTA

Genome sequencing

Definition

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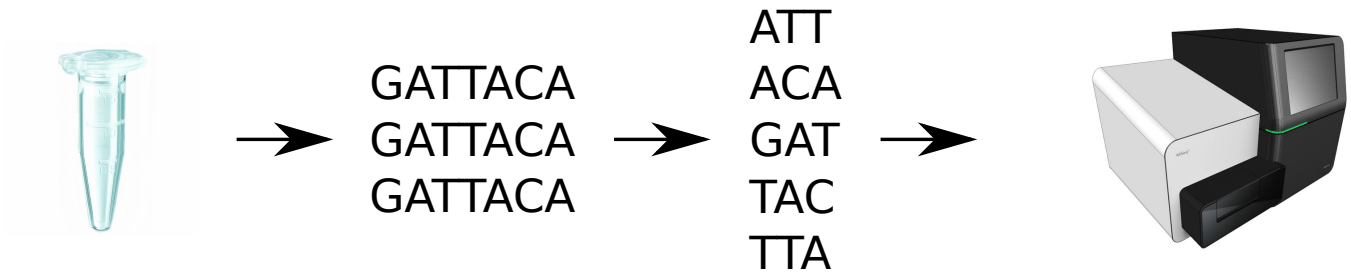
- Run it through a machine, which gives lots of A, C, G, T.



Genome sequencing

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- Extracting DNA.
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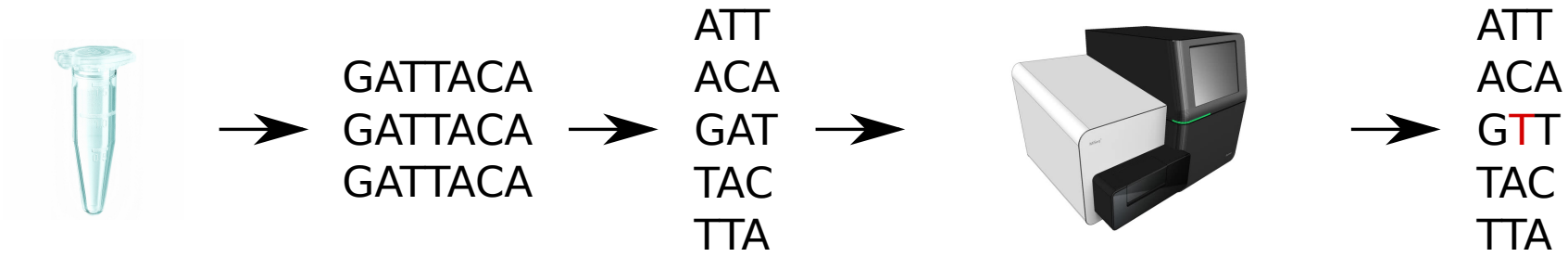
- Run it through a machine, which gives lots of A, C, G, T.

Rem. The machine can be unfaithful.



Genome sequencing

Definition

- Extracting DNA.
- Possibly cleave it.
- Run it through a machine, which gives lots of A, C, G, T.

Rem. The machine can be unfaithful.

Several flavors of sequencers

- Illumina: correct, cheap, high-throuput, short (150).
- ONT: noisy, very long (15k-100k).
- HiFi: correct, long (15k).

Genome contigs

Definition

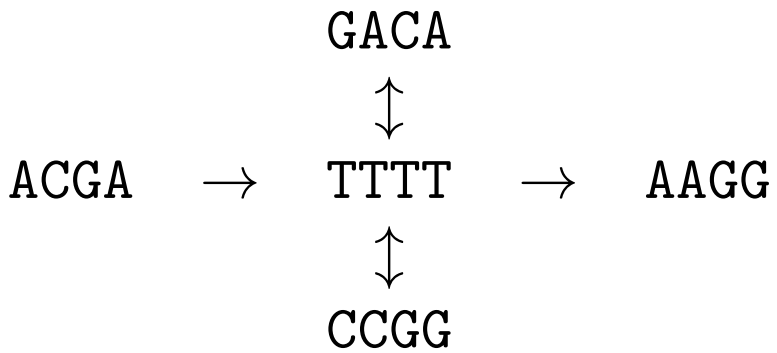
Reads can be assembled into *contigs* if they merge.

Problem: the repetitions

- A targeted *coverage* can be 60X.
- If the puzzle were perfect, we could assemble the genome.
- But repetitions make the assembly impossible.

Why assembly fails?

- Consider the genome:
  ACGATTTTGACATTTTCCGGTTTTAAGG
- Cut it into 4-letters long reads:
  ACGA, CGAT, GATT...
- Shuffle them.
- You know that:
  ACGA, GACA, CCGG are before TTTT
  GACA, CCGG, AAGG are after TTTT
- You can draw the following graph, but cannot solve it!



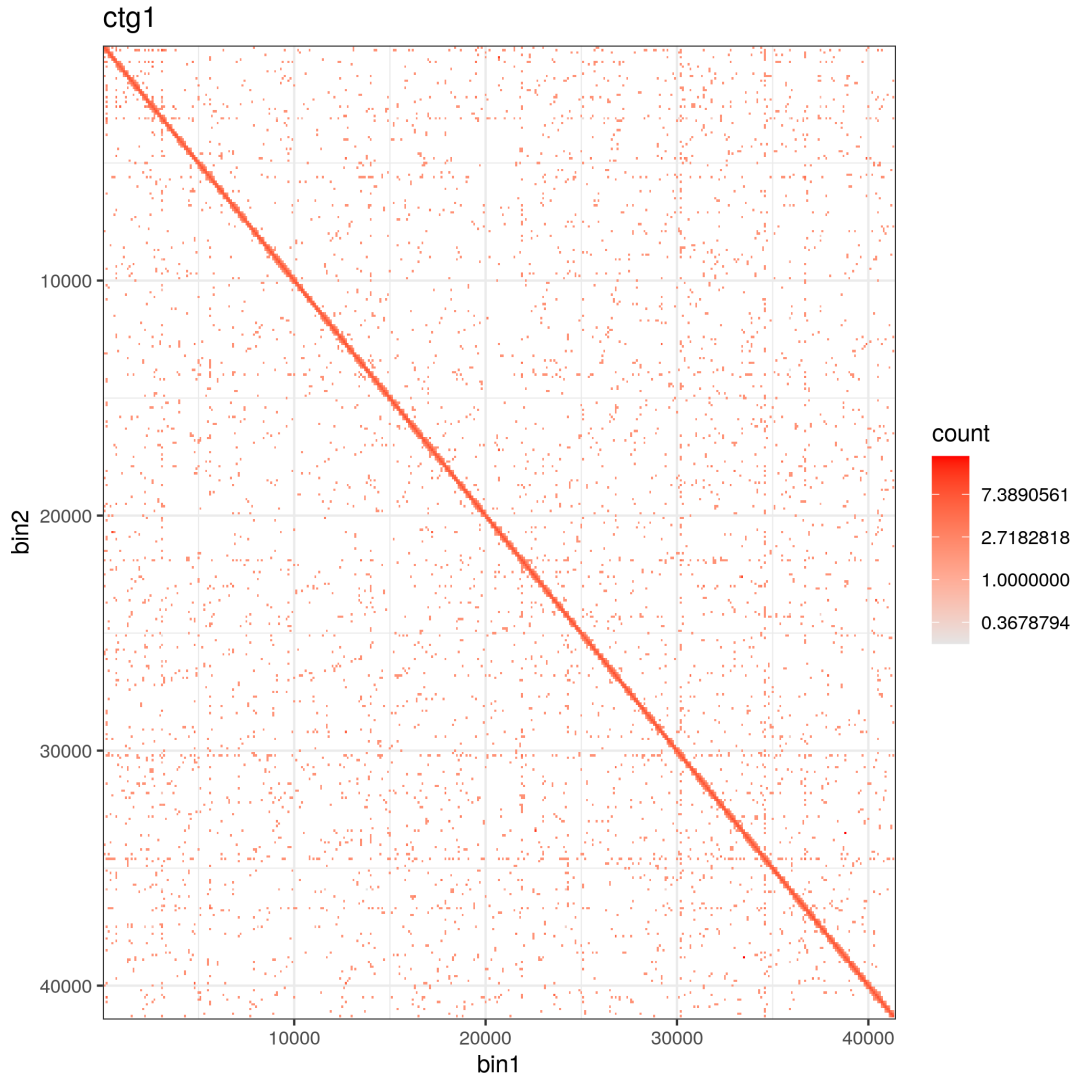
Genome scaffolding

Workaround

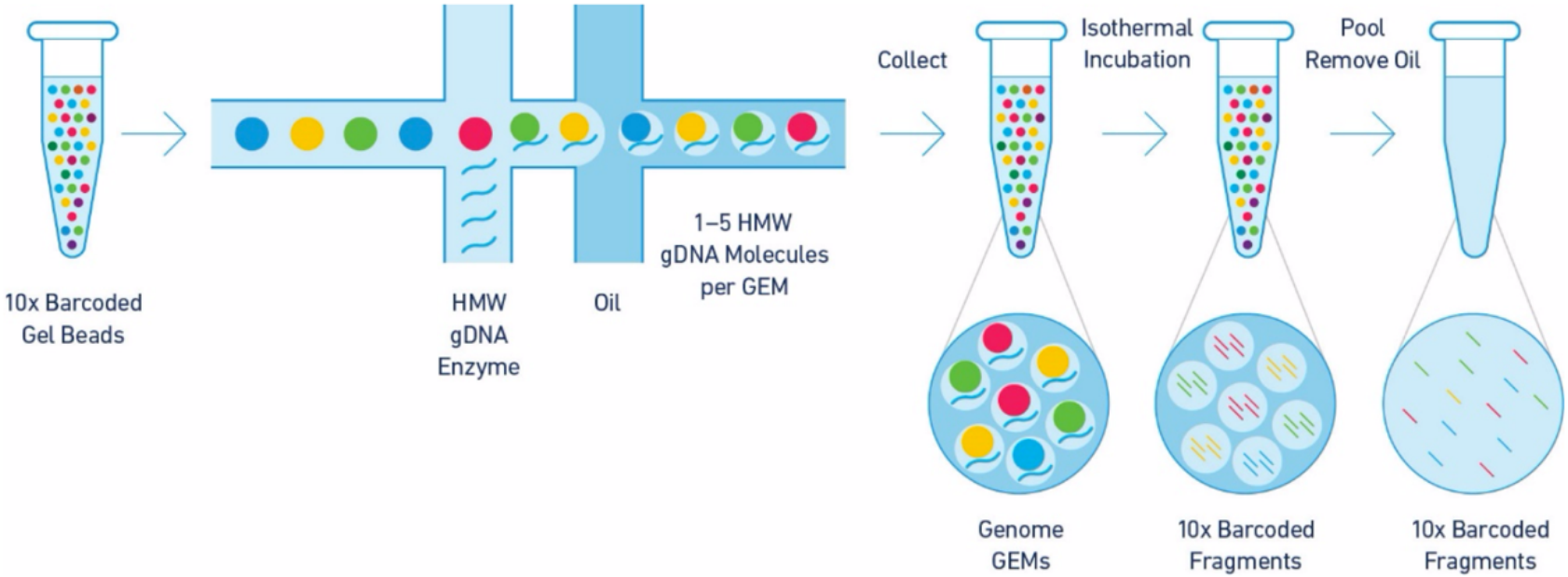
- Fragmented genomes are suboptimal for many analyses.
- If we could stitch the contigs in the right order, it would help.
- It is OK if the content of the glued positions is unknown, if the distance between the contigs is approximate.
- We can use *long-range* interactions: information which indicate that contig X is "close to" contig Y.

```
GTCAC---?---GCTAGCA
```

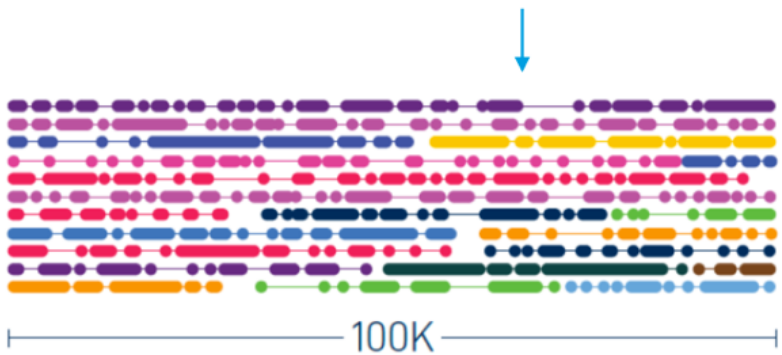
Interaction matrix



Long-range data: 10X Genomics

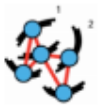
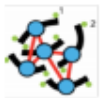
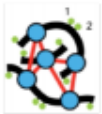
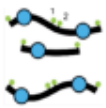
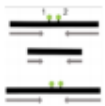


Linked-Reads

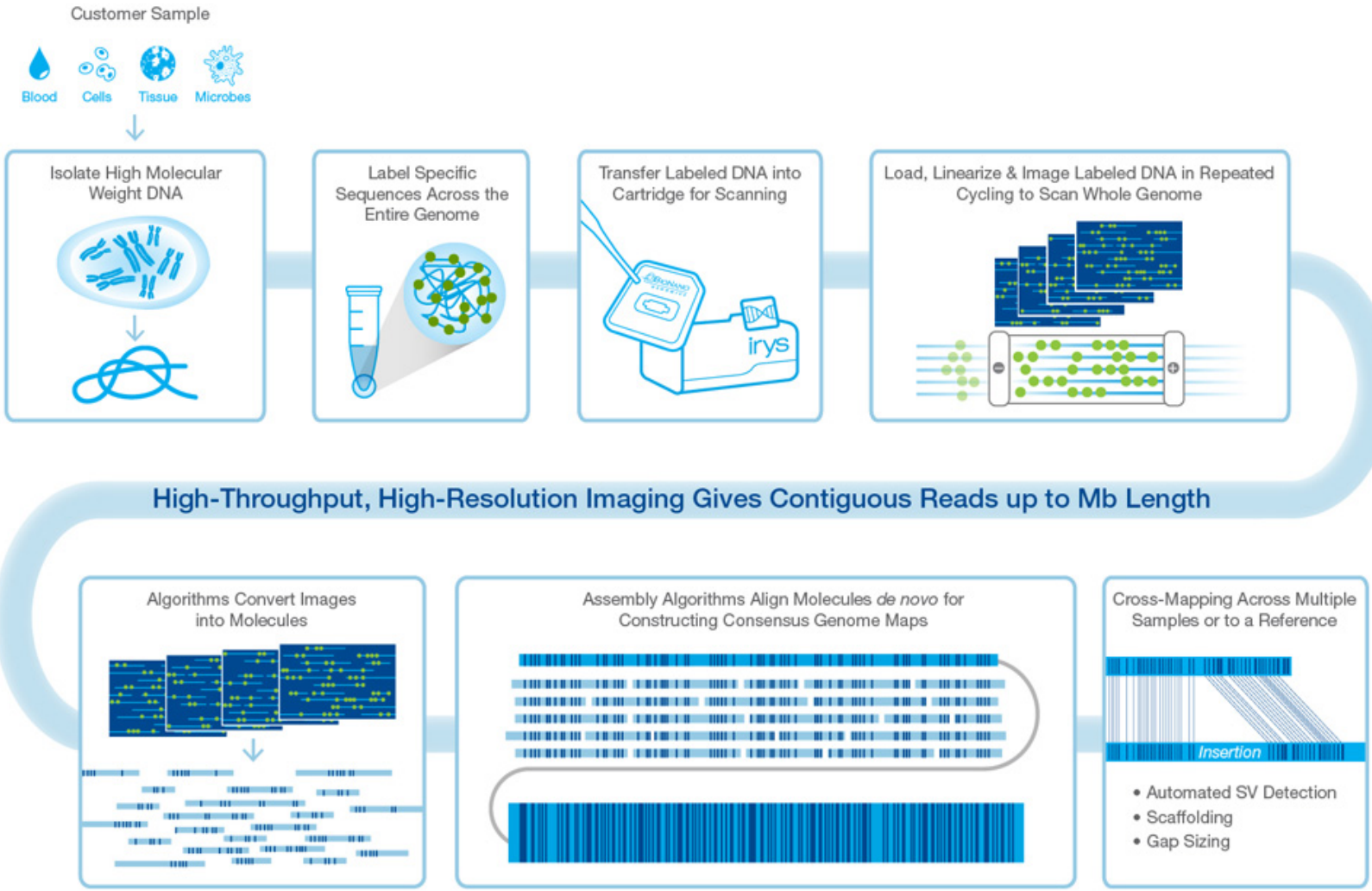


Long-range data: Hi-C

Figure 1. Dovetail™ Hi-C proximity ligation workflow

	A	Dovetail™ Hi-C libraries start with endogenous mammalian chromatin.
	B	Crosslinking (red lines) the chromatin creates a stabilized nucleosome (blue circles) scaffold.
	C	Restriction endonucleases digest the cross-linked chromatin.
	D	Biotin (green dots) mark digested DNA ends (black lines).
	E	Proximity ligation creates chimeric molecules (ex. 1 and 2).
	F	The crosslinks are reversed.
	G	DNA is purified, and enriched for ligation-containing chimeric molecules. An Illumina® library is prepared and sequenced.

Long-range data: BioNano



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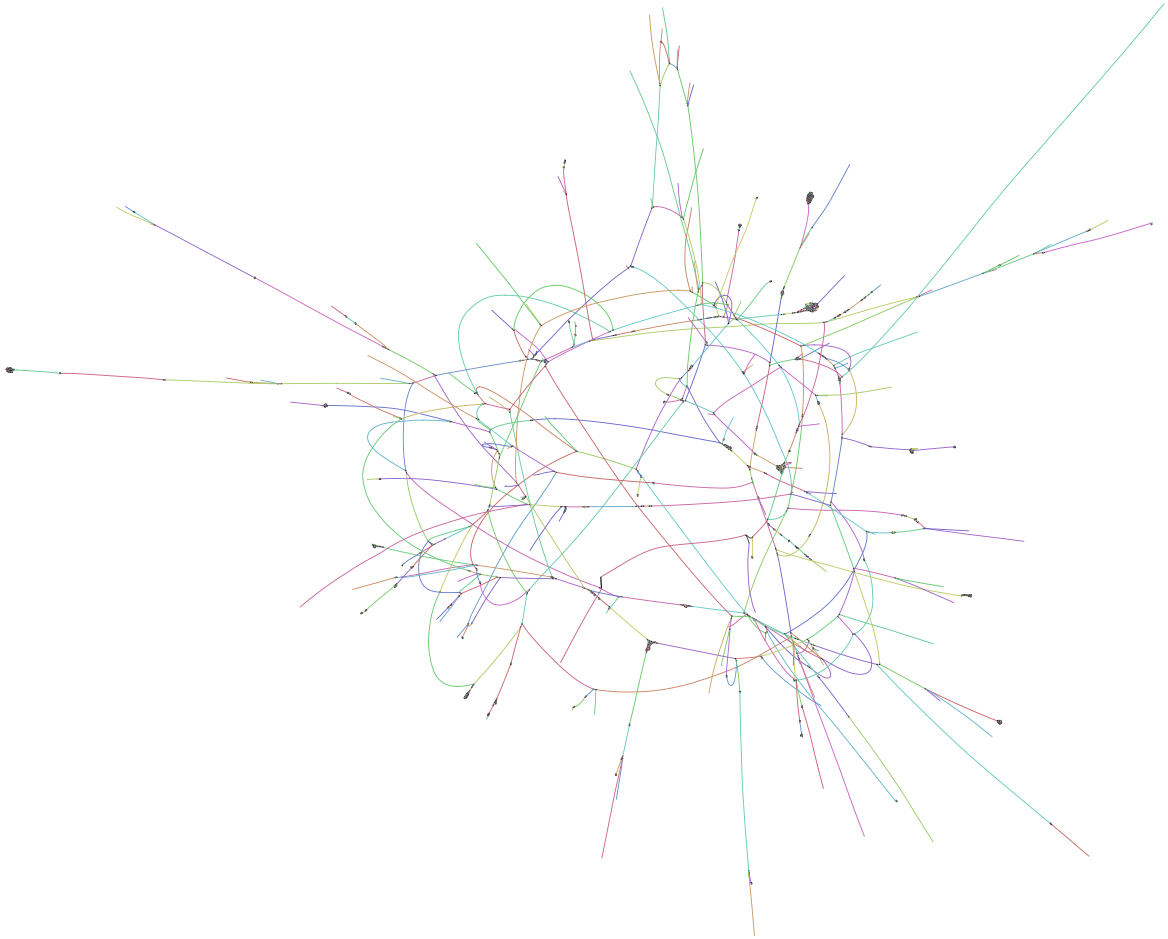
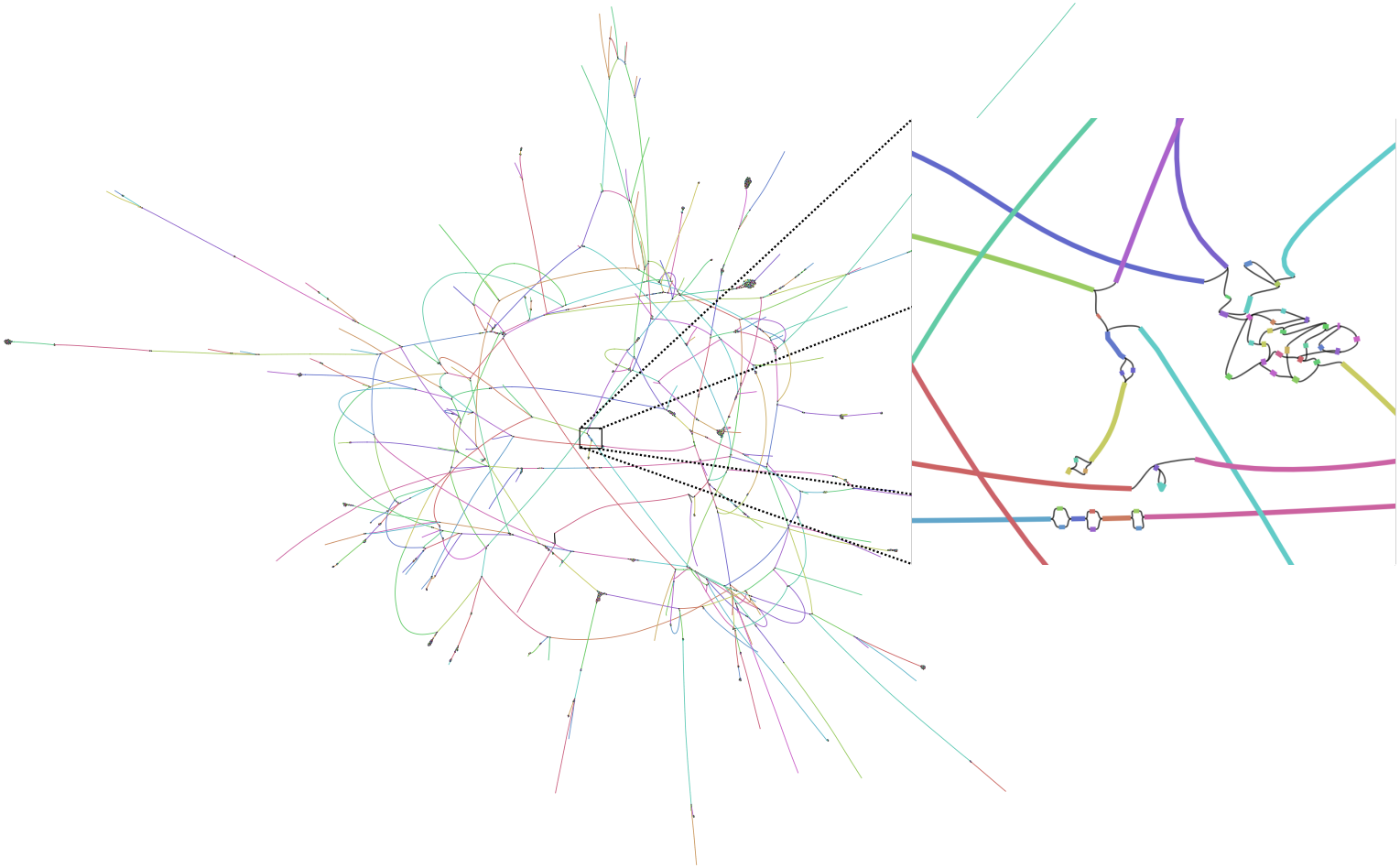
Contig assembly graphs are messy



- node: A,C,G,T sequence
- arc: if significant overlap between nodes

Contig assembly graphs are messy



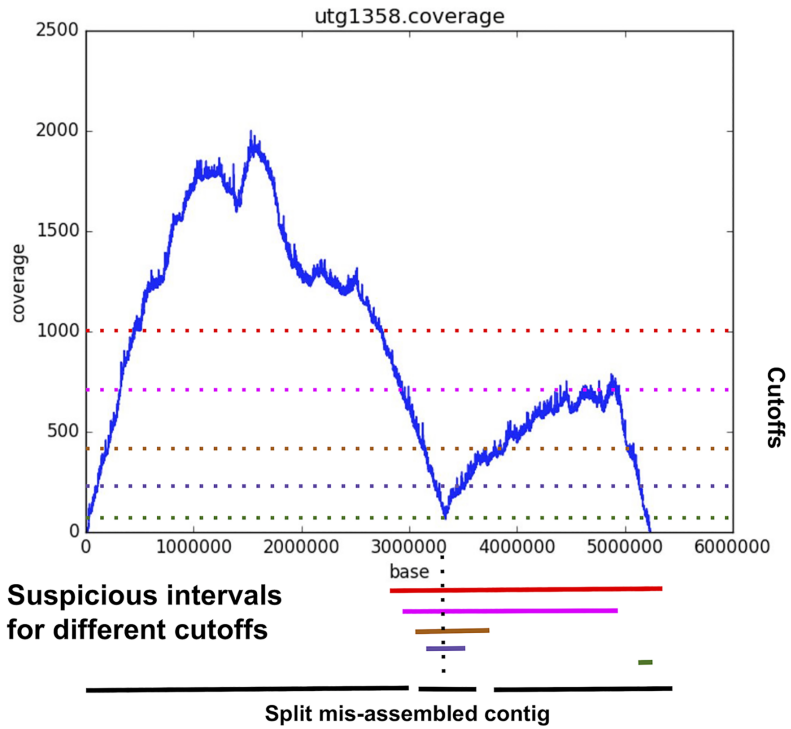
- node: A,C,G,T sequence
- arc: if significant overlap between nodes

Scaffolding methods using Hi-C reads

- align reads on contigs

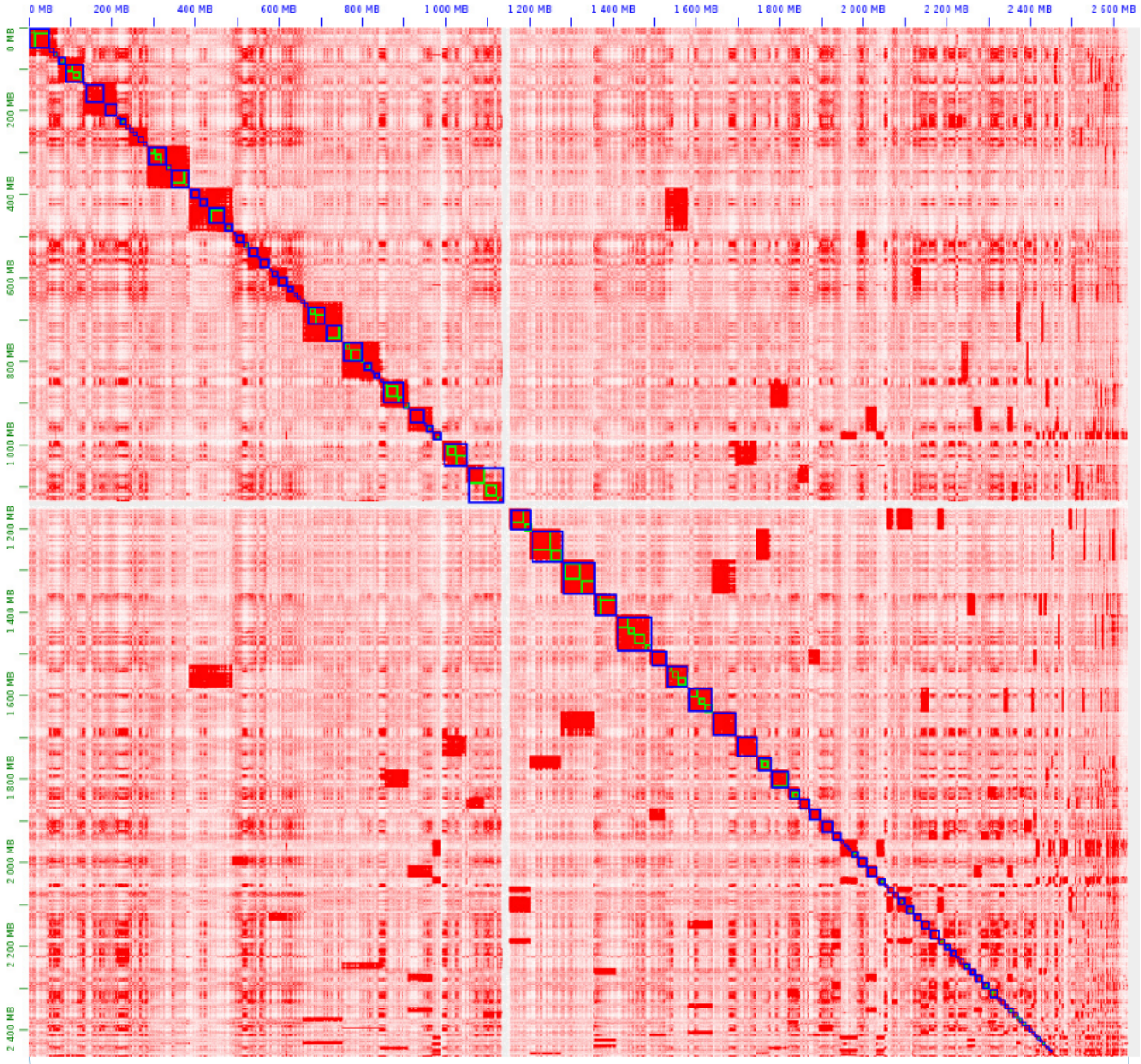
```
Read                ATTAGTTACTGATATG
                    |||| |||| | ||| \\\
Contig ....ACTACTAGATTACTTACGGATCATGCCTACGT....
```

- split contigs based on coverage drop

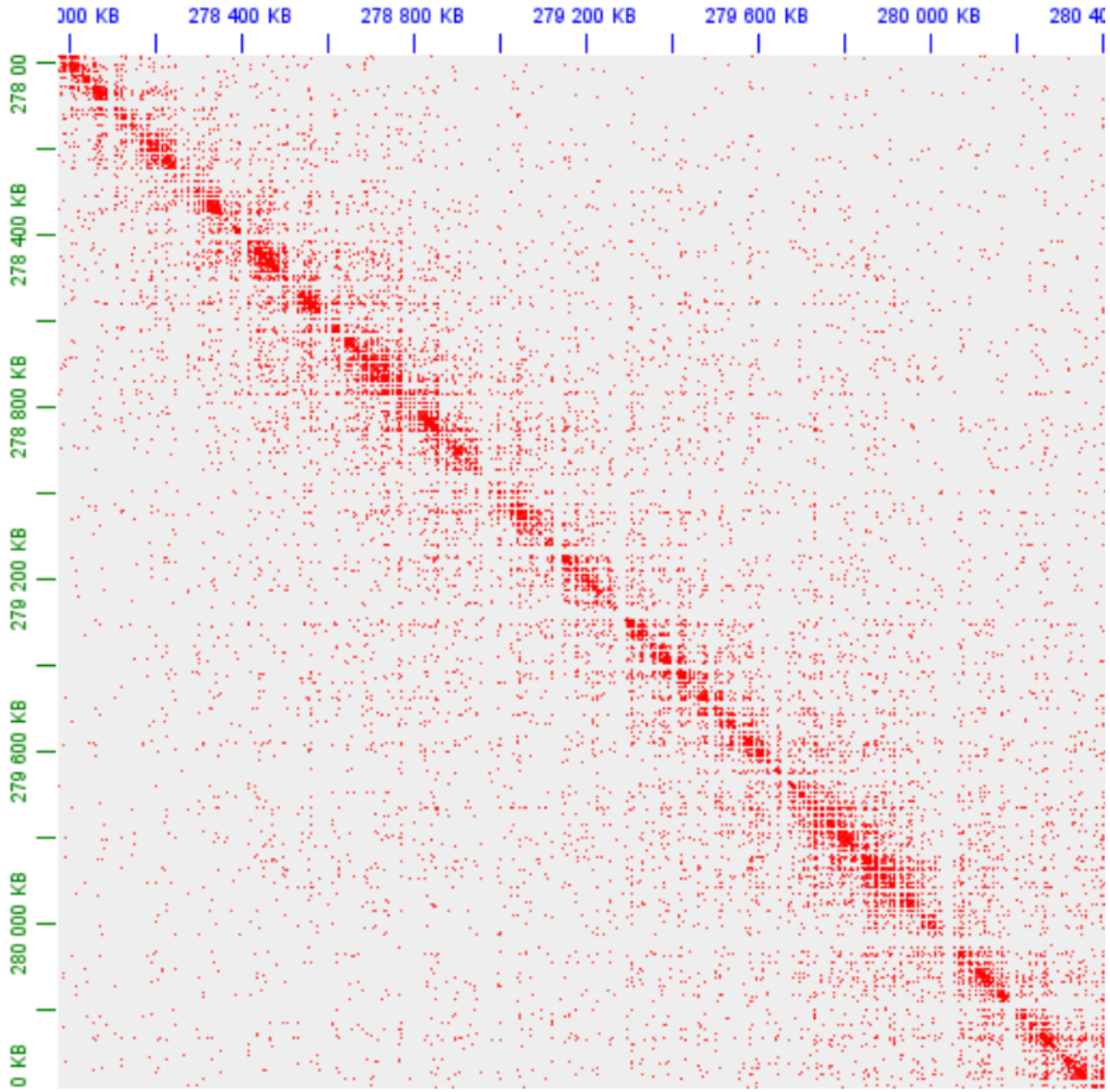


- connect contigs using contact information

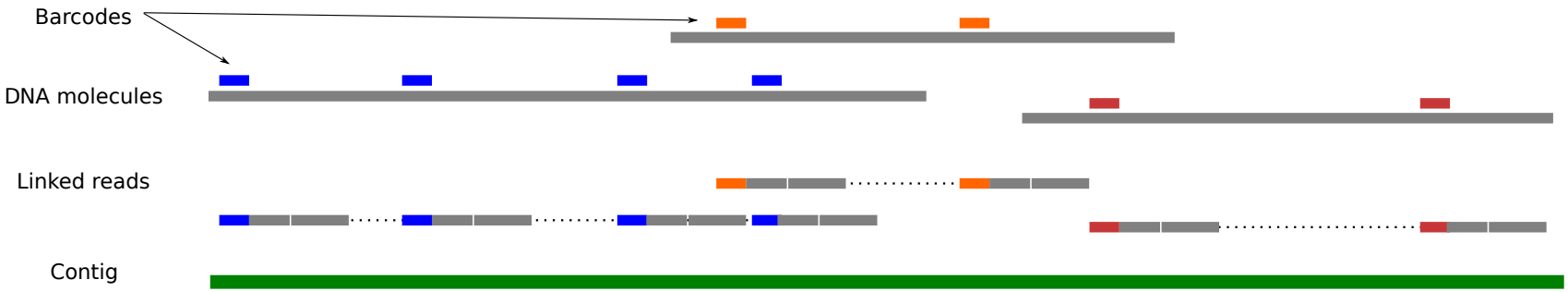
Hi-C heat map: contact information for contigs

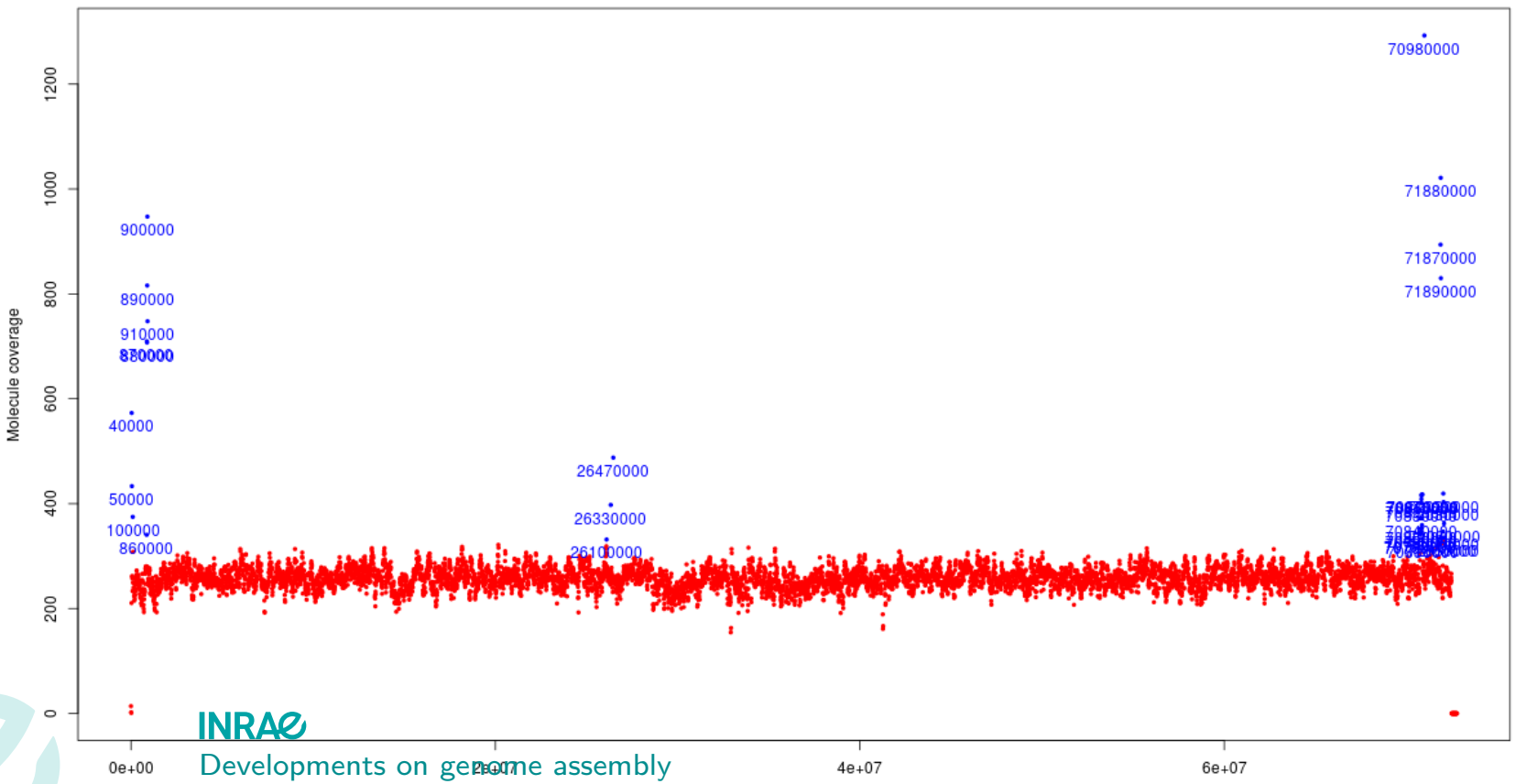


Hi-C heat map (zoom): coverage is not uniform



Split contigs with linked reads

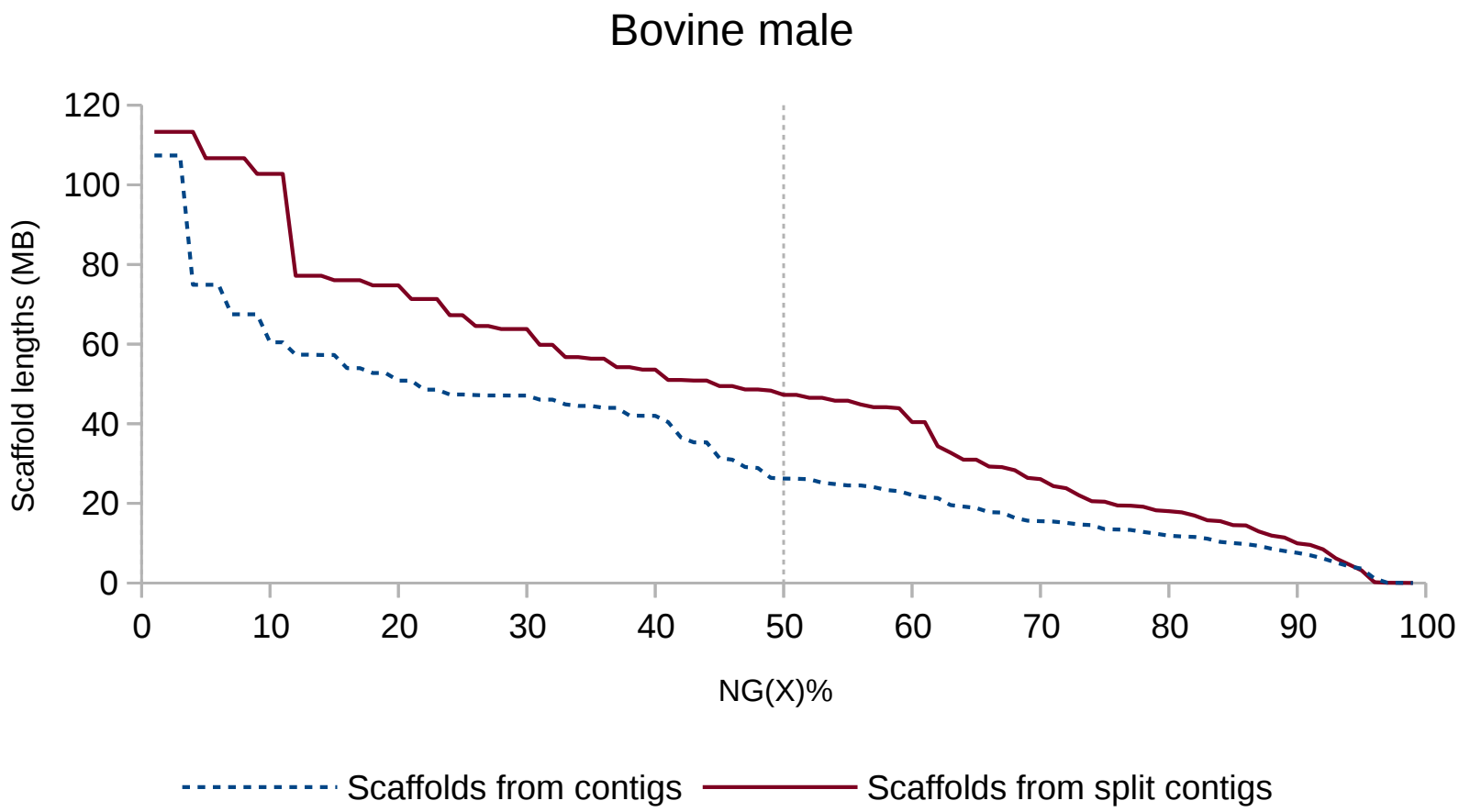




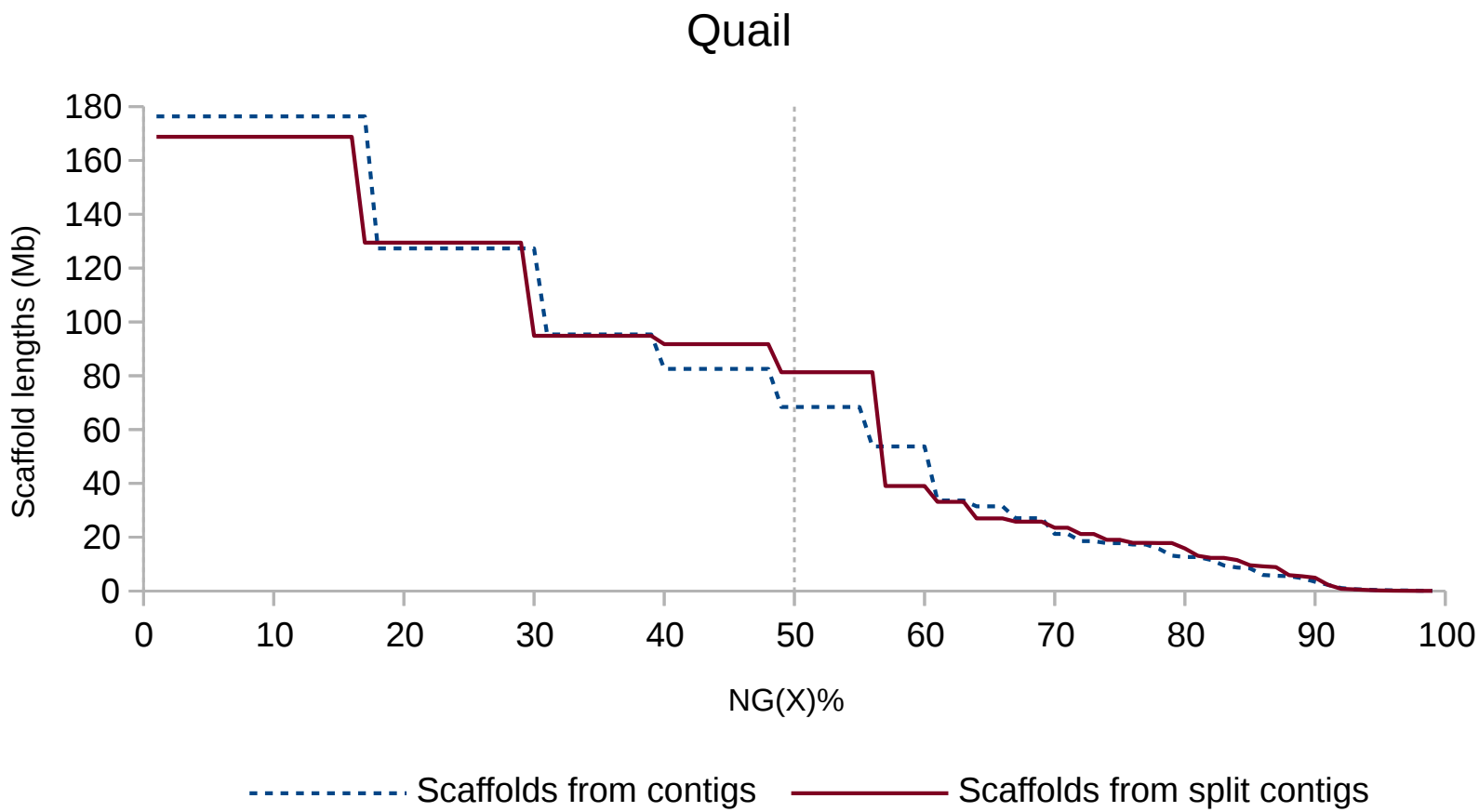
Split contigs with linked reads

- Align linked-reads on contigs
- Identify molecules (barcode, beginning and ending position, number of reads)
- Compute molecule profiles per interval (10kb)
  - Number of starting molecules
  - Number of ending molecules
  - Molecule coverage
  - Mean read density/molecule
  - Mean molecule length
- Identify outliers intervals and split contigs
- Re-connect contigs with Hi-C scaffolding methods

Scaffold split contigs with Hi-C reads



Scaffold split contigs with Hi-C reads



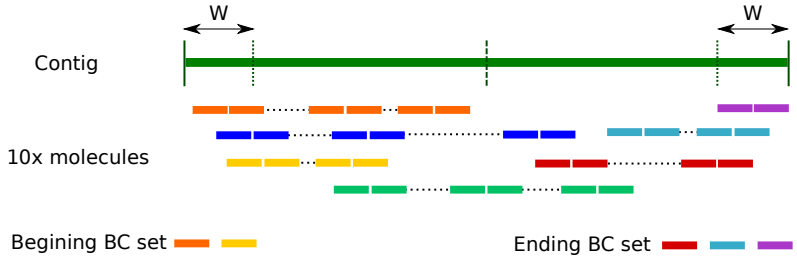
Challenges

- False positive splits
- Contig splits too short for Hi-C scaffolding
- Inversed contigs

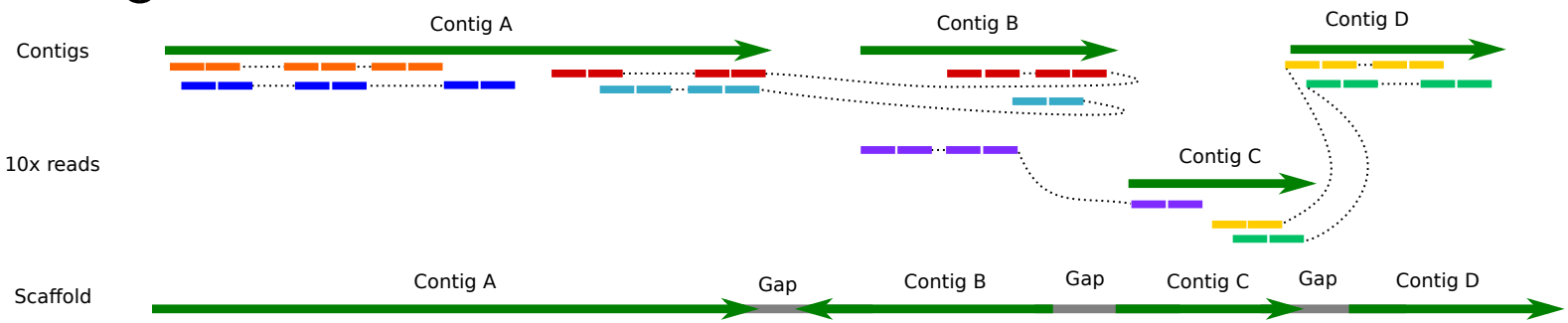
Solution: Scaffold first with linked reads

Scaffolding contigs with linked reads

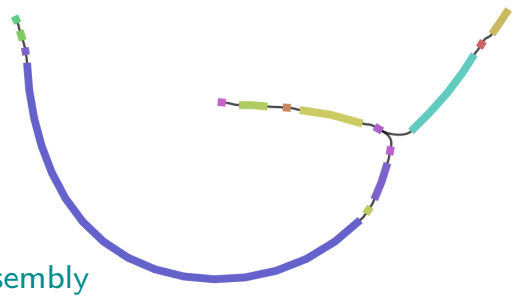
1. For each contig extremity create a representative barcode set



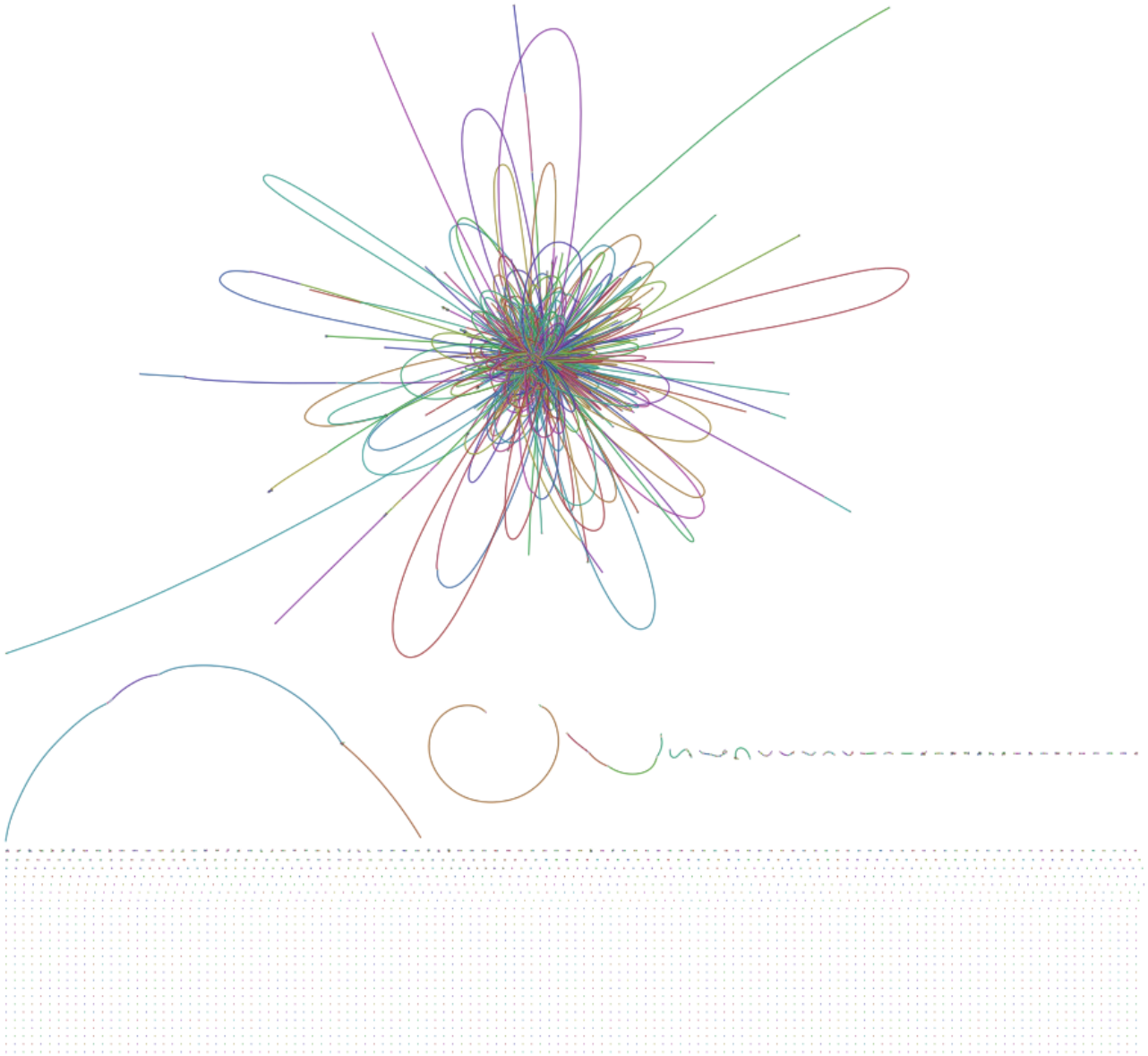
2. Connect two contigs if their representative barcode sets share enough barcodes



3. Scaffold contigs from unbranched paths



Scaffolding graph



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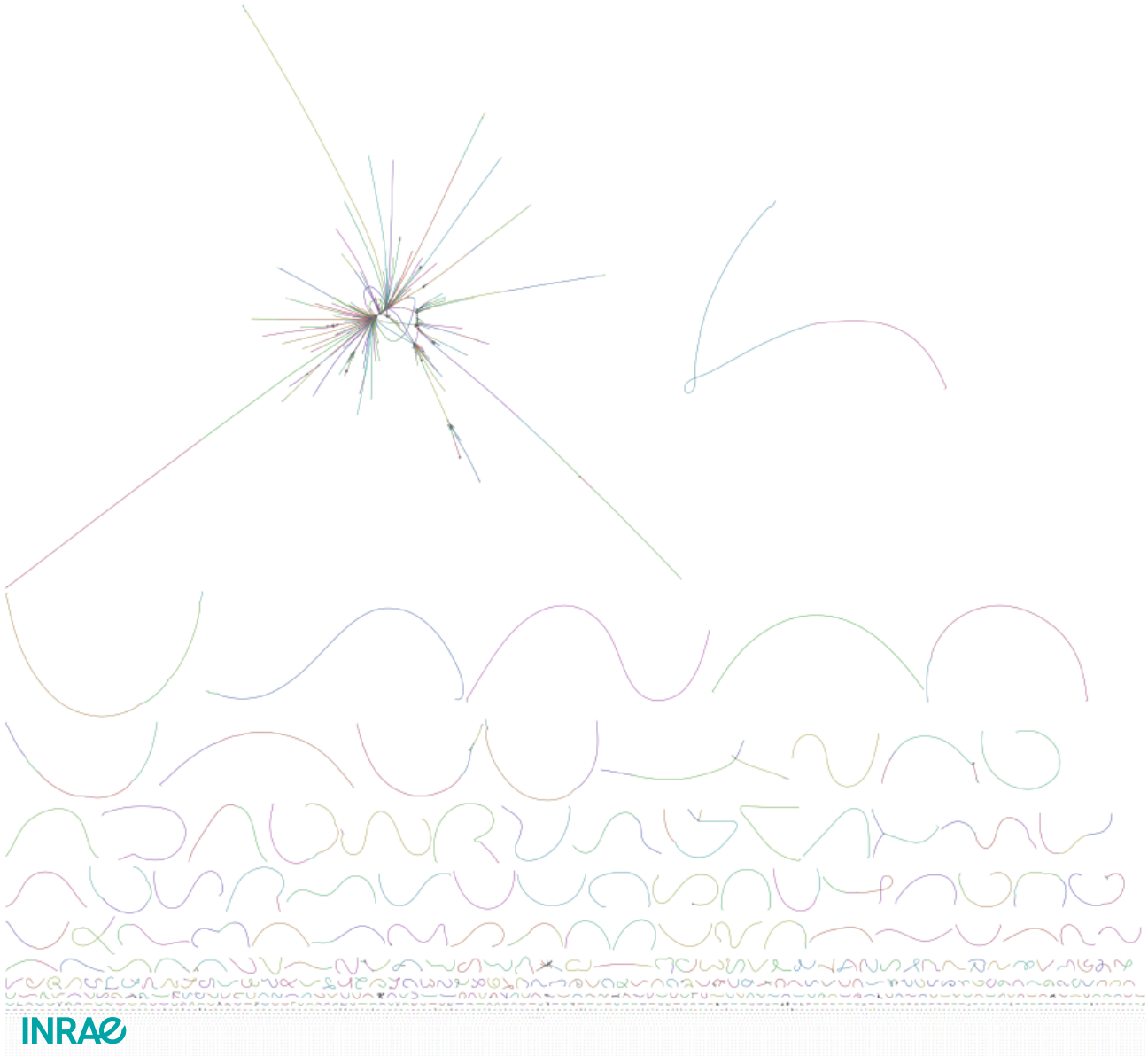
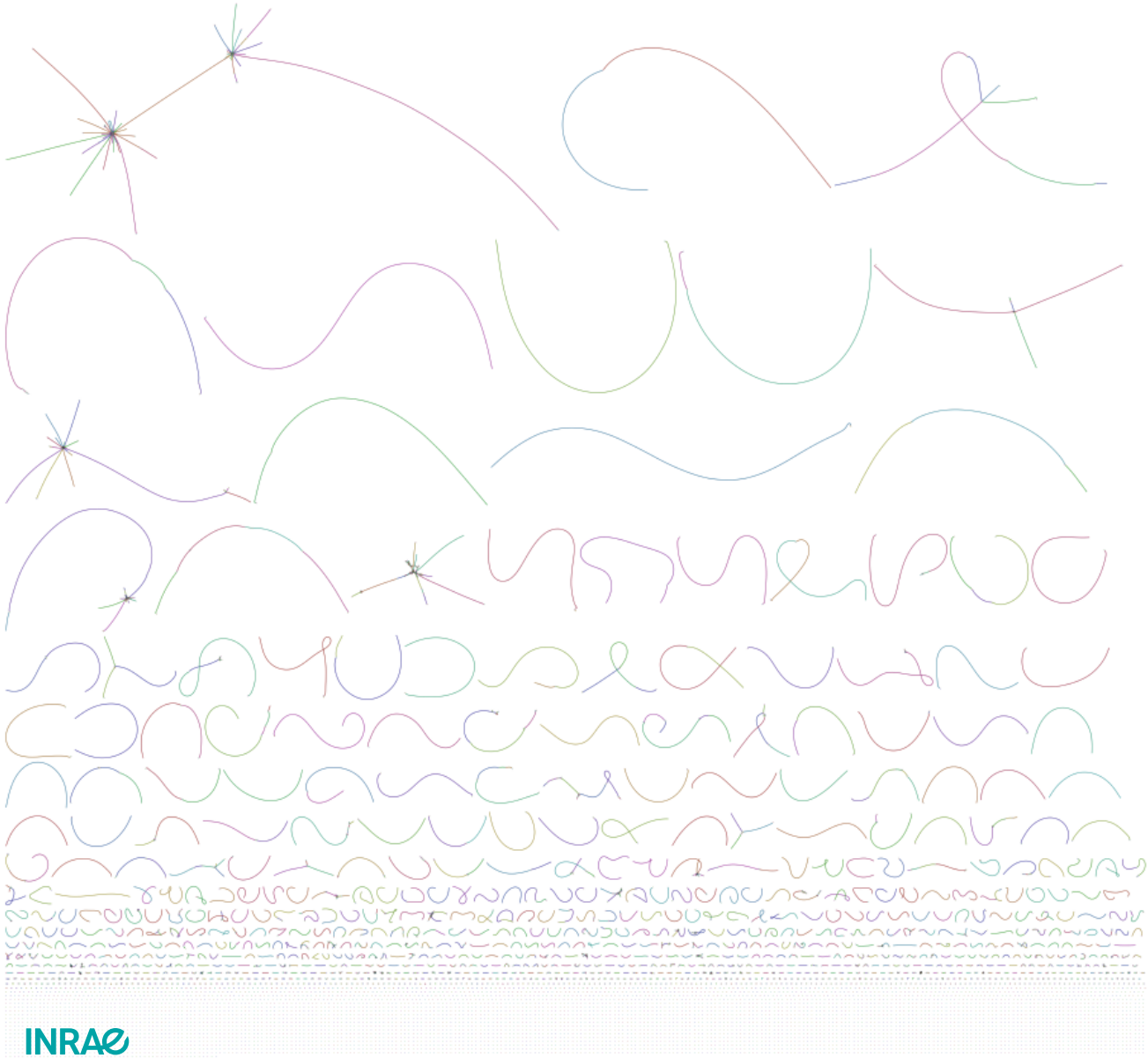
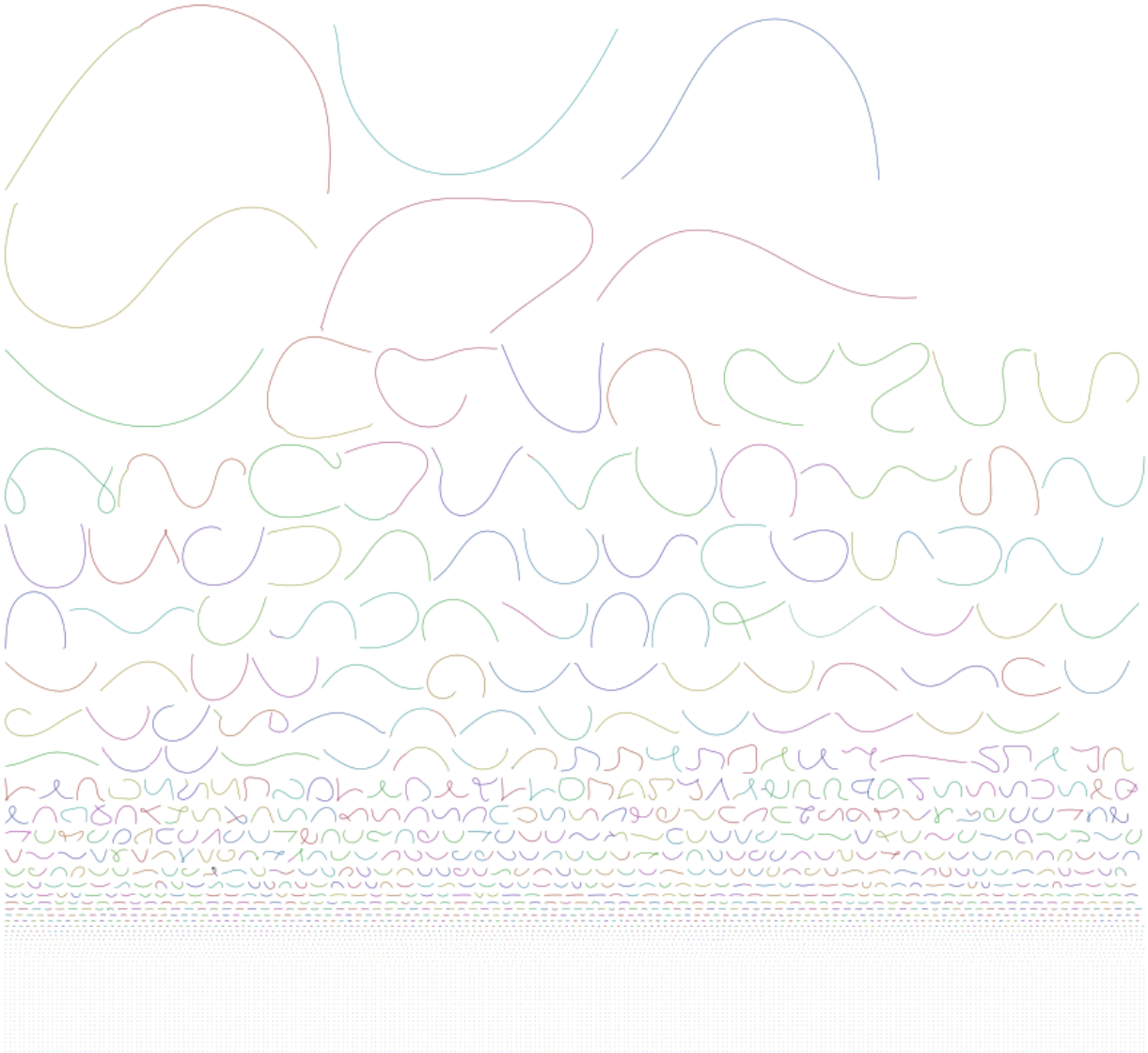


Scaffolding graph



Scaffolding graph



Challenges

- Definition of "enough" shared barcodes for very short contigs
- Read alignment in contig extremity containing repeat sequences
- Risk of introducing new connection errors

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Aim

- Current methods proceed step-wise: short range first, long range last.
- However, some choices made at step n are not consistent with data at step $n + 1$.
- It would be best to integrate the data.
- Including long reads.

Contact map

- We suppose that we "merge" all contigs.
- The contigs are chunked into *bins* of given since (such as 1k).
- A contact map is a symmetric matrix, where each cell (i, j) stores the number of times long-ranged data saw bins i and j together.

Main scaffoling steps

A split

- A split occurs when the contig step joined 2 sequences erroneously.
- They can be detected when counts are low around the diagonal.

A join

- A joins occurs when the contig step failed to join 2 sequences.
- They can be detected when counts are high in a corner.

It is thus crucial to clean noisy data!

Cleaning

Discarding low counts row/cols

- Poisson, negative binomial, logistic regressions does not work.
- Just remove all those lines with count less than mean $-$ 3 standard deviations.

Downsizing high counts row/cols

- Matrix balancing on a such big matrix does not work ($+$ some matrices are limited to the diagonal).
- Decrease the counts on the lines with count greater than mean $-$ 3 standard deviations to the average count.

A key parameter: the "molecule" size

Definition

The max range/size/distance where you expect to see an interaction. Call it m_s.

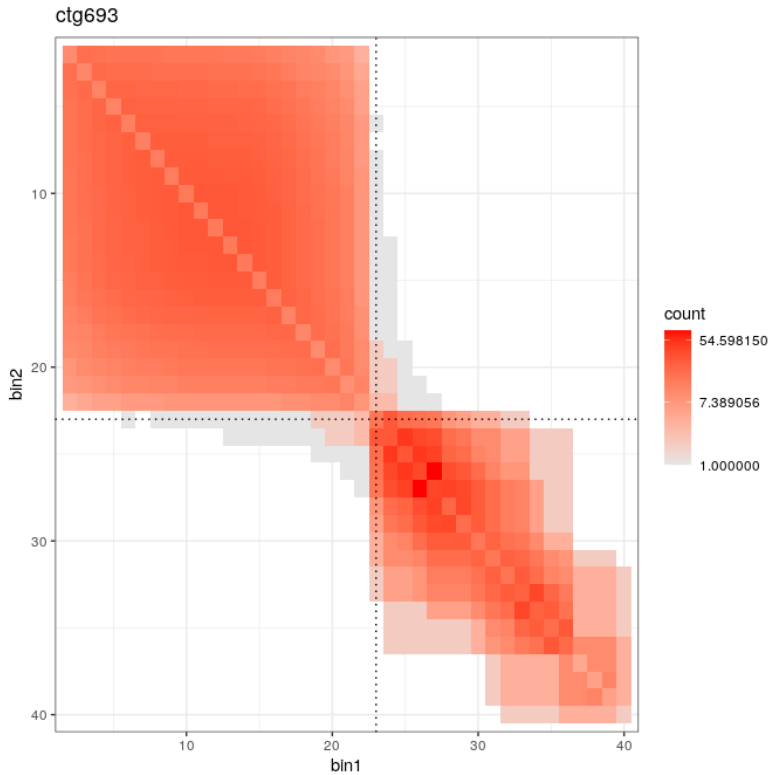
- When looking for splits: the thickness of the diagonal.
- When looking for joins: the size of the corner.
- The name comes from 10X.

The parameter can be estimated from the raw data (for long reads and 10X) or the matrix (for Hi-C).

Splitting: finding hollow triangles

Principle

- In a split at bin i, there should be no interaction between bins $[0, i-1]$ and $[i+1, +\infty]$.
- Since there is no interaction after m_s, you just look for triangular "holes".
- Splits are thus triangles such that the sum of the counts is low.
- Triangles with "too many" missing values are discarded.



Splitting: finding hollow triangles

Accounting for the diagonal strength

- Most of the counts are on the diagonal.
- Summing is more or less 1 point: the diagonal.
- Each point c_{ij} is thus transformed as: $log_2 \frac{c_{ij}}{mean(c_{i'j'} : i'-j'=i-j)}$.

Splitting: finding hollow triangles

Finding a suitable threshold

- The distribution of triangles should be centered around 0.
- Positive triangles are noise, and supposed to be the background distribution.
- A kind of p-value can be given to negative triangles, comparing the negative with the positive distributions.

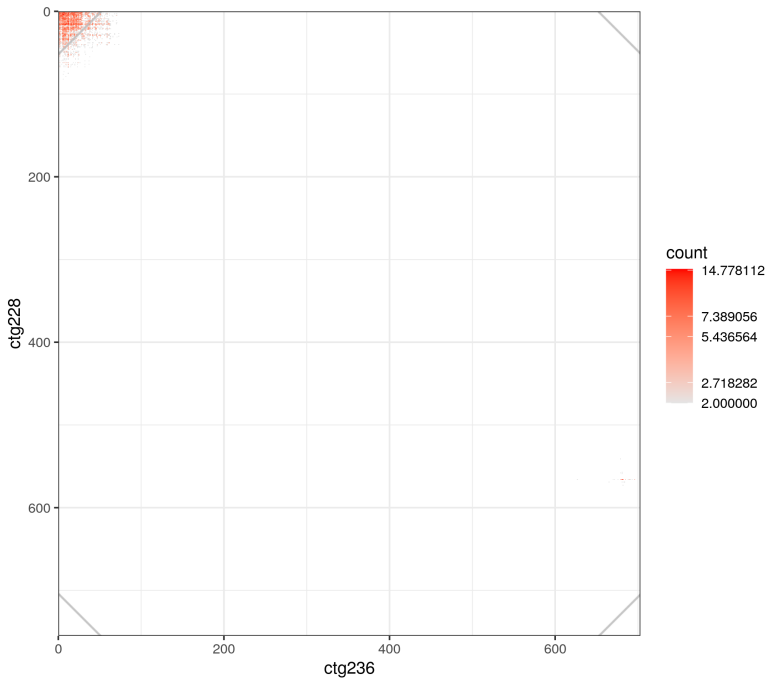
Splitting: merging results

- Splits from one dataset are compared with other datasets.
- If the corresponding triangles are positive, splits are discarded.

Joining: finding full triangles

Principle

- Count distribution of one corners are compared to the count distribution of
  - the other corners,
  - the "interior".
- The min p-value is kept.



Joining: merging results

- Joins are merged, and sorted by p-value.
- Contigs are joined greedily.

Conclusion

- Benchmarking.
- Benchmarking.
- Benchmarking.