Introduction to single-cell genome assembly

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Outline: introduction

- Assembly basics
- Assembly metrics
- Single-cell data specific problems
- Available assemblers
- How SPAdes works
- Sample
- Today's exercise

De novo genome assembly: what every biologist should know **Monya Baker**
Nature Methods **9**, 333–337 (2012) doi:10.1038/nmeth.1935

Assembly puzzle

http://www.scienceinschool.org

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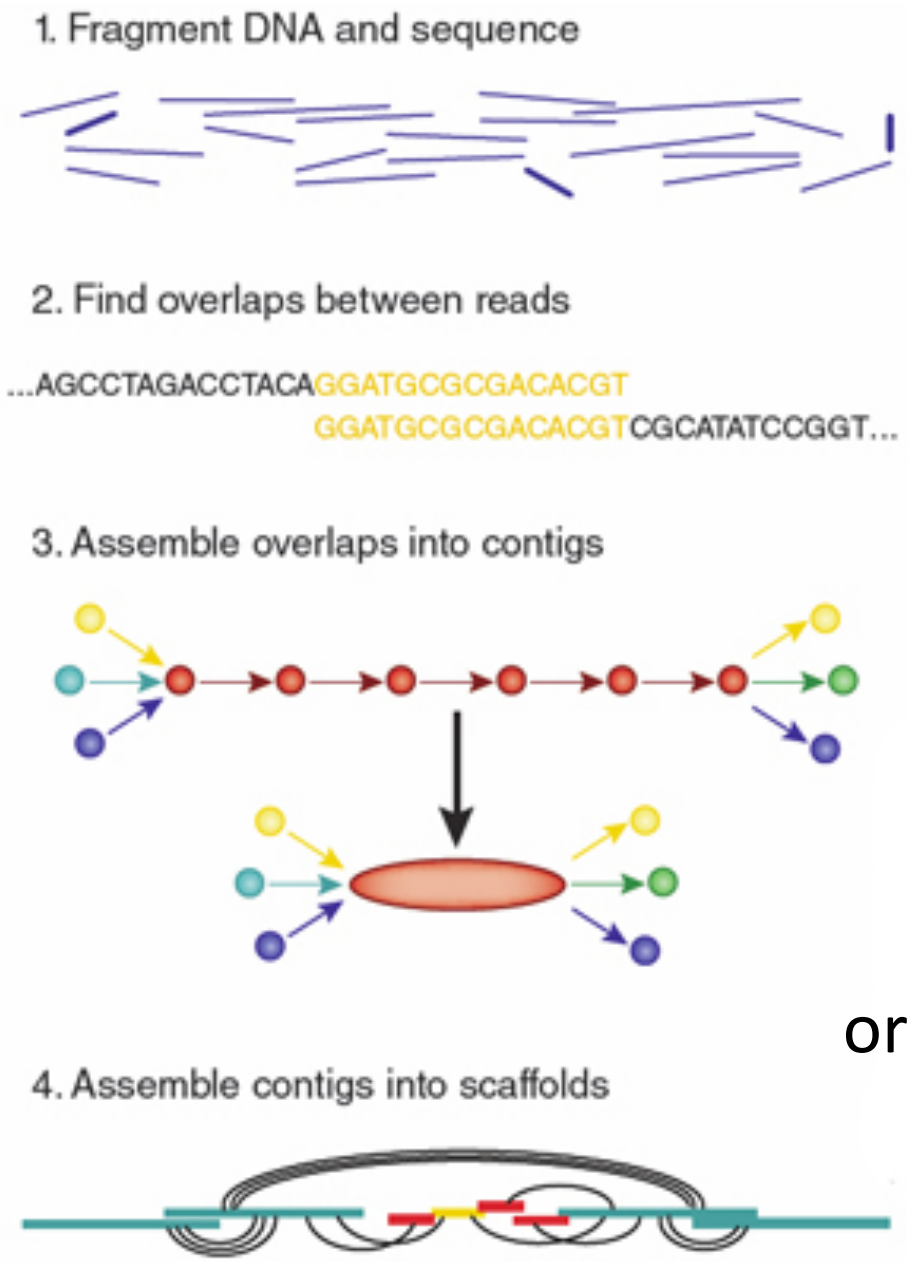
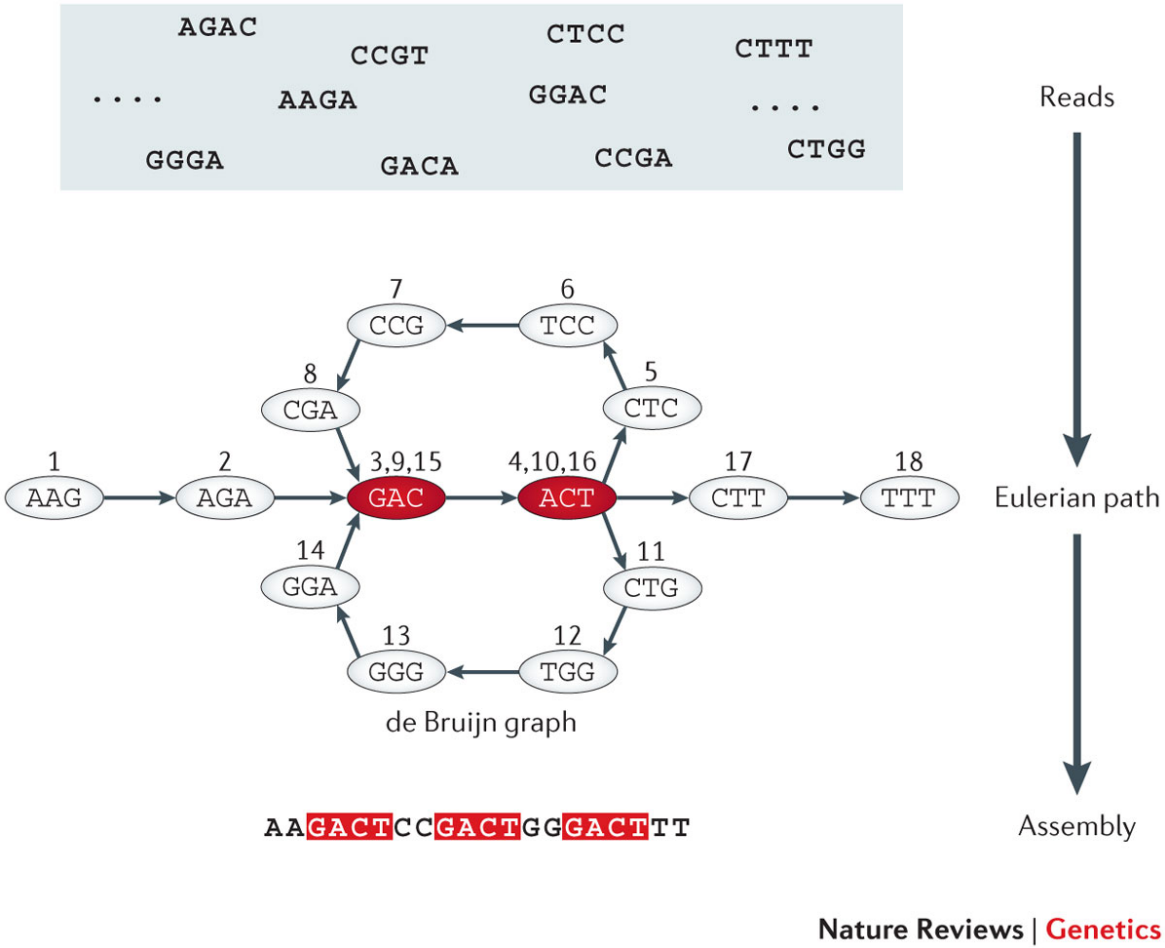


Contigs = continuous sequence

Scaffolds = ordered contigs with gaps

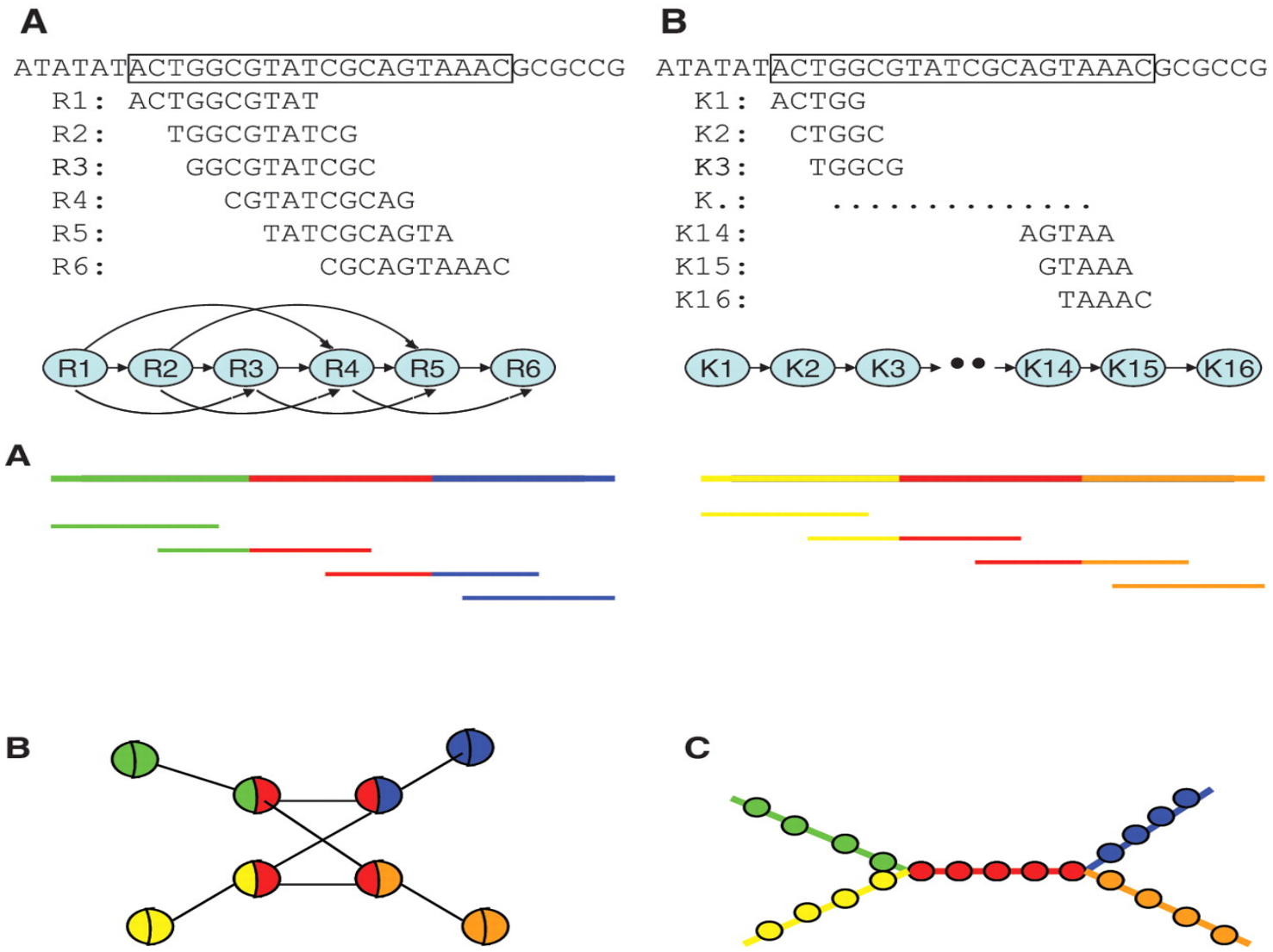
De novo genome assembly: what every biologist should know **Monya Baker** *Nature Methods* **9**, 333–337 (2012) doi:10.1038/nmeth.1935

de Bruijn graph assembly



Nat Rev Genet. 2013 May;14(5):333-46. doi: 10.1038/nrg3433.
Computational solutions for omics data. Berger B1, Peng J, Singh M.

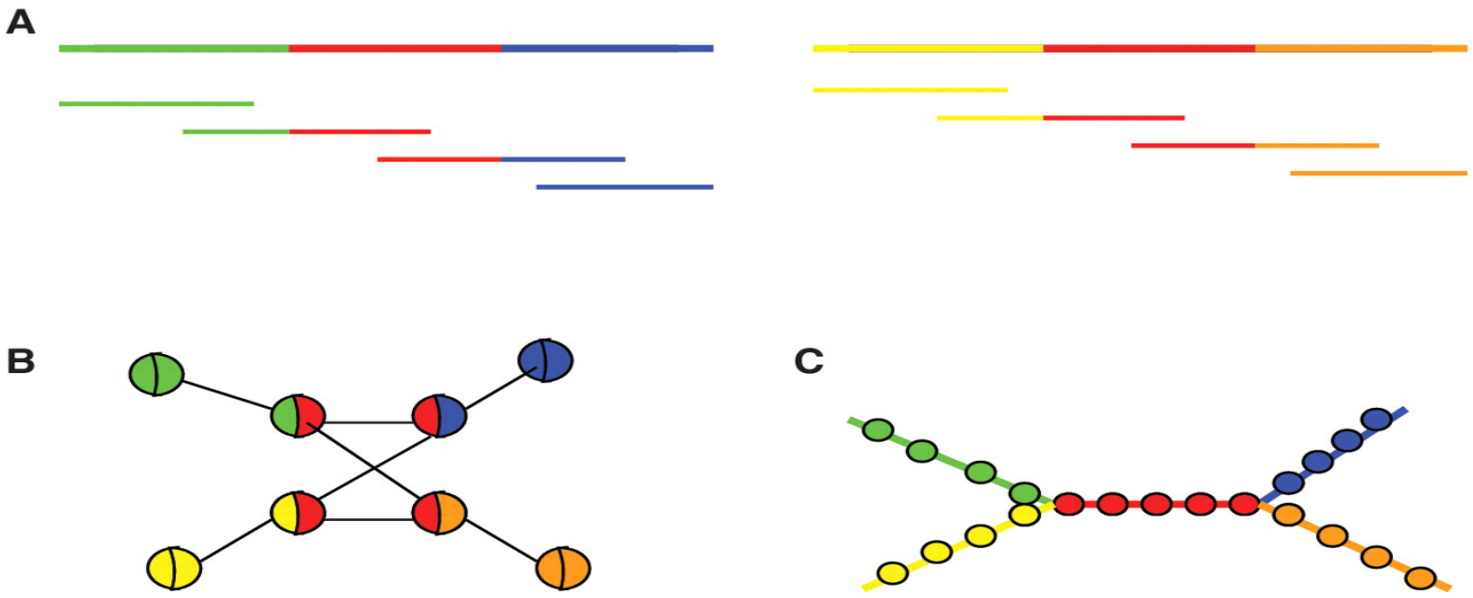
Overlap vs kmer graphs



Slide courtesy of Francesco Vezzi, SciLife Lab

Assembly difficulties

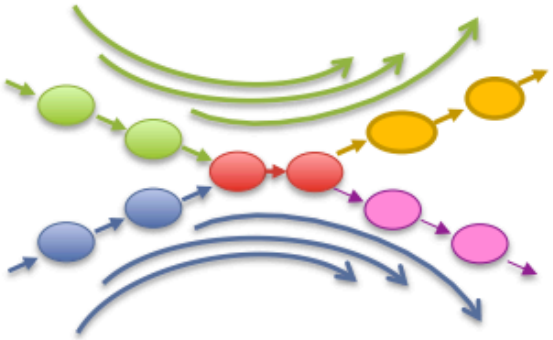
REPEATS



Slide courtesy of Francesco Vezzi, SciLife Lab

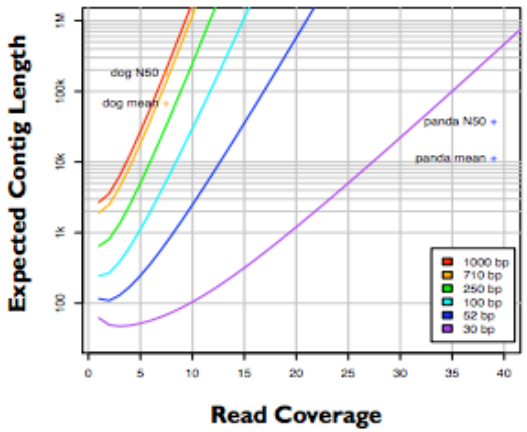
Ingredients for a good assembly

Read Length



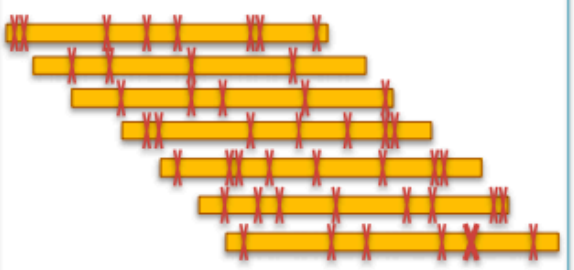
Reads & mates must be longer than the repeats

Coverage



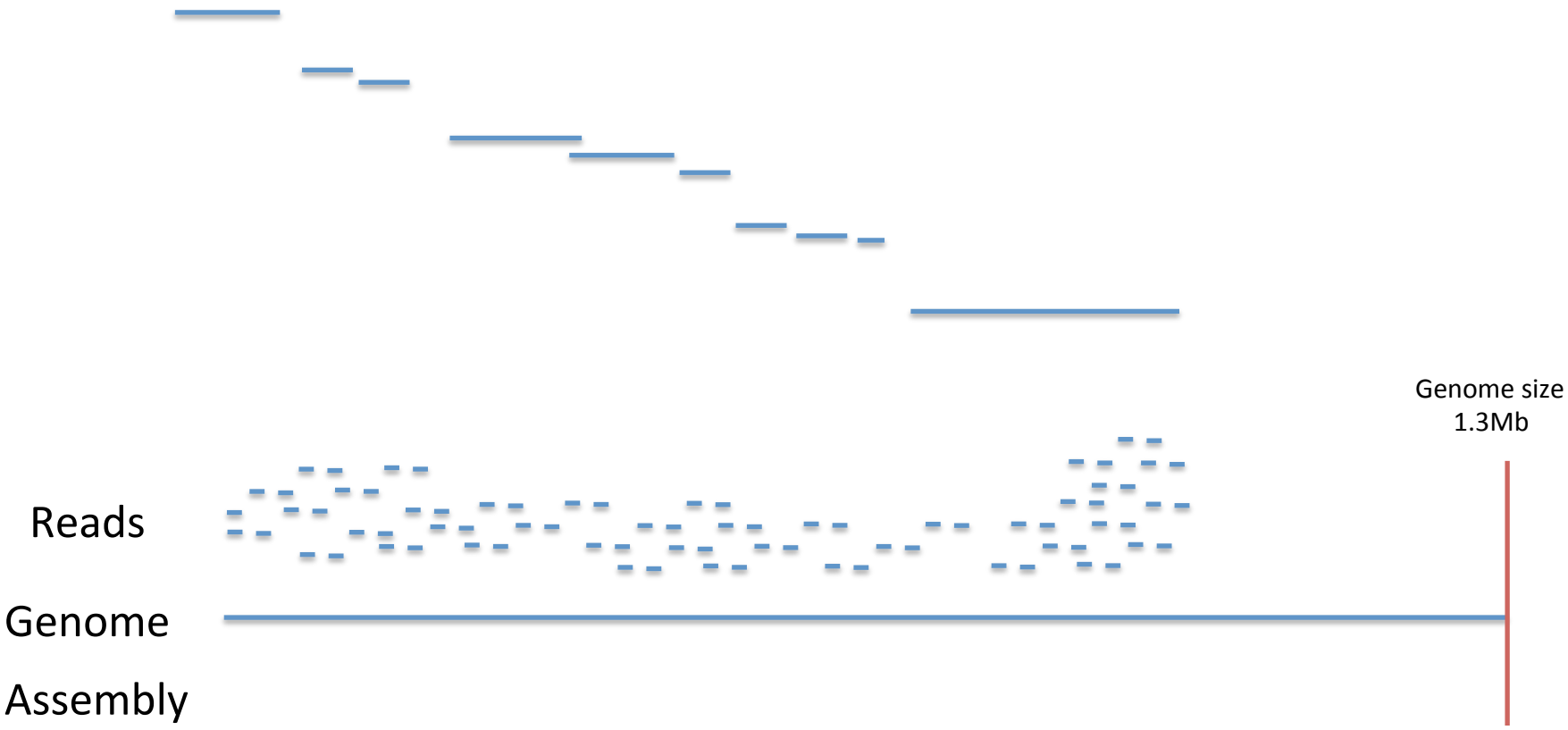
High coverage is required

Quality



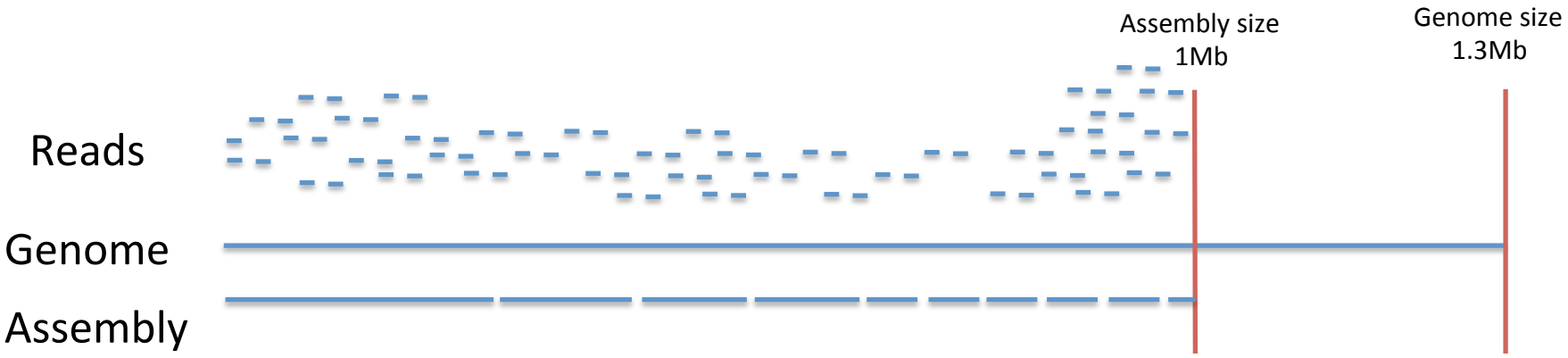
Errors obscure overlaps

Slide courtesy of Francesco Vezzi, SciLife Lab


Genome size
1.3Mb
Reads
Genome
Assembly

Assembly metrics

- assembly size
- number of contigs, largest contig
- N50



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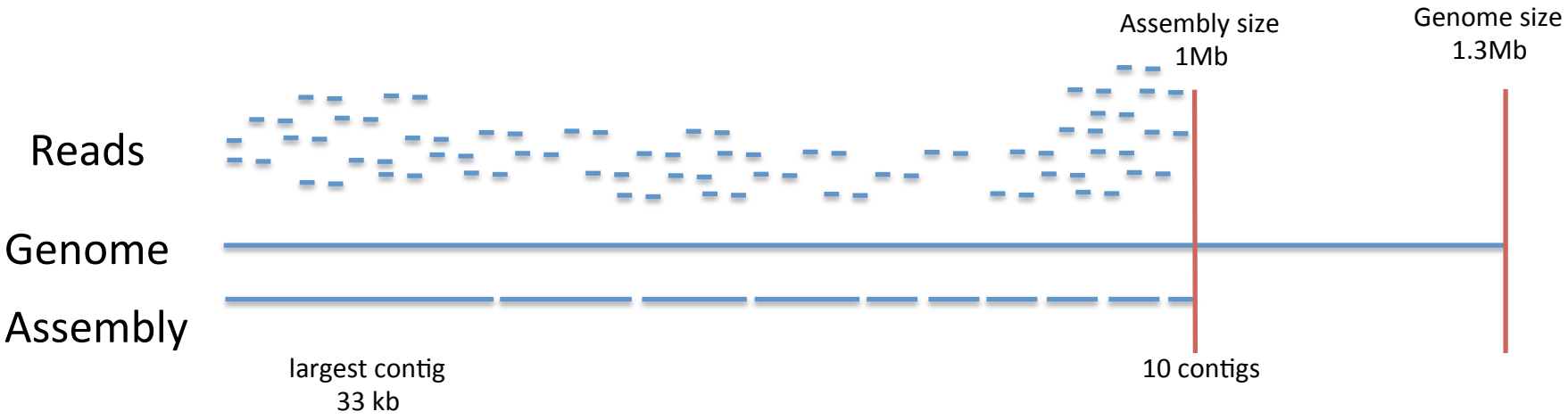

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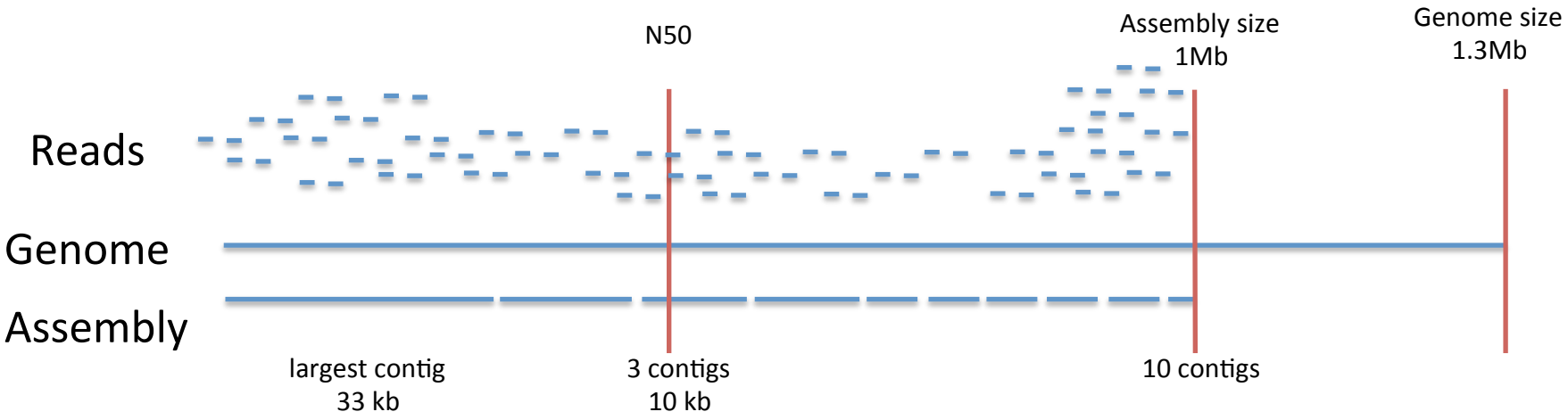

Assembly metrics

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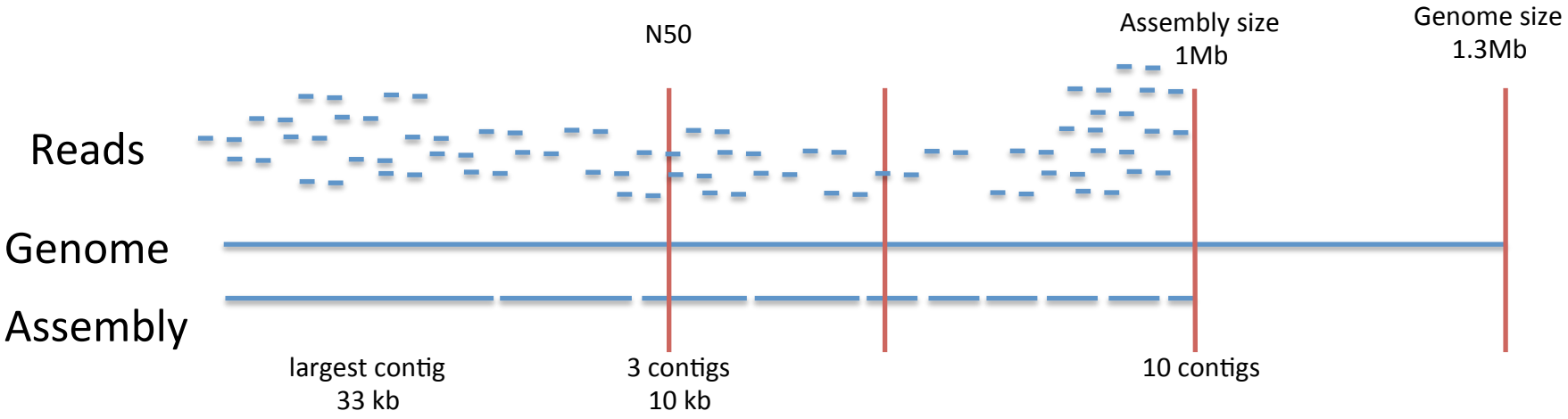


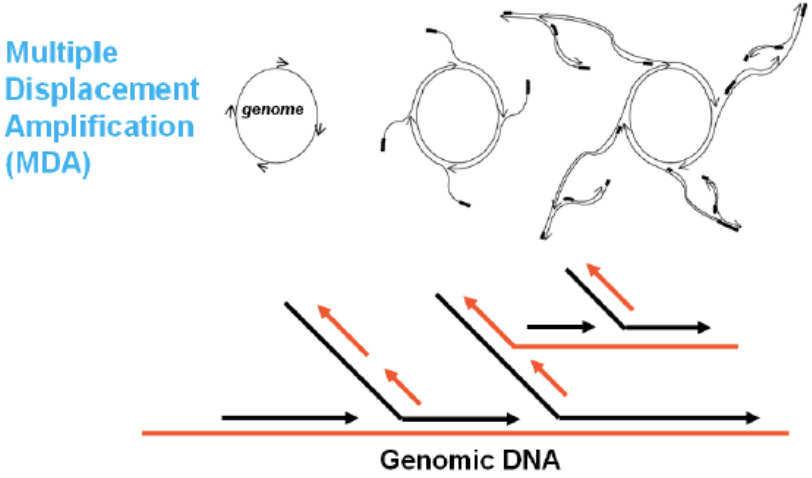
Outline: single cell assemblies

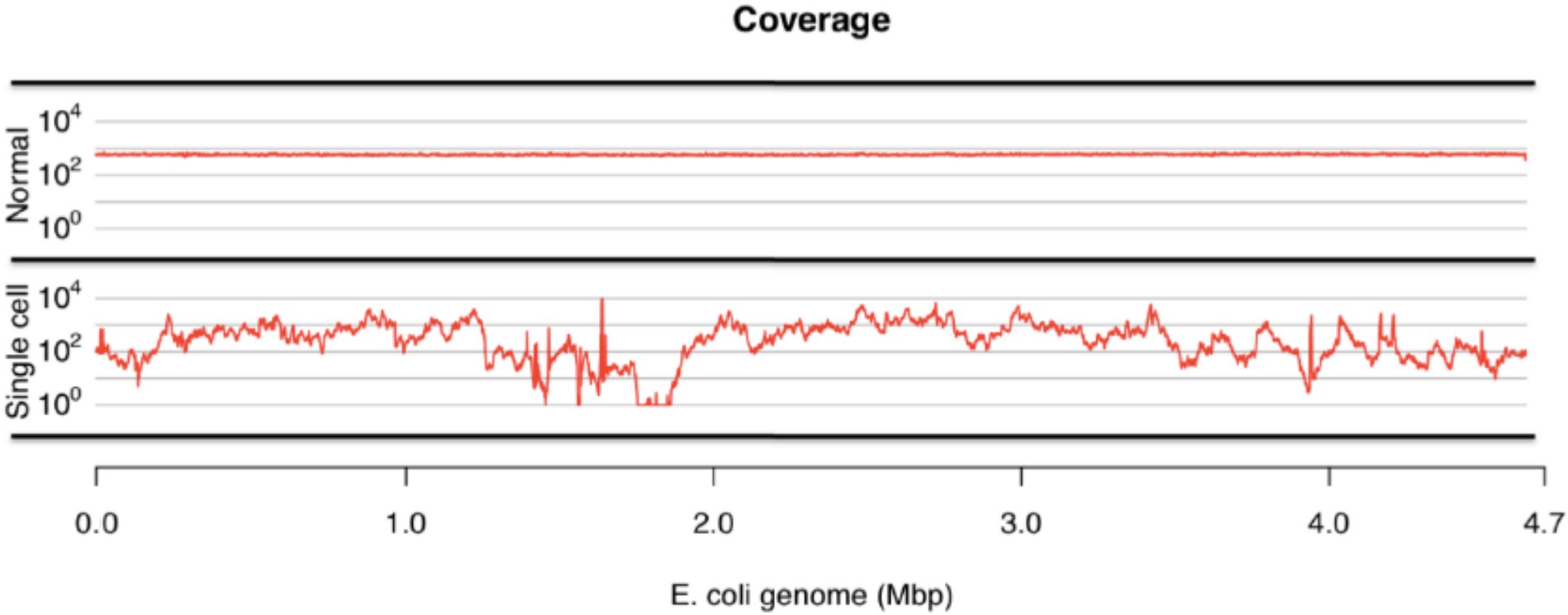
- Assembly basics
- Assembly metrics
- Single-cell data specific problems
- Available assemblers
- How SPAdes works
- Sample
- Today's exercise

Problems with single-cell data

MDA artefacts

- Chimeras
- Uneven coverage





How does this affect assembly?

- de Bruijn graph sensitive to k-mer quality
- Bad quality k-mers from low-coverage regions
  - Erroneous graph connections → misassemblies
  - Or gaps due to removal of low-coverage areas
- Specialized single-cell genome assemblers are needed

Single-cell genome assemblers available currently

- E+V-SC (Euler+Velvet-SC) (2011)
  - Euler and Velvet modification
  - Not for pairs
  - single k-mer
- IDBA-UD (2012)
  - Error correction
  - Multiple k-mers
  - paired-end reads
- SPAdes (2012)
  - Error correction
  - Multiple k-mers
  - paired-end reads
  - Also tries to solve chimera problems

Why use SPAdes?

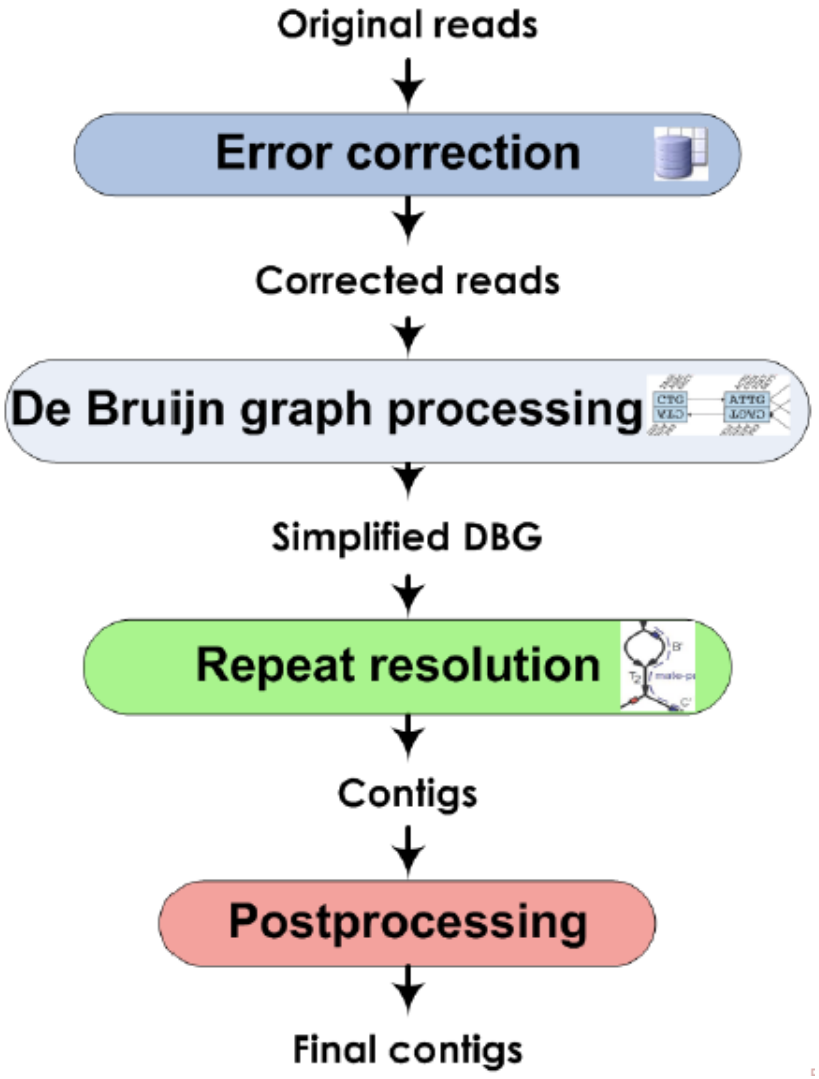
(better assembly results)

Assembly	NG50	# of contigs	Largest contig	Total length	Misassemled contigs	mismatch (bp per 100kbp)	indels (bp per 100kbp)	Mapped genome (%)	# genes
A5	14399	745	101584	4441145	8	12.01	0.17	89.88	3444
ABySS	68534	179	178720	4345617	6	3.32	1.68	88.268	3704
CLC	32506	503	113285	4656964	2	5.53	1.42	92.291	3768
EULER-SR	26662	429	140518	4248713	17	10.87	35.67	84.898	3416
Ray	45448	361	210820	4379139	17	6.29	2.83	88.372	3636
SOAPdenovo	1540	1166	51517	2958144	1	1.87	0.11	57.672	1766
Velvet	22648	261	132865	3501984	2	2.19	1.23	73.765	3080
E+V-SC	**32051**	**344**	**132865**	**4540286**	**2**	**2.33**	**0.73**	**91.744**	**3771**
IDBA-UD contigs	**98306**	**244**	**284464**	**4814043**	**8**	**5.09**	**0.27**	**95.21**	**4045**
IDBA-UD scaffolds	**109057**	**229**	**284464**	**4813609**	**8**	**5.14**	**0.77**	**95.199**	**4052**
SPAdes3.1 contigs	**109059**	**238**	**268493**	**4797090**	**1**	**3.29**	**0.45**	**94.936**	**4036**
SPAdes1.1 scaffolds	**110081**	**233**	**268493**	**4799481**	**1**	**4.02**	**0.64**	**94.959**	**4041**

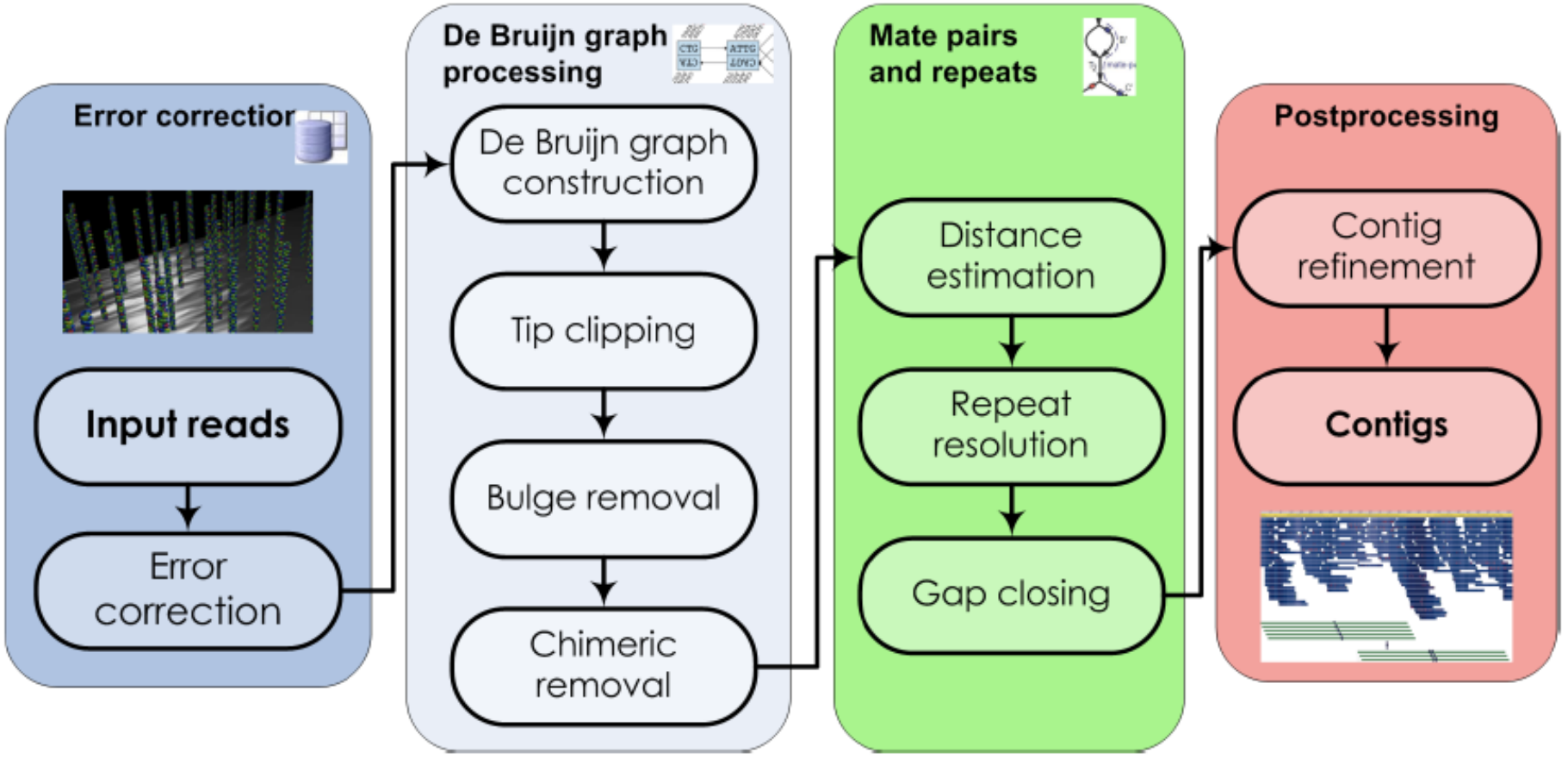
Using *E. coli* single-cell

How does SPAdes achieve this?

- Error correction of reads before assembly
  - *Uses novel algorithm: BayesHammer*
  - *This reduces erroneous k-mers that could mess up assembly*
- Use of multiple k-mers to construct assembly graph
  - *Improved resolution of assembly graphs*
- Uses mate pairs to improve de Bruijn graph construction
  - *Paired de Bruijn graphs ("Rectangle Graphs")*
    - *helps to resolve repeats*
    - *Helps with contig scaffolding*
- Removal of chimeric connections in graph
  - *Less mis-assemblies in the contigs*
- Final correction of errors in contigs (using bwa)
  - *Improved contig quality*
- All these steps in a single command
  - *Other tools need multiple tools to do same procedures*



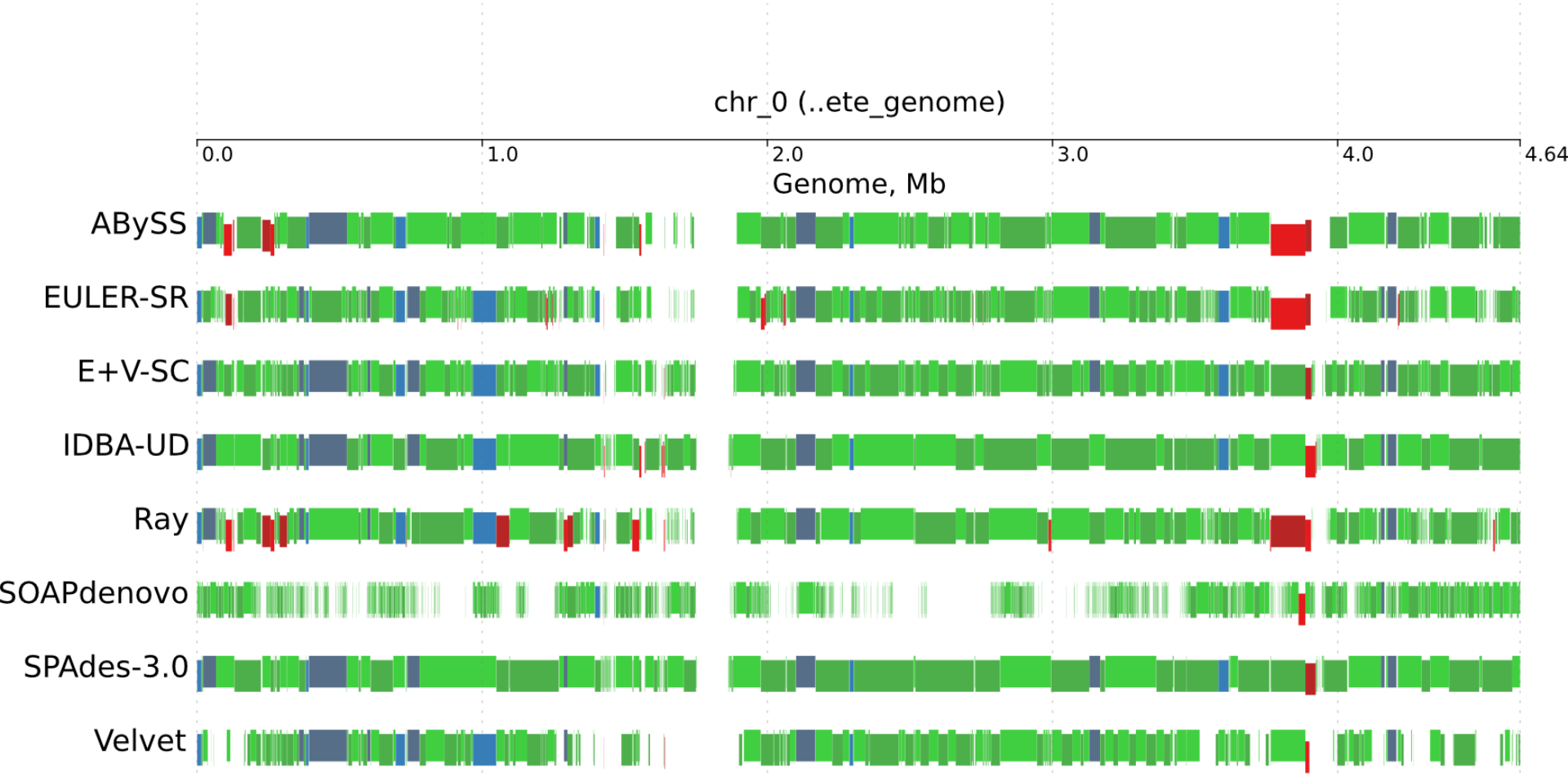
More details of each step



A few things to consider when using SPAdes

- SPAdes currently only works on Illumina data
  - Other NGS data won't work
- HiSeq data
  - 100-150 bp paired end reads
    - Shorter k-mers
    - Faster assembly
- MiSeq data
  - 250-300 bp paired end reads (longer)
    - Larger k-mers
      - assembly takes longer if smaller k-mers are used
    - User may need to optimize k-mer selection to produce optimal assembly
- In general, it works better with short, high quality reads
- Can also be used for multi-cell genomic data

Why use SPAdes?
(better genome coverage)



Acknowledgements

- Jimmy Saw (single cell analysis)
- Anders Lind (Coverage/chimera checks)
- Joran Martijn (MEGAN analysis)
- Lionel Guy (Genome completeness estimates)

Outline: practical part

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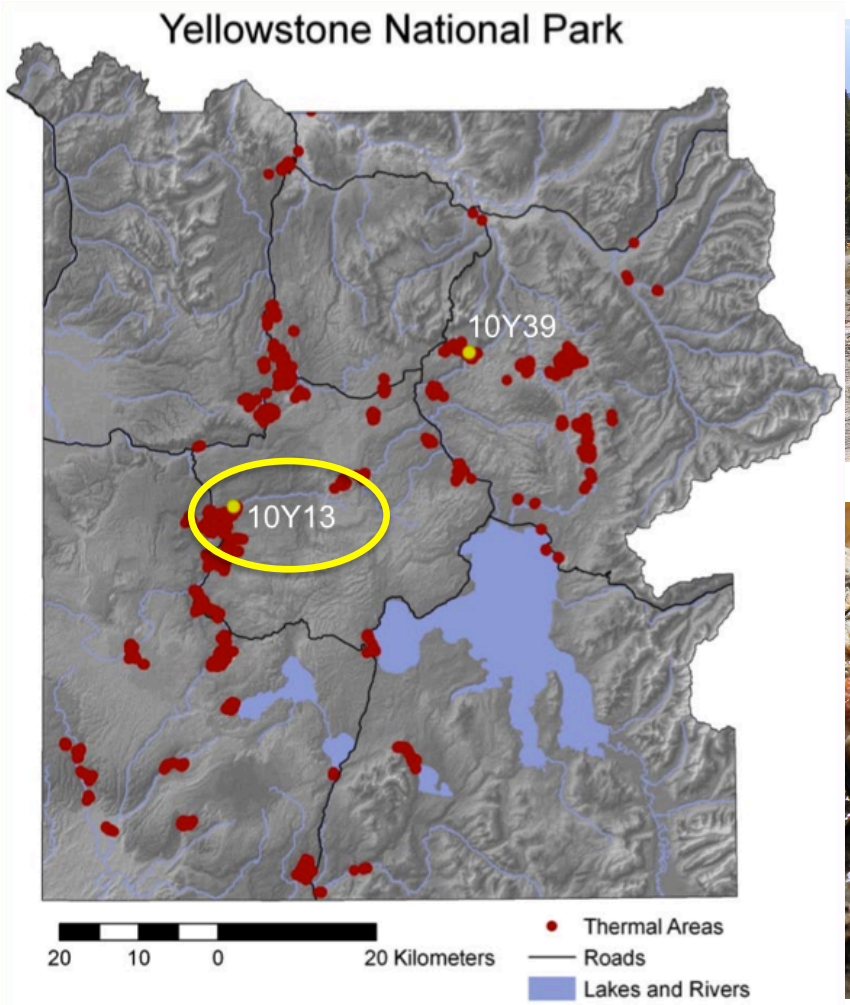
Sample



Culex Basin
pH 8.6, T=68.8°C

Images on courtesy of Cristina Takacs-Vesbach and Dan Coleman

Datasets to be used

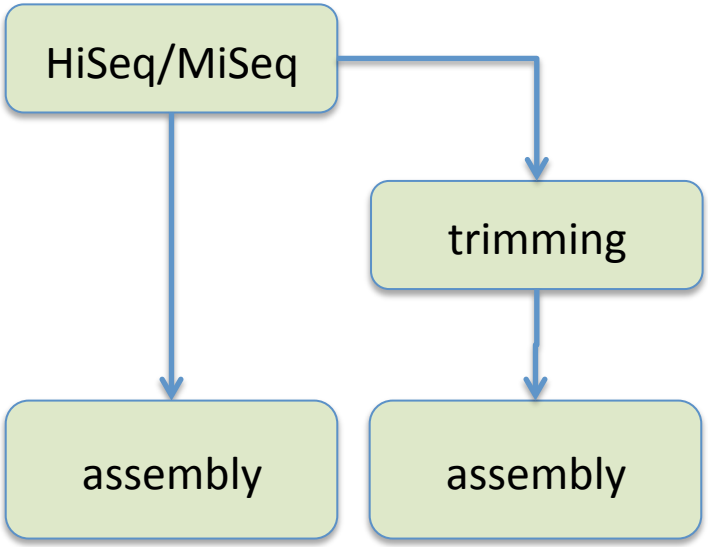
From same SAG

- **Dataset1**
  - Paired end **HiSeq** data for **G5**
  - G5_Hiseq_R1_001.fastq
  - G5_Hiseq_R2_001.fastq
- **Dataset2**
  - Paired end **MiSeq** data for **G5**
  - G5_Miseq_R1_001.fastq
  - G5_Miseq_R2_001.fastq

12 assemblies per group

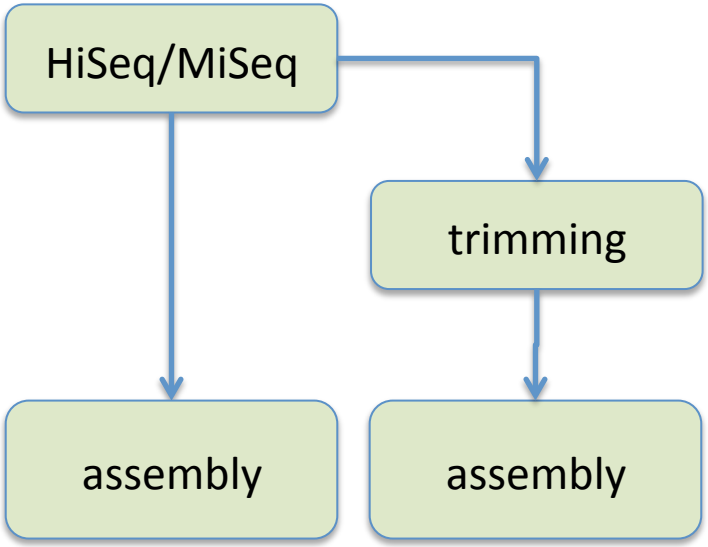
- **6 assemblies**
  - 3 assemblies with original data
  - 3 assemblies with trimmed data
- **6 assemblies**
  - 3 assemblies with original data
  - 3 assemblies with trimmed data

Overview of exercises today



1. General instructions
2. Familiarizing with data (QC)
3. Single-cell genome assemblies using SPAdes (HiSeq data)

Overview of exercises today



1. General instructions
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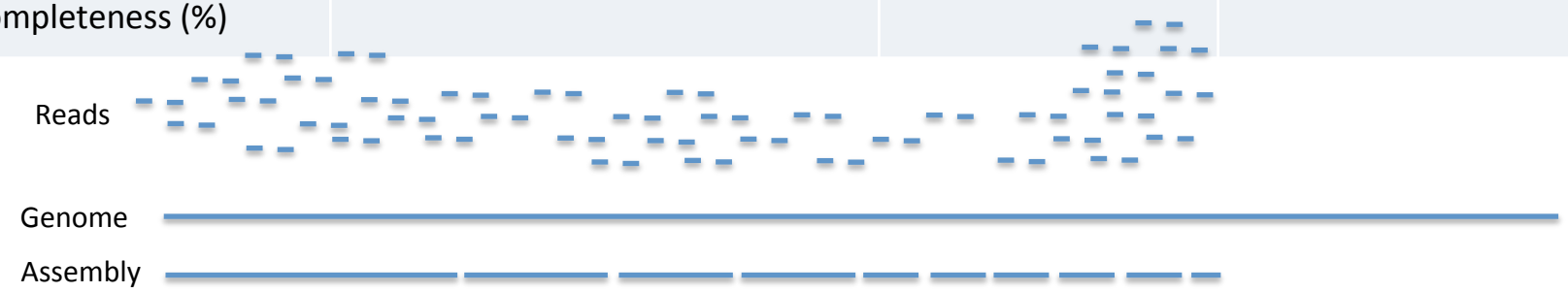
3 programs:

- Spades
- IDBA-UD
- Ray

Exercise: compare assemblies

Table 1	HiSeq data (Original data)		
	Spades	IDBA-UD	Ray
Number of reads			
Assembly time			
Number of contigs			
Total assembly size			
Largest contig			
N50			
G+C%			
Number of ORFs			
Completeness (%)			



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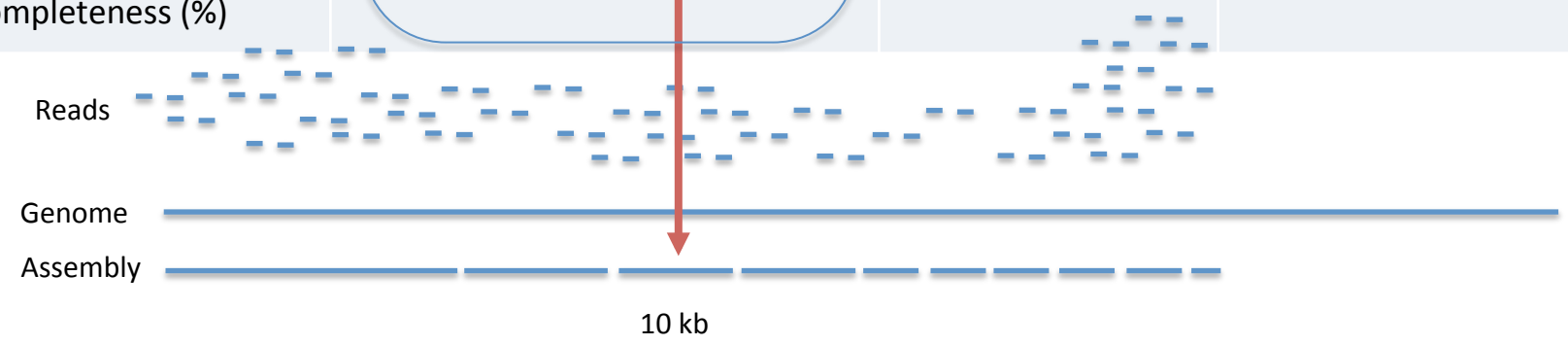

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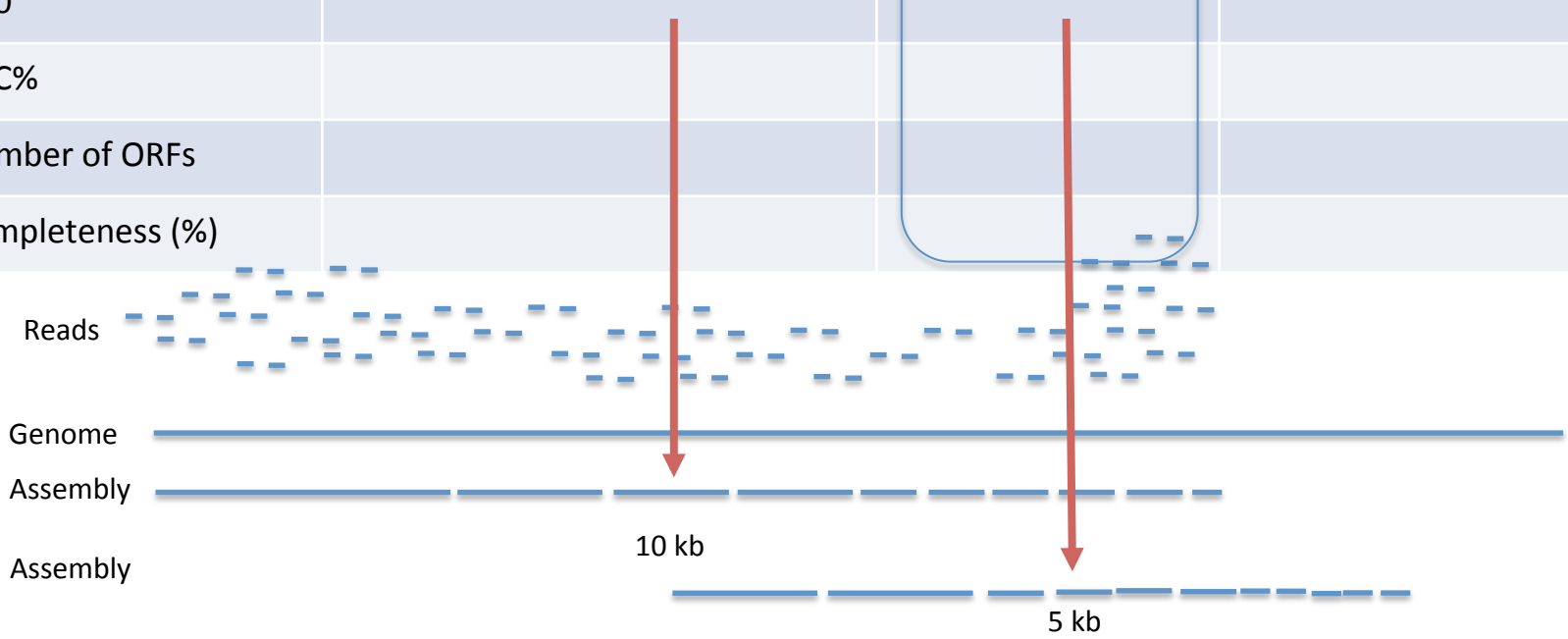
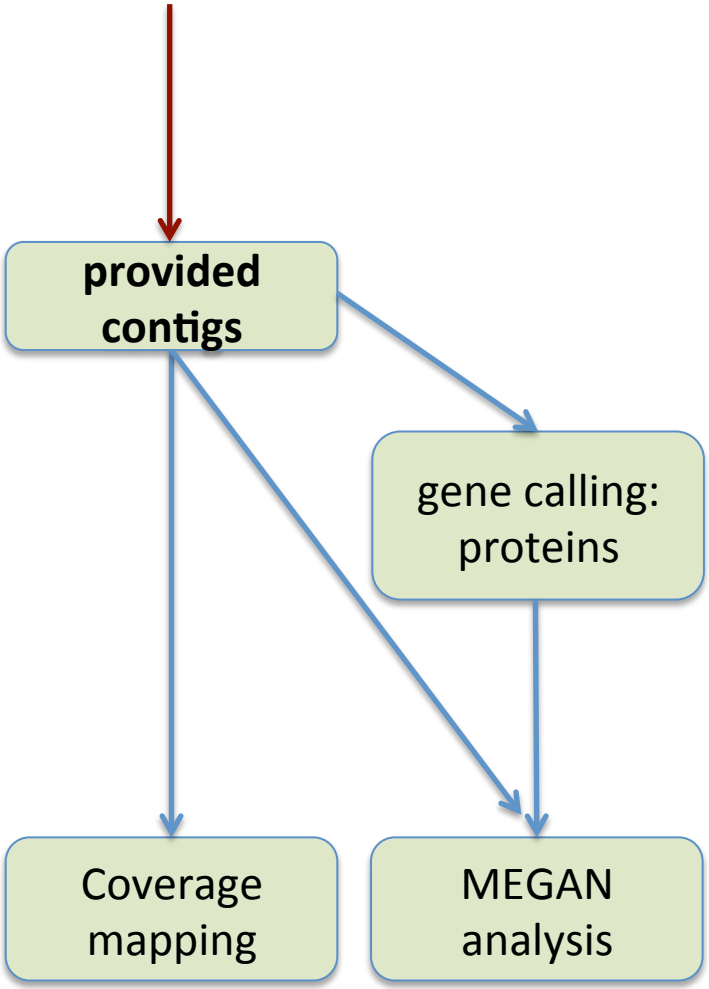


Overview of exercises today



1. General instructions
2. Familiarizing with data (QC)
3. Single-cell genome assemblies using SPAdes (HiSeq data)

PROVIDED CONTIGS

4. Assessing read coverage and chimera checking (with Artemis)
5. Checking for contaminants (with MEGAN)

Datasets to be used

From same SAG

- **Dataset1**
  - Paired end **HiSeq** data for **G5**
  - G5_Hiseq_R1_001.fastq
  - G5_Hiseq_R2_001.fastq
- **Dataset2**
  - Paired end **MiSeq** data for **G5**
  - G5_Miseq_R1_001.fastq
  - G5_Miseq_R2_001.fastq

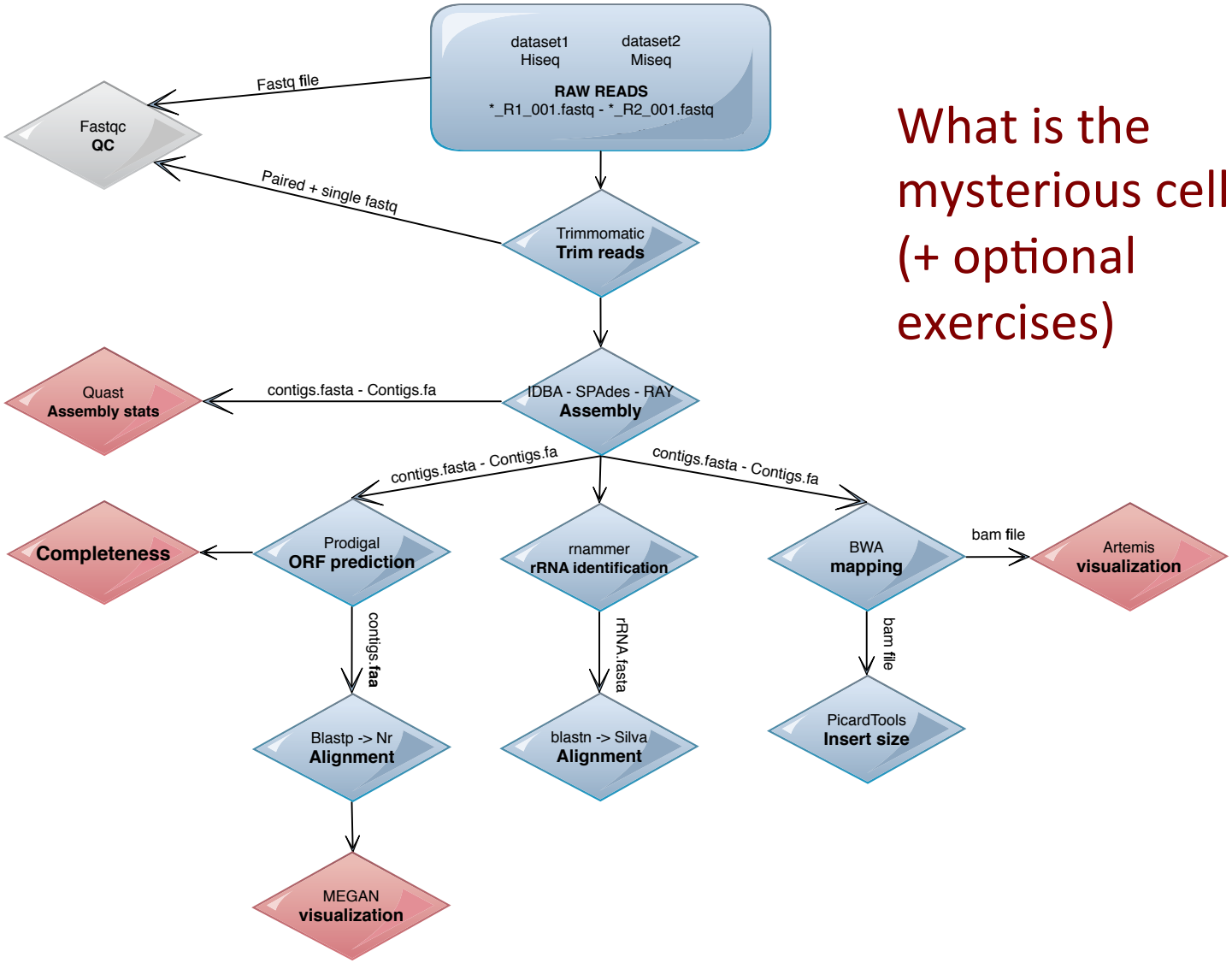
Mysterious SAG

- **Dataset3**
  - Paired end MiSeq data for **N21**
  - N21_Miseq_1.fastq
  - N21_Miseq_2.fastq

12 assemblies per group

- **6 assemblies**
  - 3 assemblies with original data
  - 3 assemblies with trimmed data
- **6 assemblies**
  - 3 assemblies with original data
  - 3 assemblies with trimmed data
- **choose assembly yourself**
  - Use same settings as before
  - Try optimizing assembly (program, kmer, flags, …)

Overview of exercises today



What is the mysterious cell? (+ optional exercises)

Organization into groups

- Groups
  - Put your names in google doc
  - Decide who does which assembly
- Morning session
  - Playing with the data individually (familiarize)
  - Each person runs 3 assemblies (total 12 per group)
- Afternoon session (individually or in groups/pairs)
  - Coverage and chimera checking analyses
  - MEGAN analysis
  - Choose the steps to find out what the mysterious SAG is
  - Choose optional exercises if you have time