# RADseq: from tissue to data

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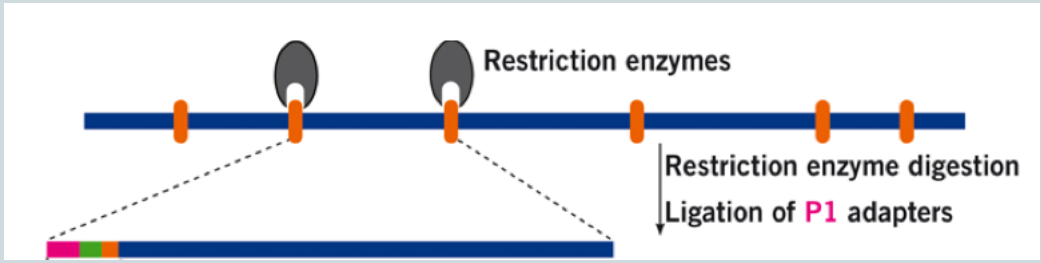
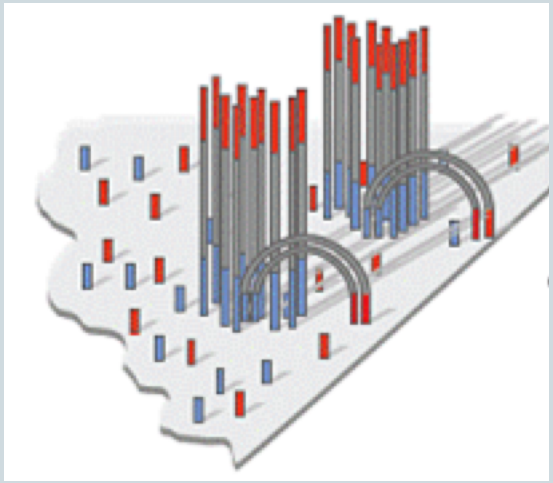
JOSH VAN BUSKIRK

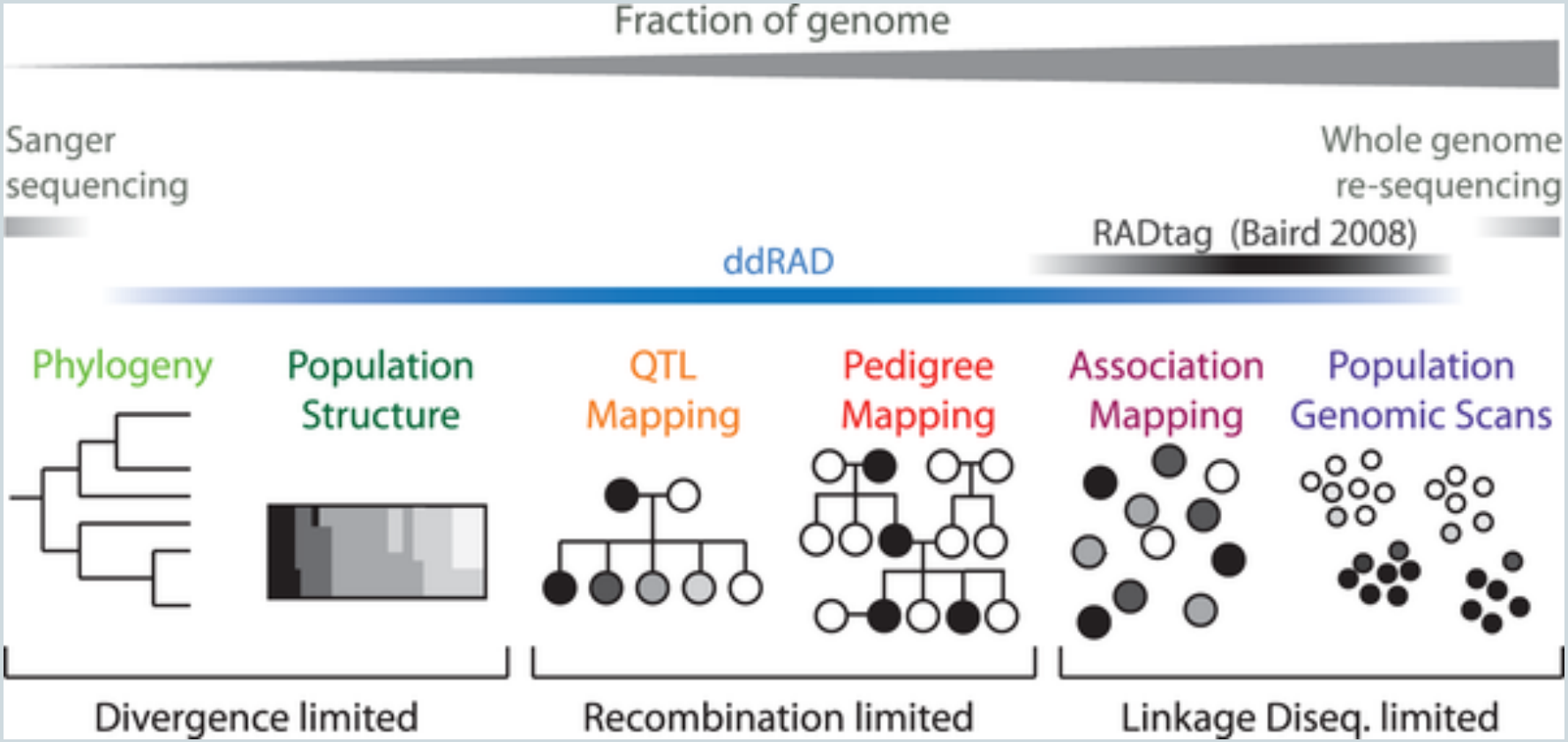
# What is RADseq?



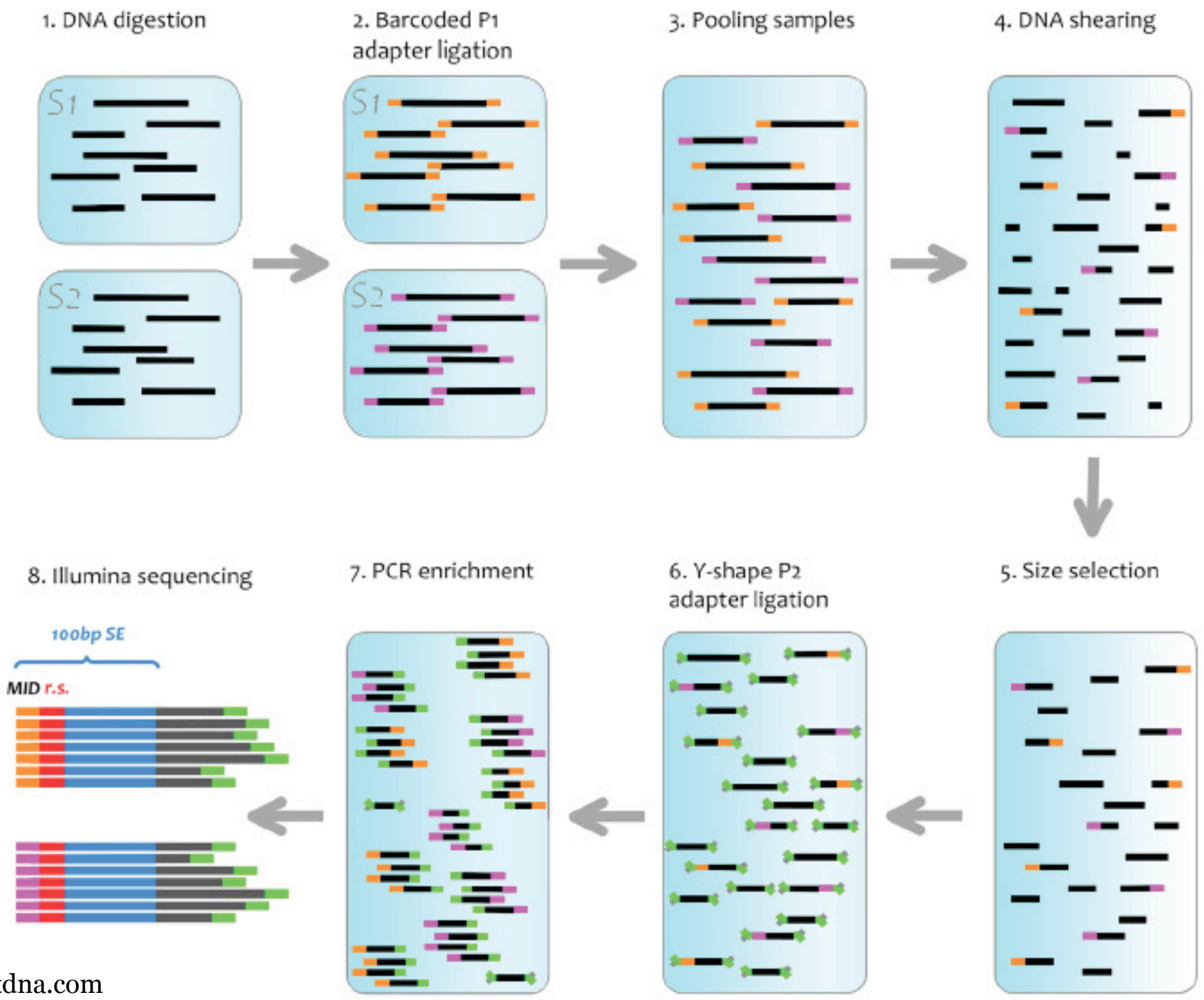



```
ggt ggc tgg ggt cag
ggt ggc tgg ggc cag
ggt ggc tgg ggc cag
ggt ggc tgg ggt cag
ggt ggc tgg gga cag
gga ggc tgg ggt caa
```

# What is RAD good for?




Peterson *et al.* 2012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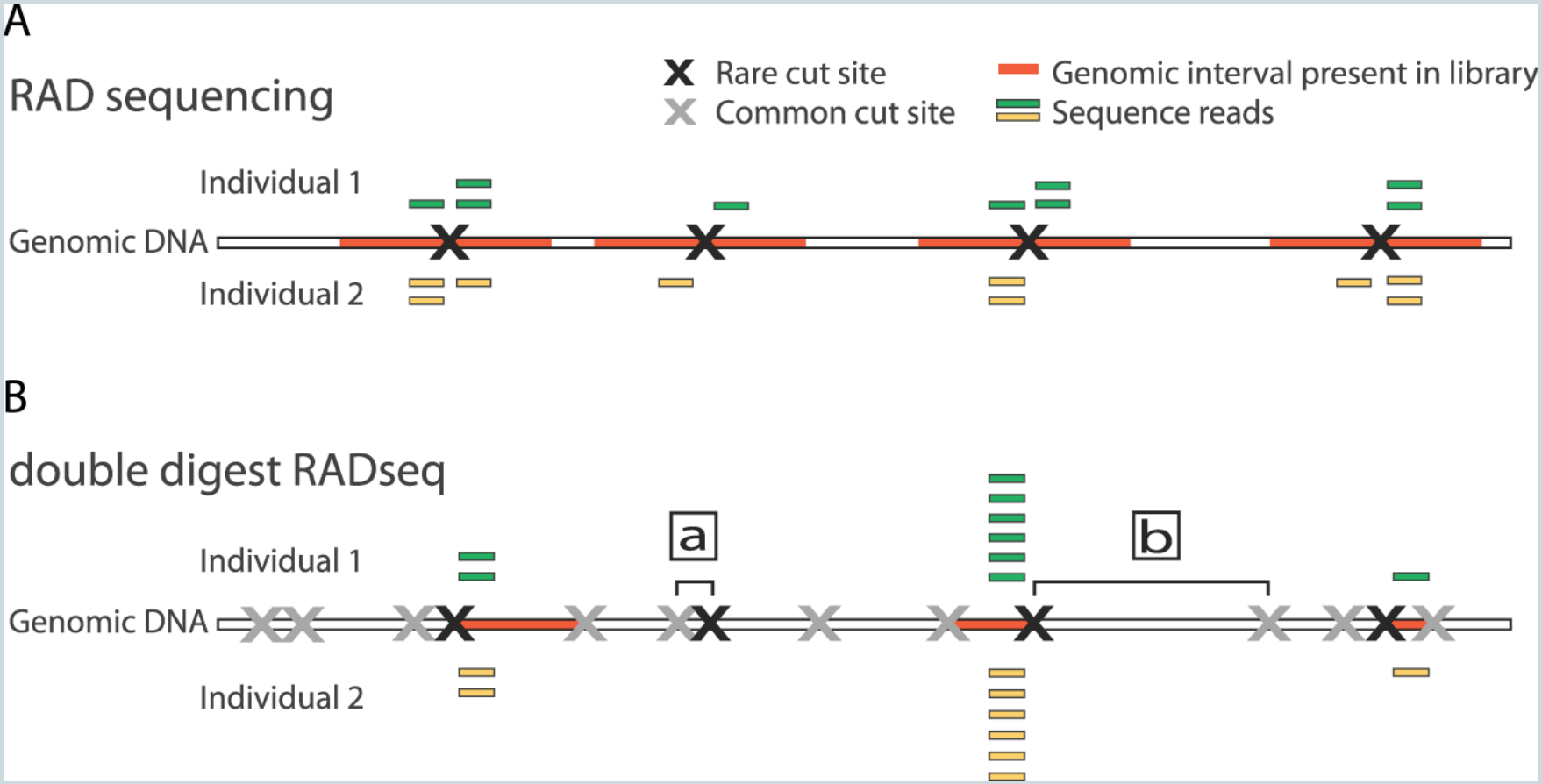
6. Y-shape P2 adapter ligation
7. PCR enrichment
8. Illumina sequencing
100bp SE
MID r.s.

# Many different types…

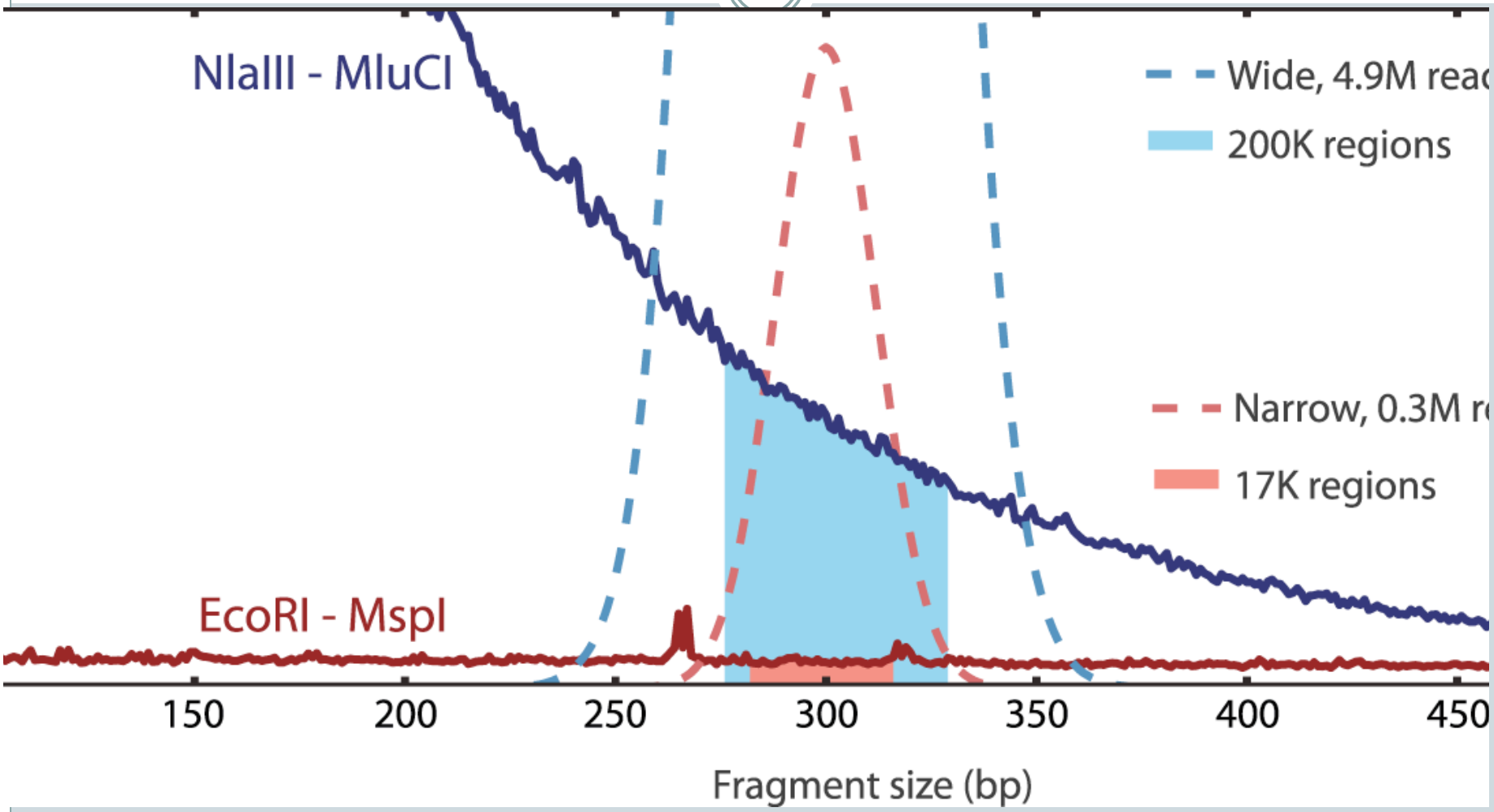
- **mbRAD** (Miller *et al.* 2007; Baird *et al.* 2008)
- **ddRAD** (Peterson *et al.* 2012)
- ezRAD (Toonen *et al.* 2013)
- 2bRAD (Wang *et al.* 2012)
- …GBS


Review: Davey *et al.* 2011
Demystifying the RAD fad: Puritz *et al.* 2014

# mbRAD vs ddRAD

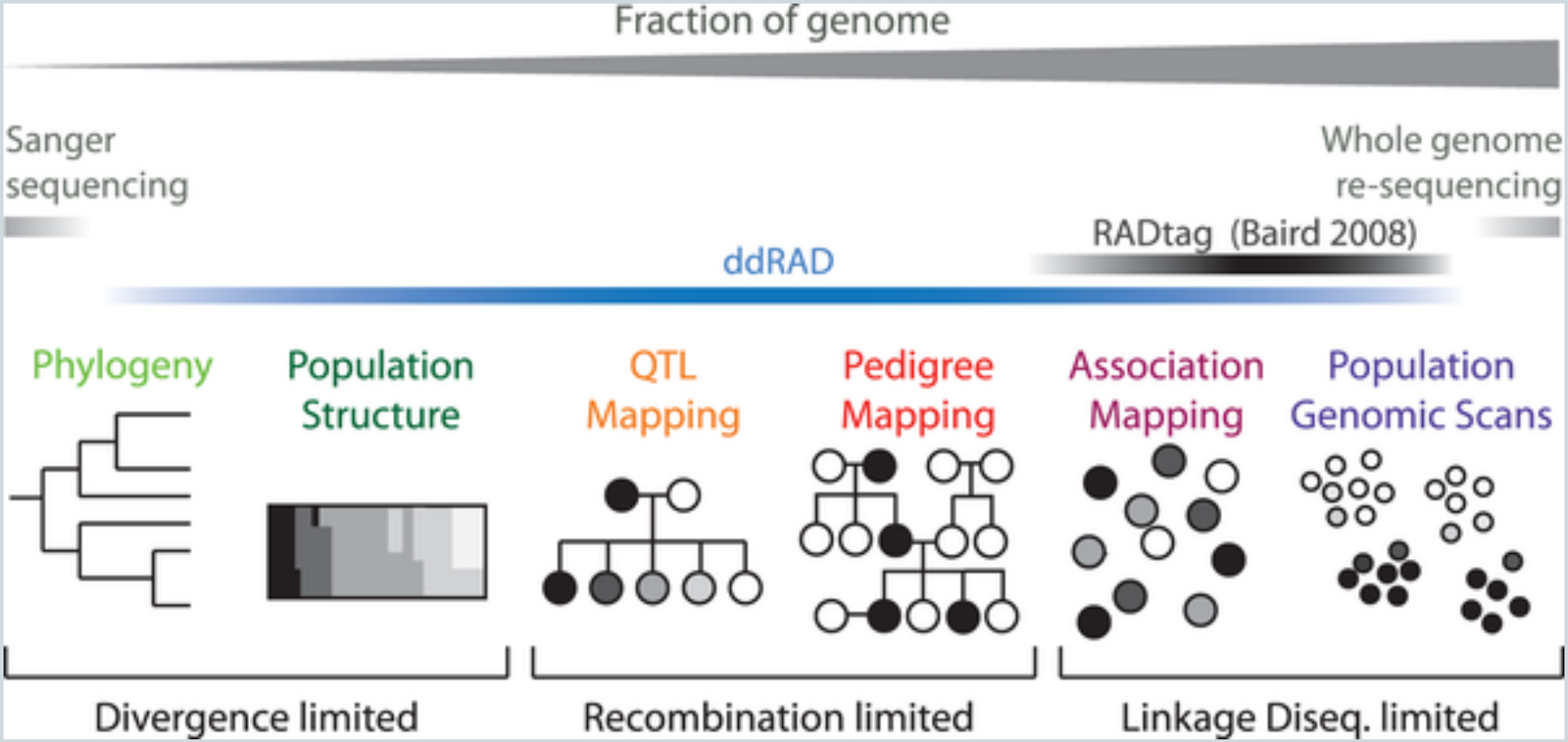


# ddRAD – flexibility in the number of loci




Peterson *et al.* 2012

# What is RAD good for?




Peterson *et al.* 2012

# Many different types…

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- …GBS


So… which one suits me best?

Paired-end vs single-end sequencing?

Pooled vs individual labeling?

Experimental design?

Davey *et al.* 2011
Demystifying the RAD fad (Puritz *et al.* 2014)

# Experimental design

- What is the question?
  - Population genetics
  - Phylogenetics
  - Looking for selection
- What is your organism/system?
- Available genomic resources?
- Budget

# Experimental design

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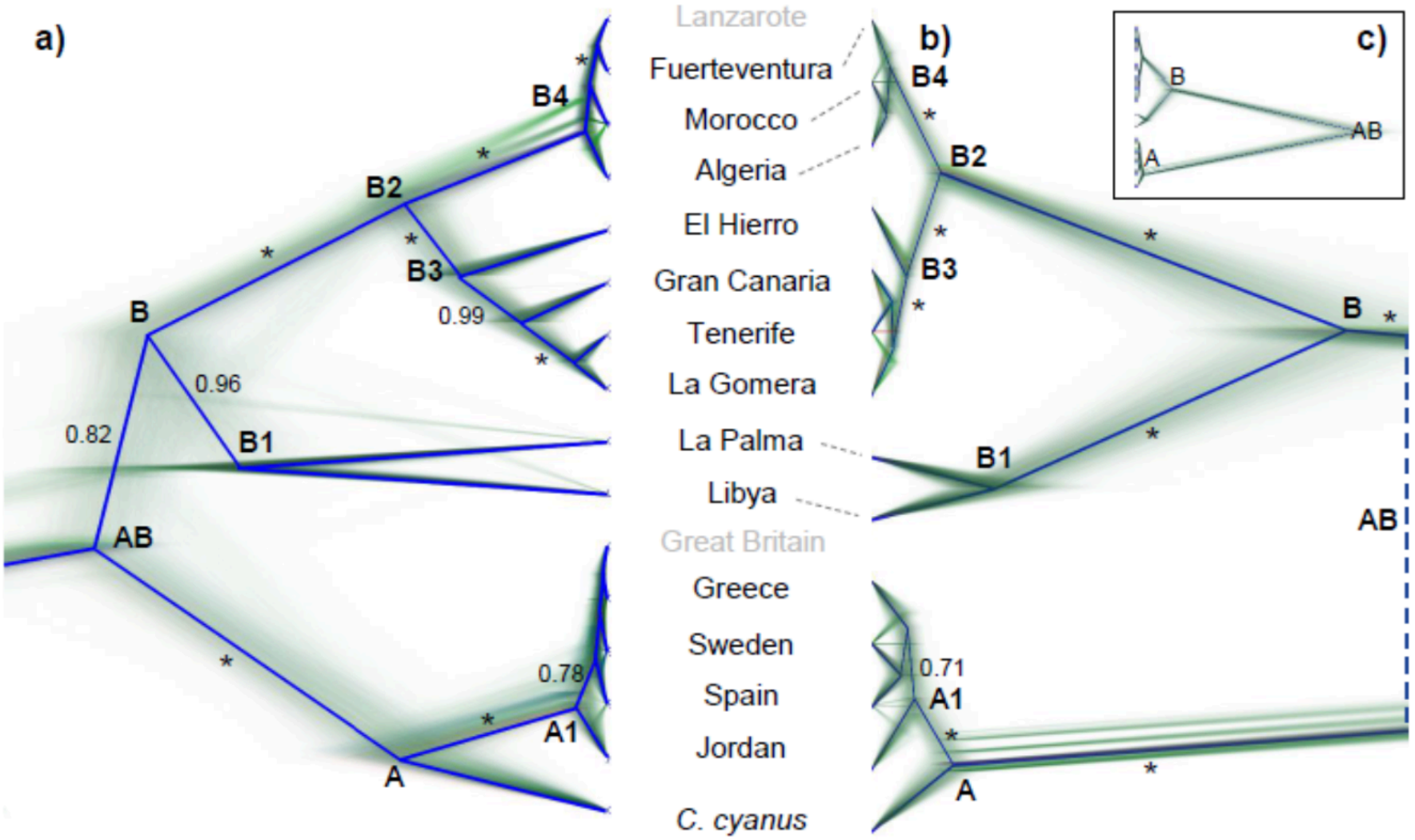
# Experimental design: Question?

- Pop genetics & phylogenetics:
  - **Controversial/discussed points:**
    - Pooled vs individual labels (e.g. Emerson *et al.* 2014)
    - Coverage needed? (e.g. Davey *et al.* 2013)
    - Markers vs individuals

# Experimental design: Question?

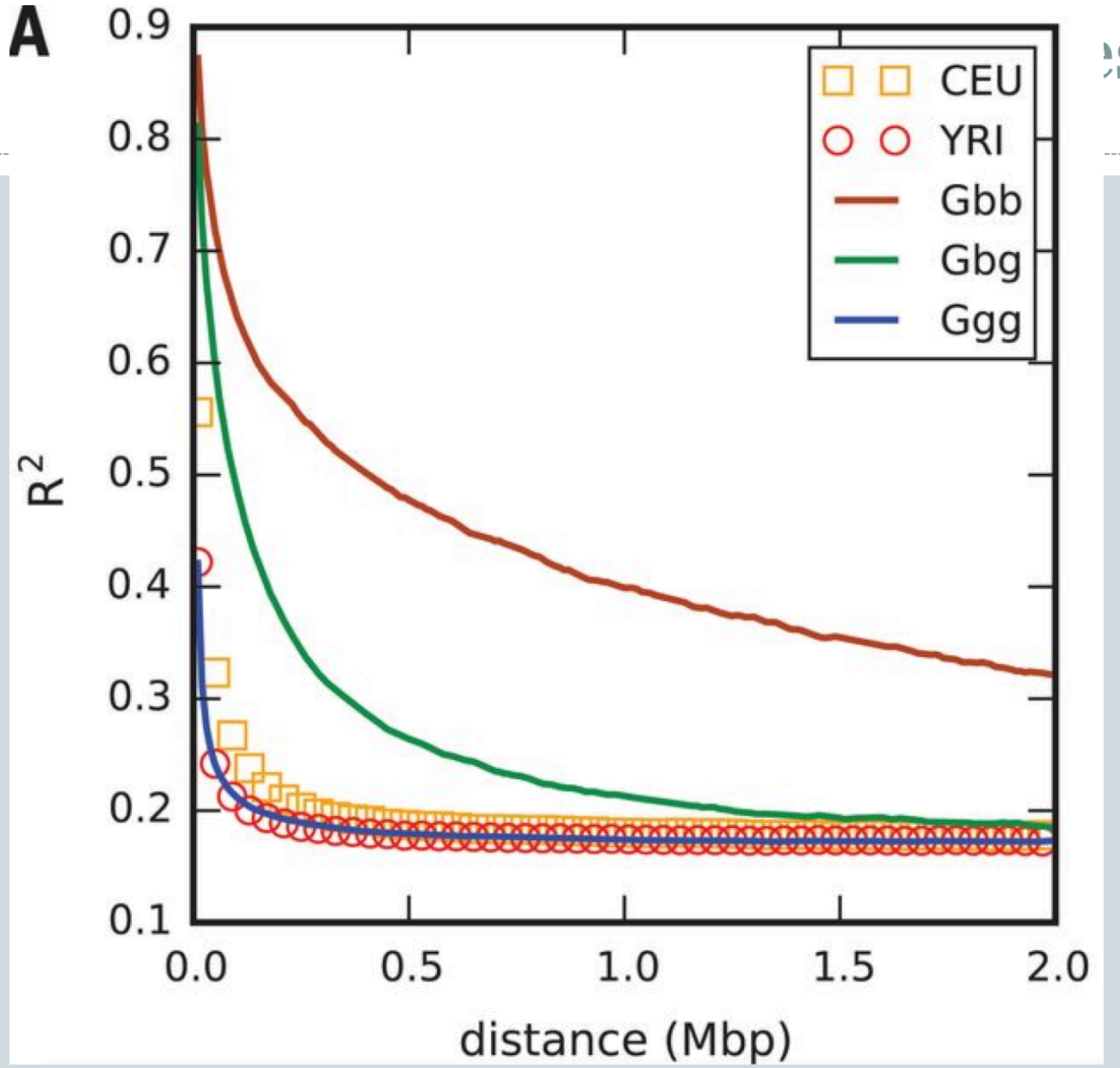



Stervander *et al.* 2015

# Experimental design: Question?

- Marker density:
  - Which restriction enzymes?
- Looking for selection?
  - LD
    - How big are my LD blocks?

# estion?




Mountain Gorilla

Xue *et al.* 2015

# Experimental design

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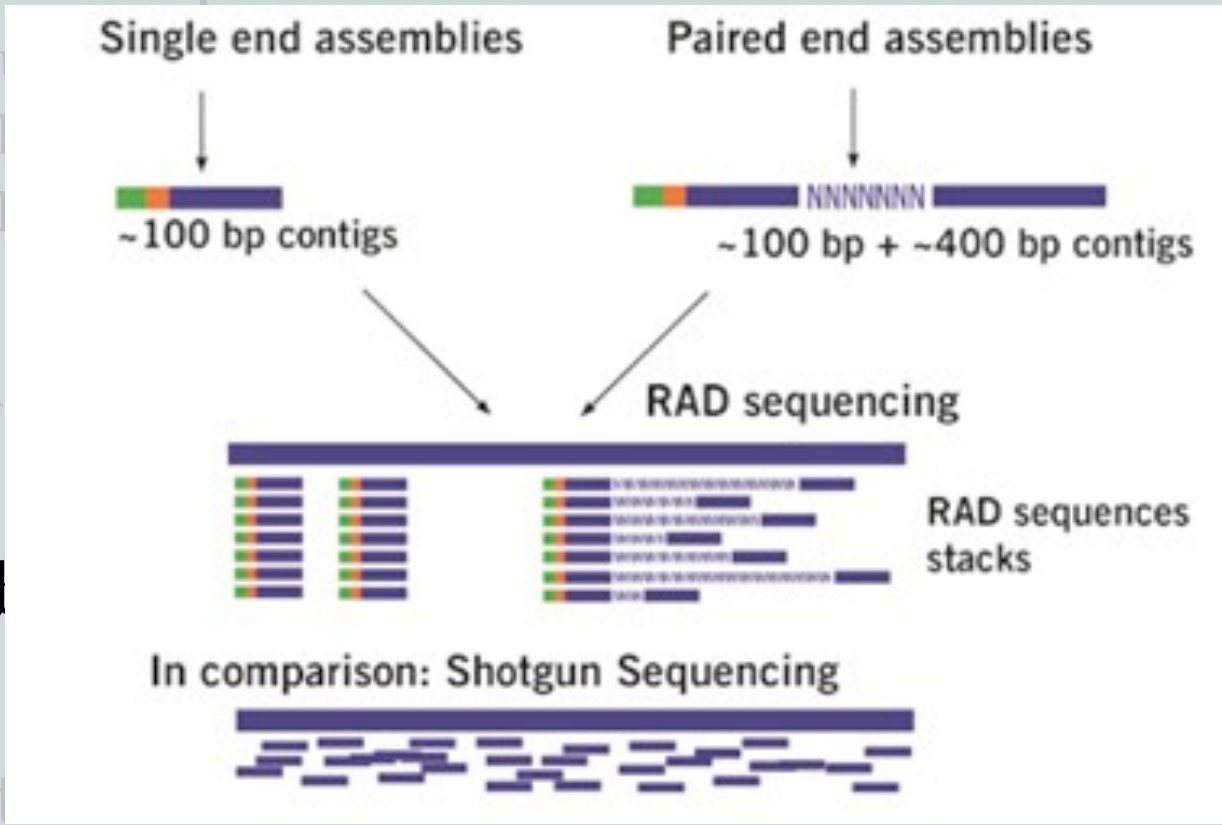

# Experimental design

- What is the question?
  - Population genetics
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# Lab & Sequencing facilities

- GDC
  - Adapter aliquots (RAD & ddRAD)
  - Covaris
  - Caliper
  - BioAnalyzer
  - Qubit
  - qPCR
  - MiSeq
  - Help & advice!
- FGCZ
  - HiSeq (8 lanes = 1 flowcell; 250Mil reads per lane)
  - MiSeq (1 lane; 44-50Mil reads)

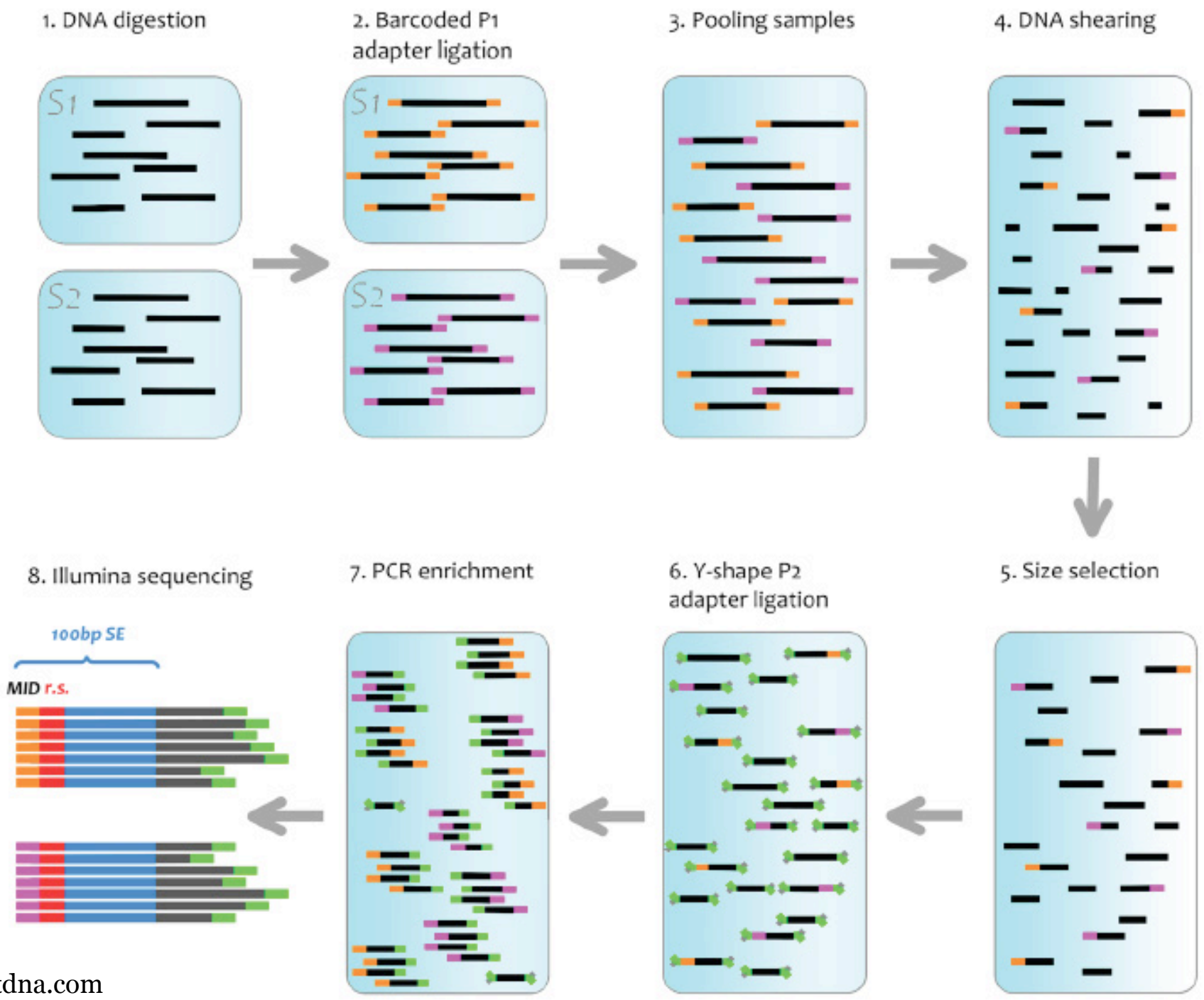
# Wet lab

**Lab effort**:

- Need high quality DNA
- Large quantities of DNA
- Taxa specific optimisation

**Optimisation:**

- Restriction digest variation between samples.
- PCR bias
- Variation in size selection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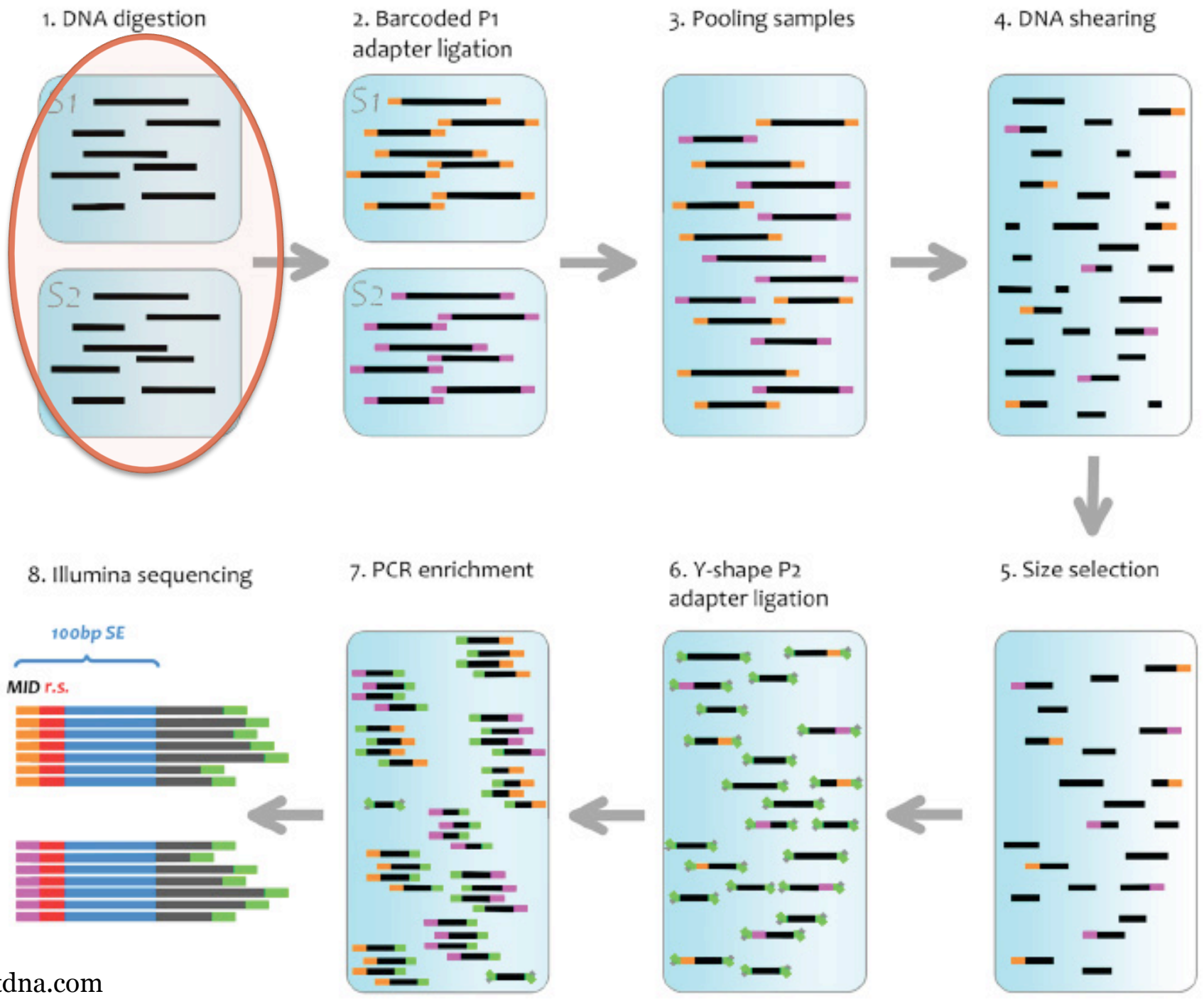
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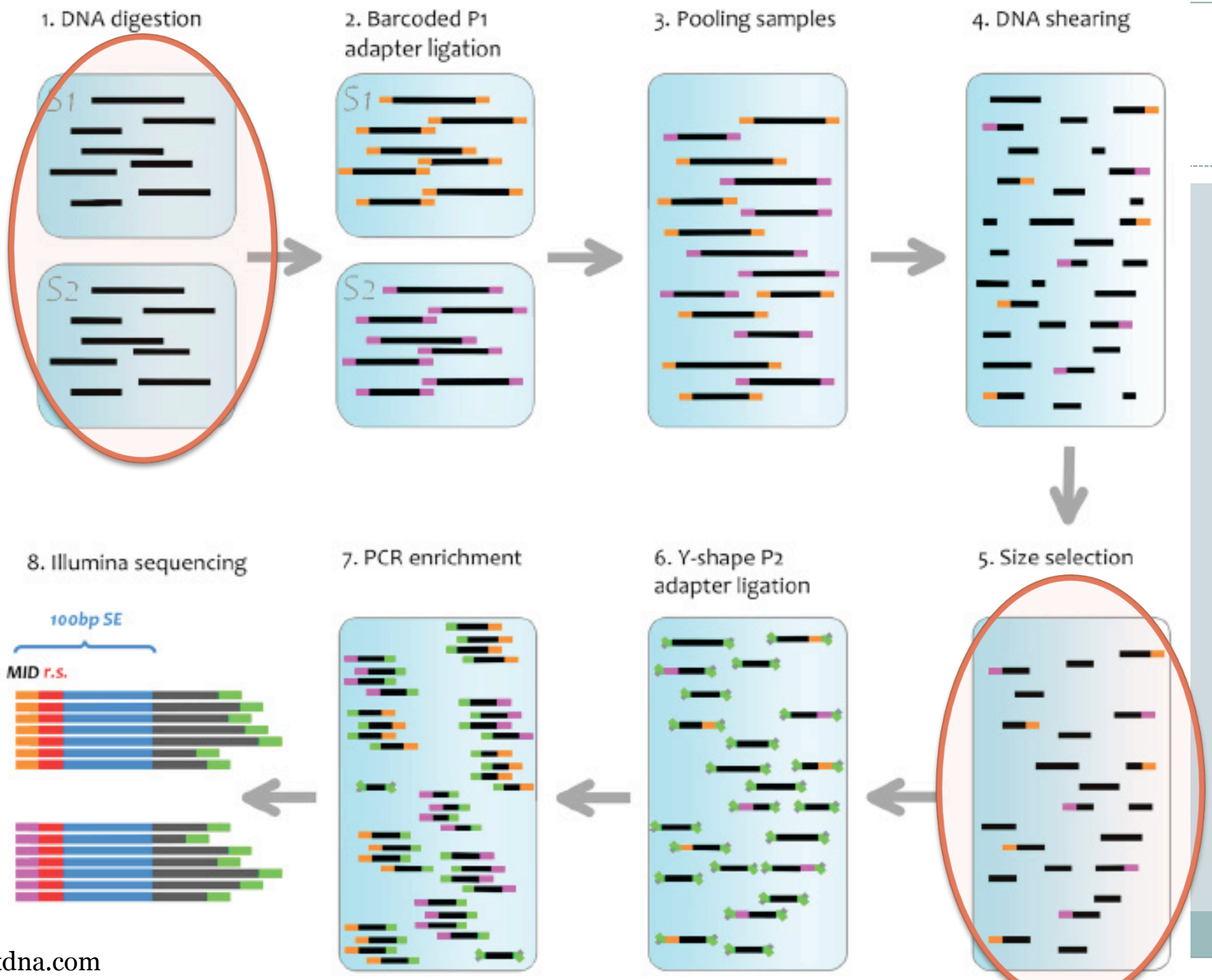
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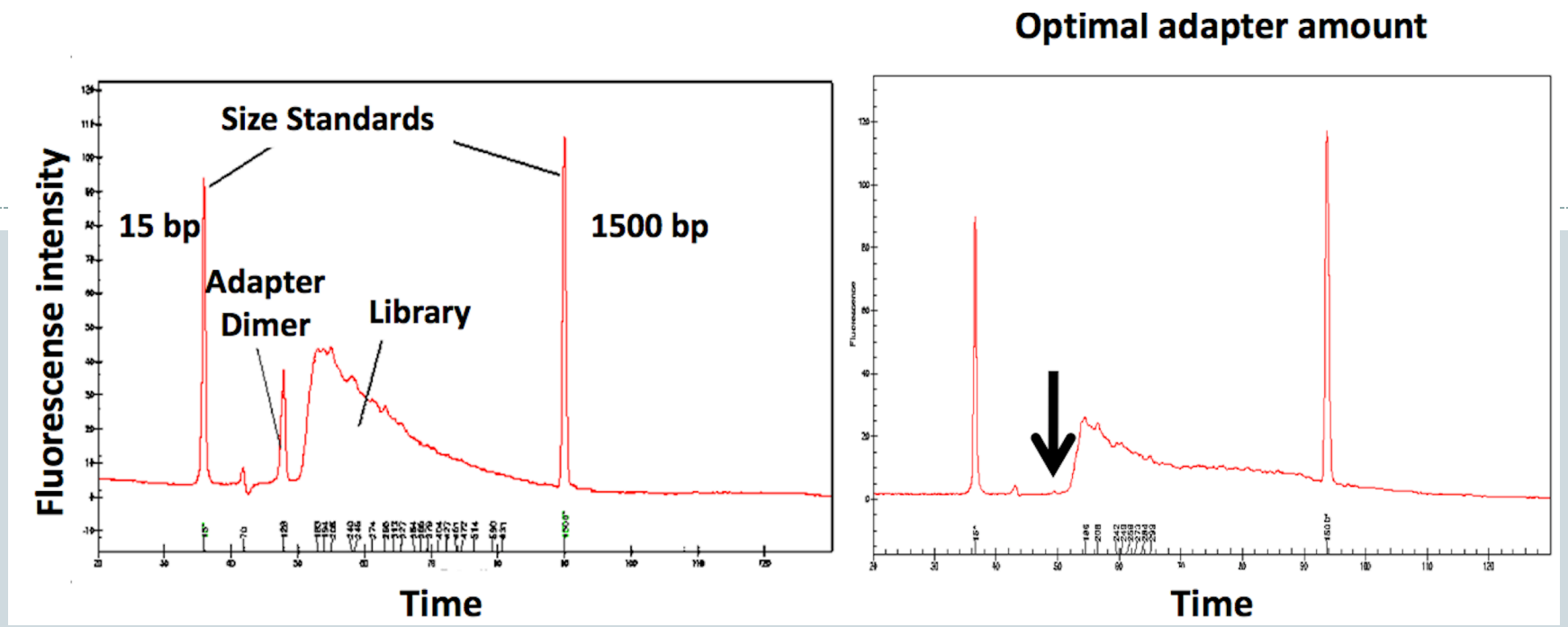
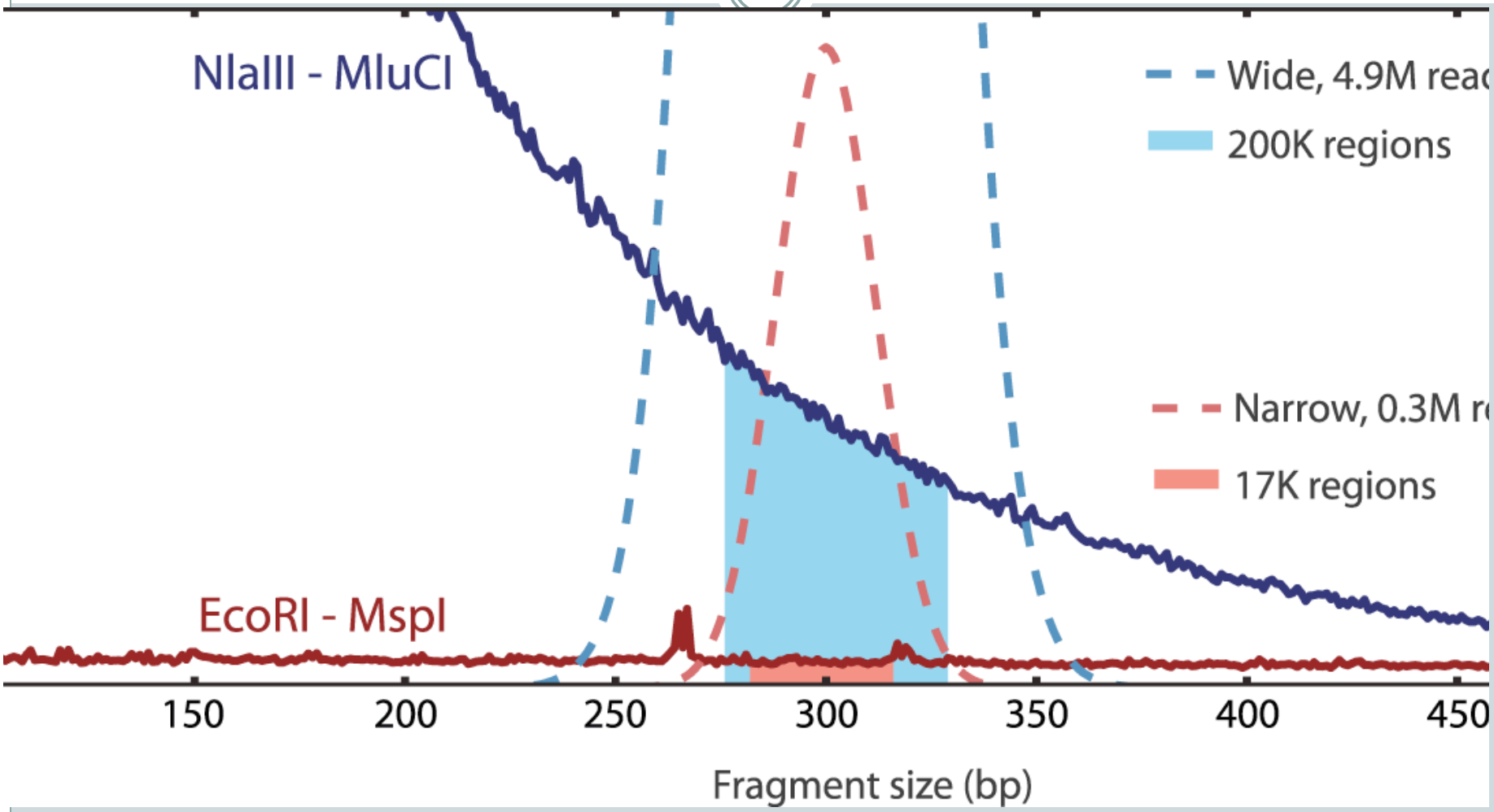
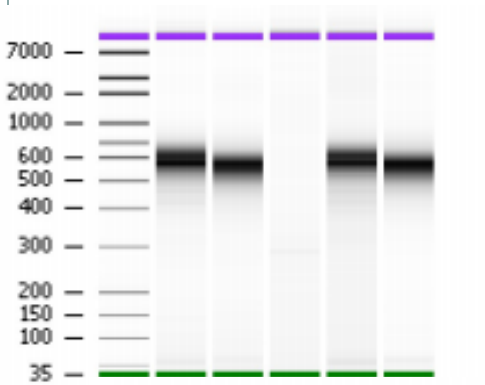
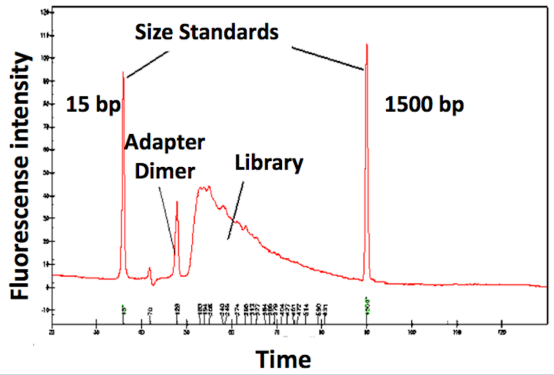

Size Standards
15 bp
1500 bp
Adapter
Dimer
Library
Fluorescense intensity
Time
Optimal adapter amount
Time

# ddRAD – importance of size selection

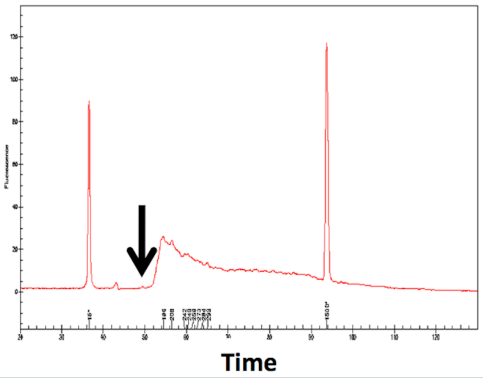



Peterson *et al.* 2012

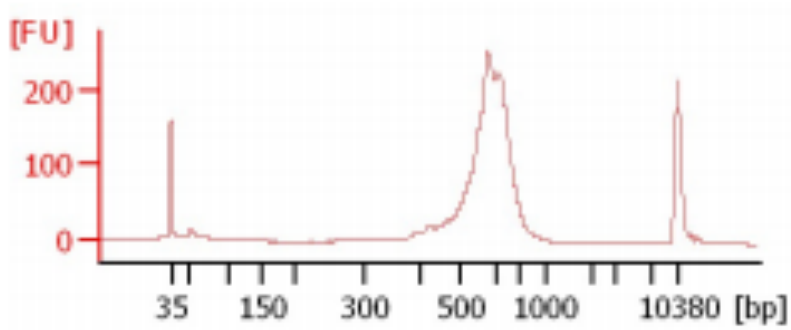


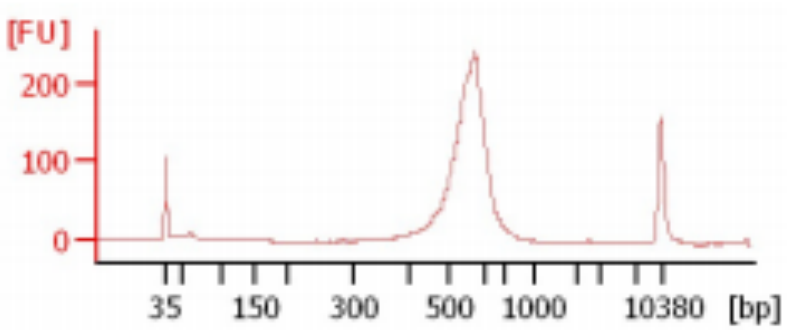

Optimal adapter amount



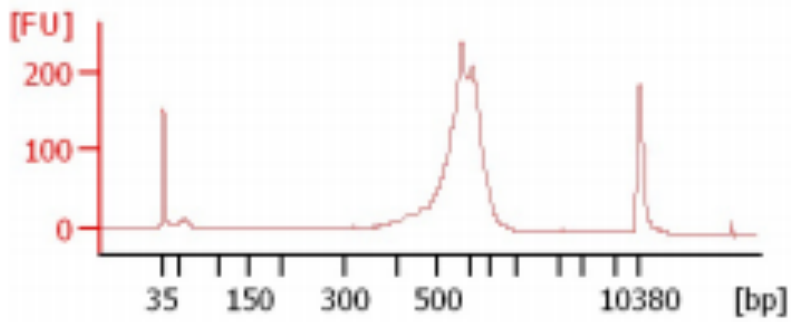

Dsyl 3




Nep 1




Dsyl 3

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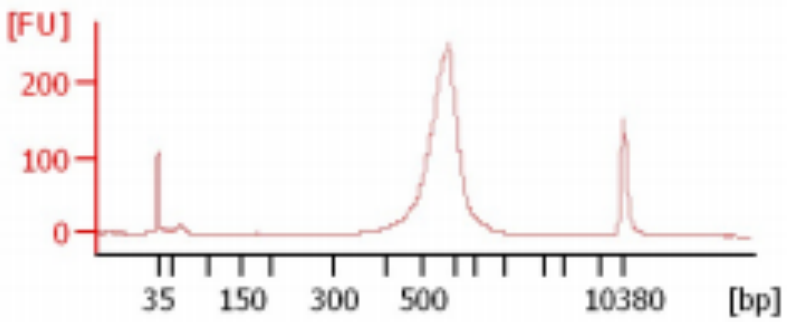

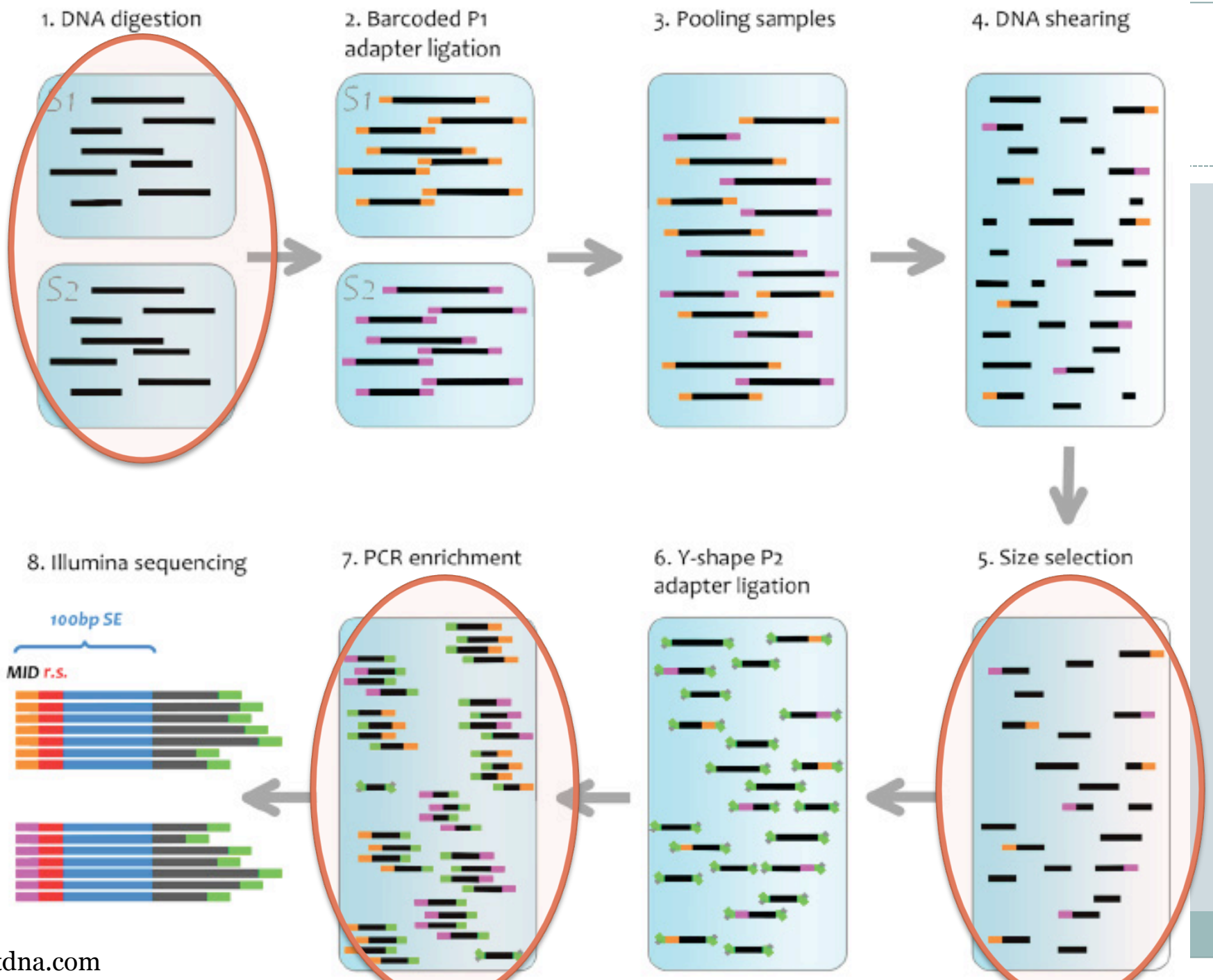
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100bp SE
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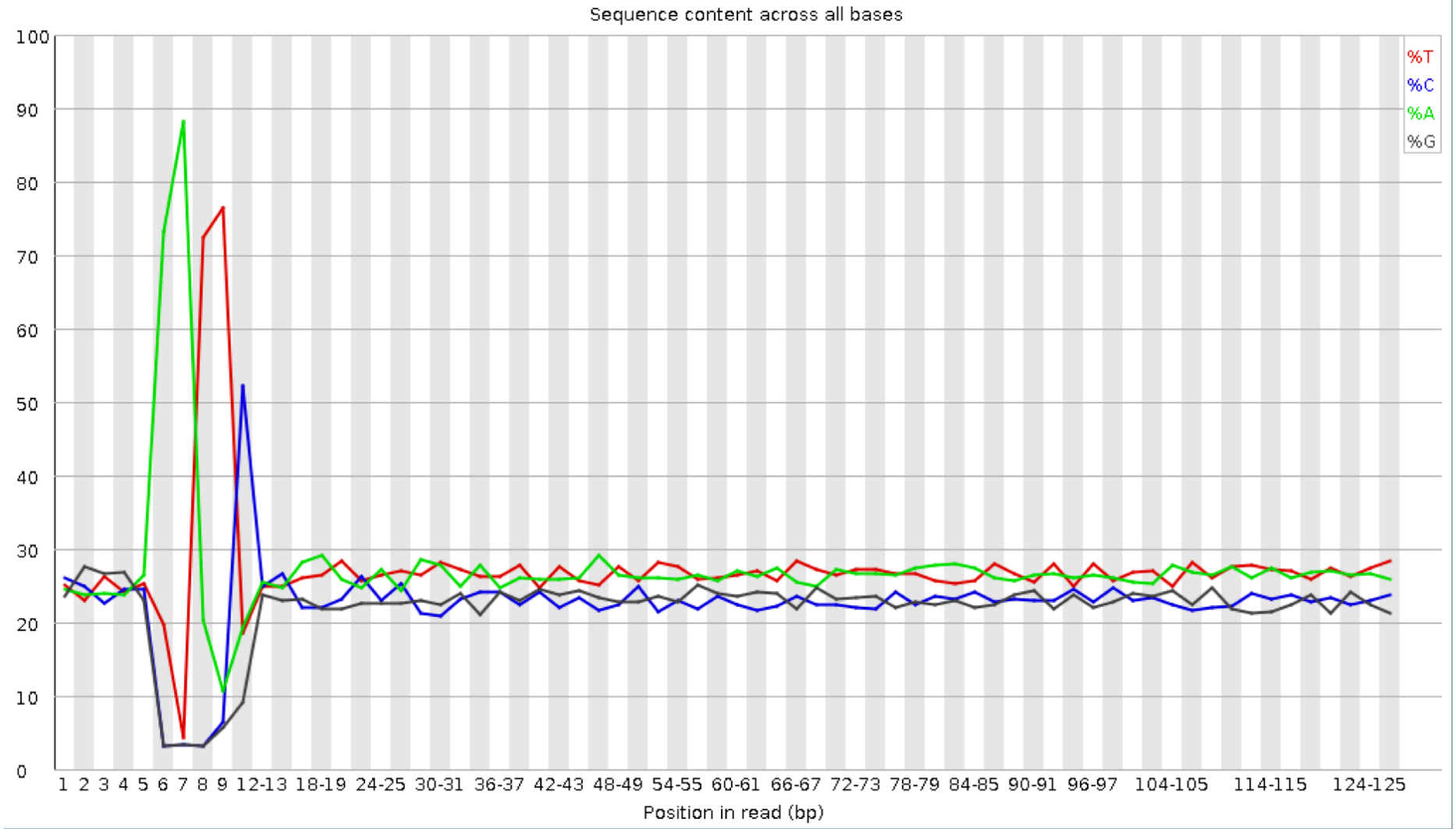
# Lab & Sequencing facilities

FGCZ

- HiSeq  (250Mil reads; 100-150bp)
- MiSeq (100Mil reads; 150-300bp)

# Lab & Sequencing facilities



# Lab & Sequencing facilities

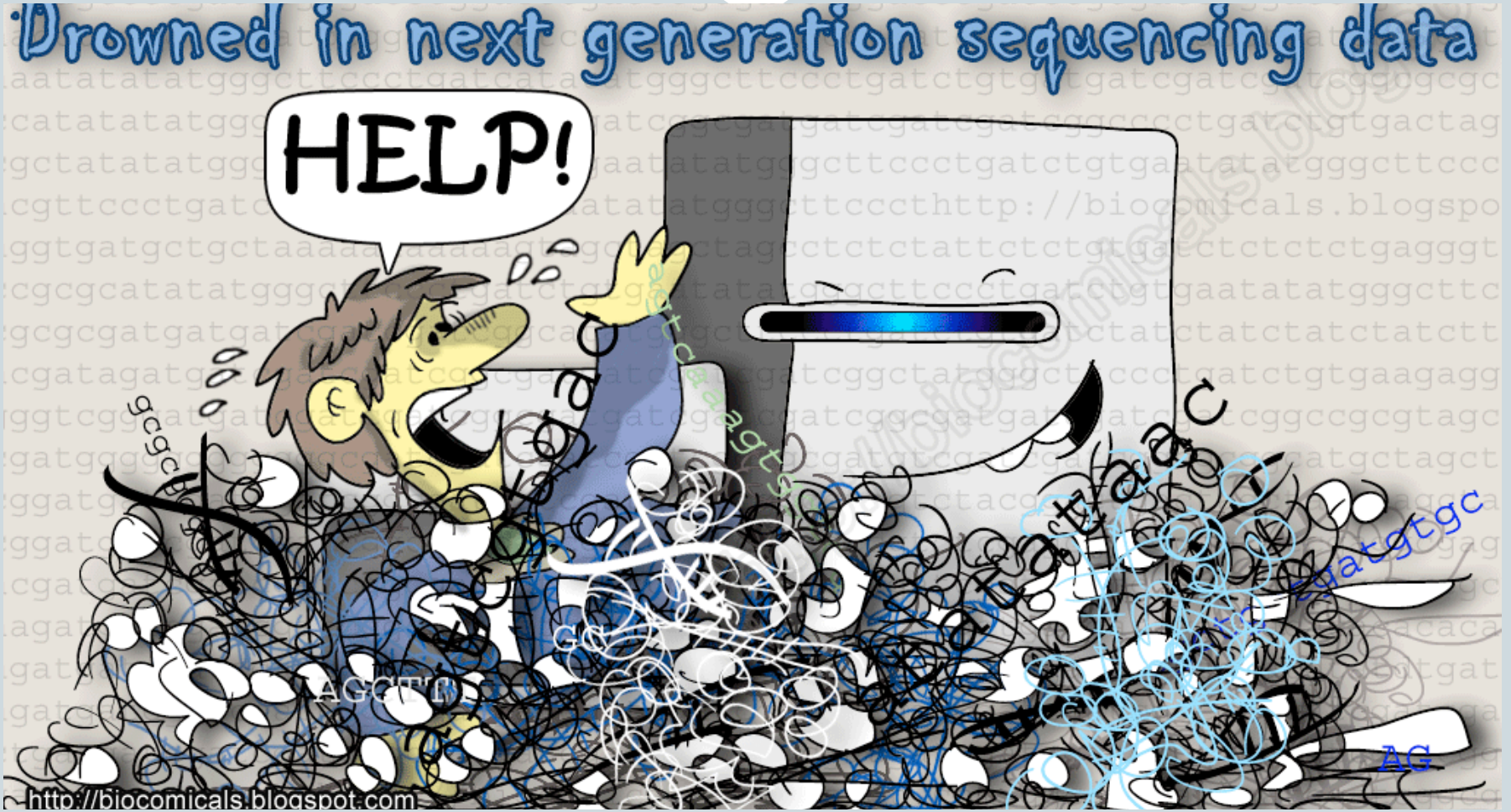
FGCZ

- HiSeq (250Mil reads; 100-150bp)
- MiSeq (100Mil reads; 150-300bp)

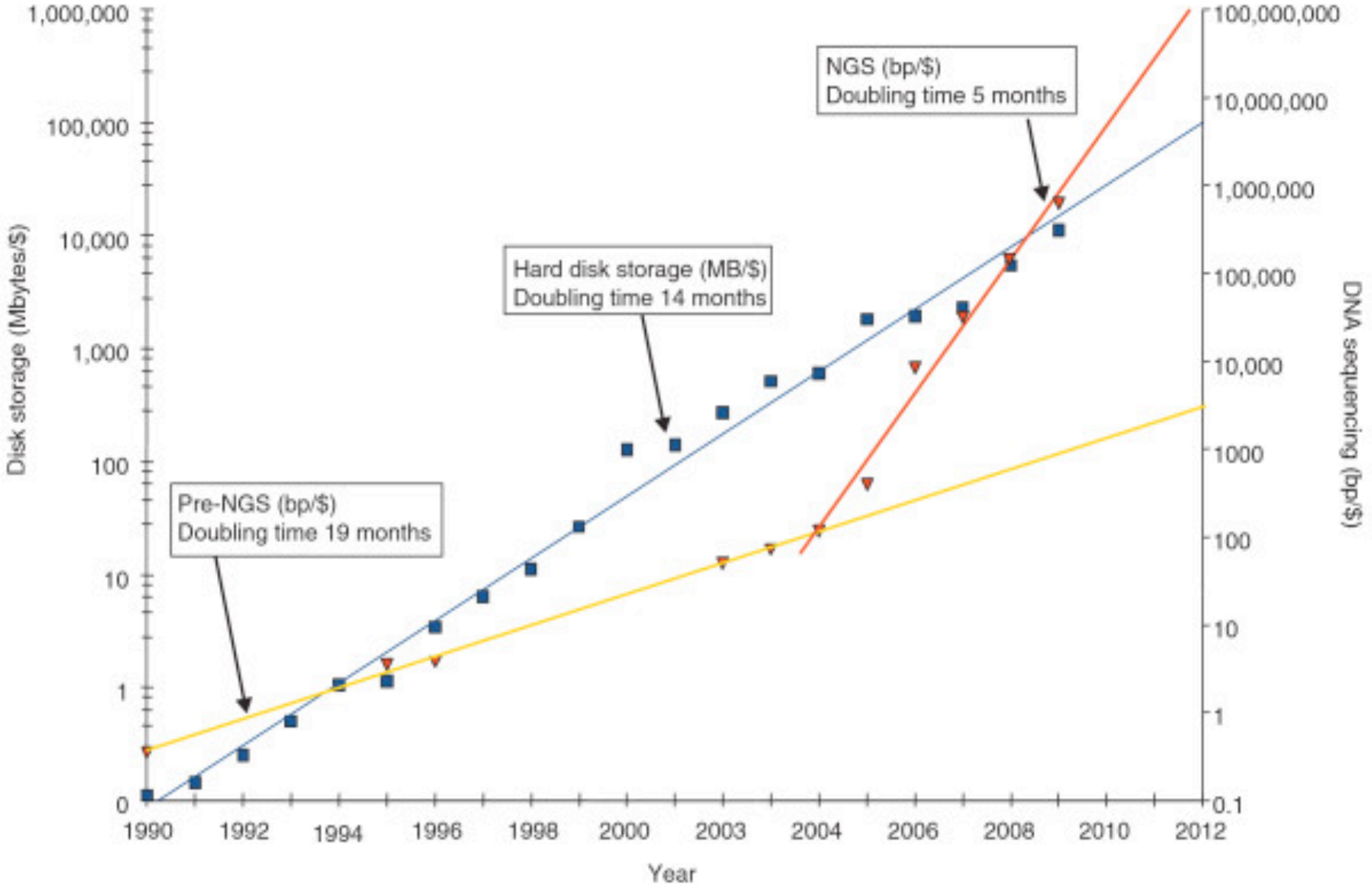
Final nr of reads & coverage:

- -20% for PhiX

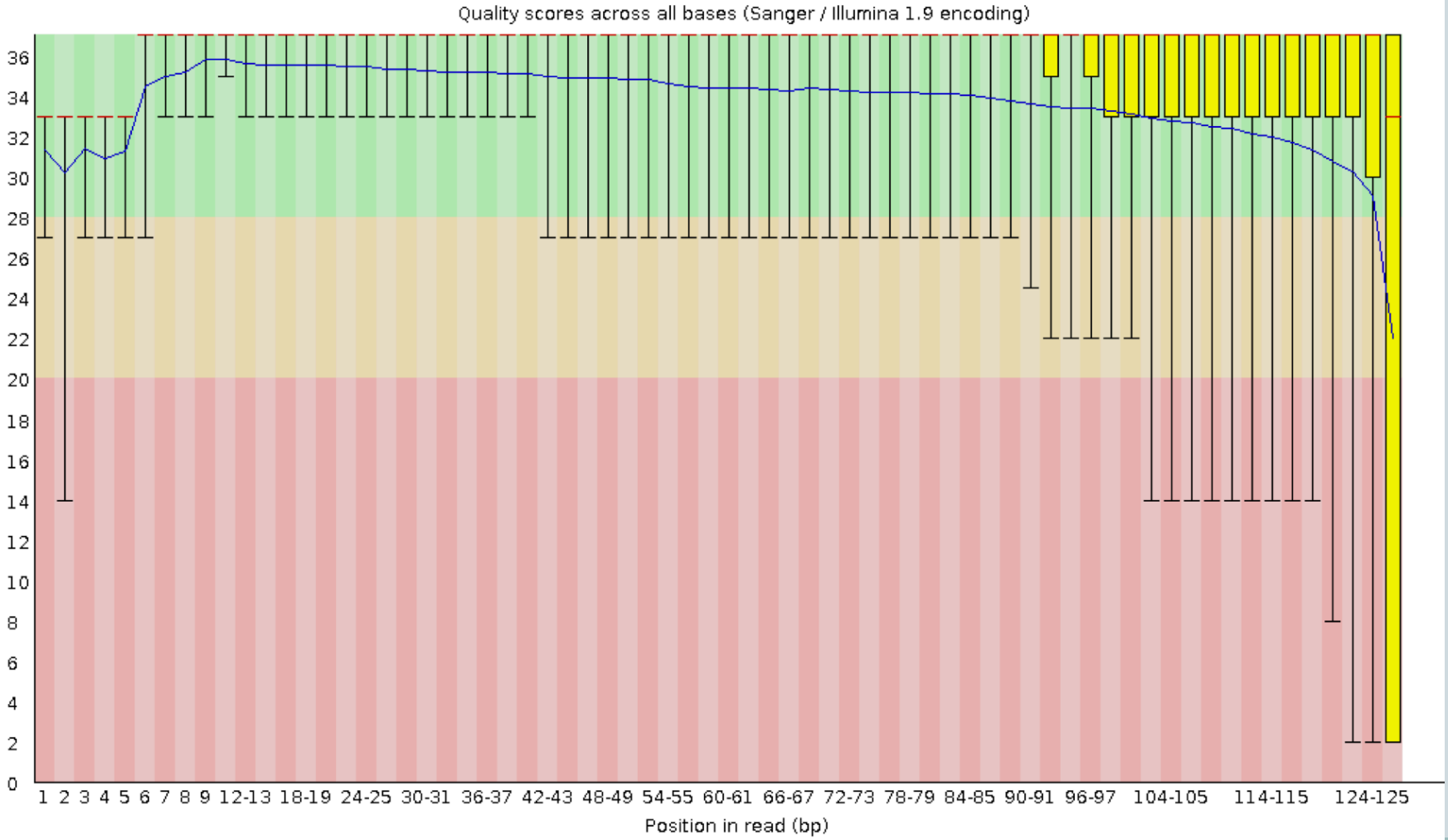
# … 2-6 weeks later



# Large data


NGS (bp/$)
Doubling time 5 months
Hard disk storage (MB/$)
Doubling time 14 months
Pre-NGS (bp/$)
Doubling time 19 months
Disk storage (Mbytes/$)
DNA sequencing (bp/$)
Year
1,000,000
100,000
10,000
1,000
100
10
1
0
100,000,000
10,000,000
1,000,000
100,000
10,000
1000
100
10
1
0.1
1990
1992
1994
1996
1998
2000
2003
2004
2006
2008
2010
2012

# Data!


Quality scores across all bases (Sanger / Illumina 1.9 encoding)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
1 2 3 4 5 6 7 8 9 12-13 18-19 24-25 30-31 36-37 42-43 48-49 54-55 60-61 66-67 72-73 78-79 84-85 90-91 96-97 104-105 114-115 124-125
Position in read (bp)

# RAD bioinformatics pipeline

1. Demultiplex sequences
   - Based on barcode + cutsite + mutation(s)
2. Clean data
   - Poor quality reads
   - Shorter reads
   - Remove adapter dimer
3. Find loci
   1. Within sample clustering
   2. Between sample clustering
   3. Call SNPs
4. Filter SNPs

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**Optimise**

**Optimise**

# RAD bioinformatics pipeline

- Stacks (Catchen et al. 2013)
  - First available pipeline
  - Designed for RAD but handles other methods
- dDocent (Puritz et al. 2014)
  - Simple, customisable bash backbone for bioinformatics
  - Designed for ddRAD & ezRAD
  - FreeBayes or GATK
- pyRAD (Eaton 2014)
  - Analysis pipeline written in Python
  - Many different RAD types
  - Clustering using Usearch or Vsearch
- aftrRAD (Sovic et al. 2015)
  - Newest
  - Blend between stacks and pyrad

# 1. Demultiplex data

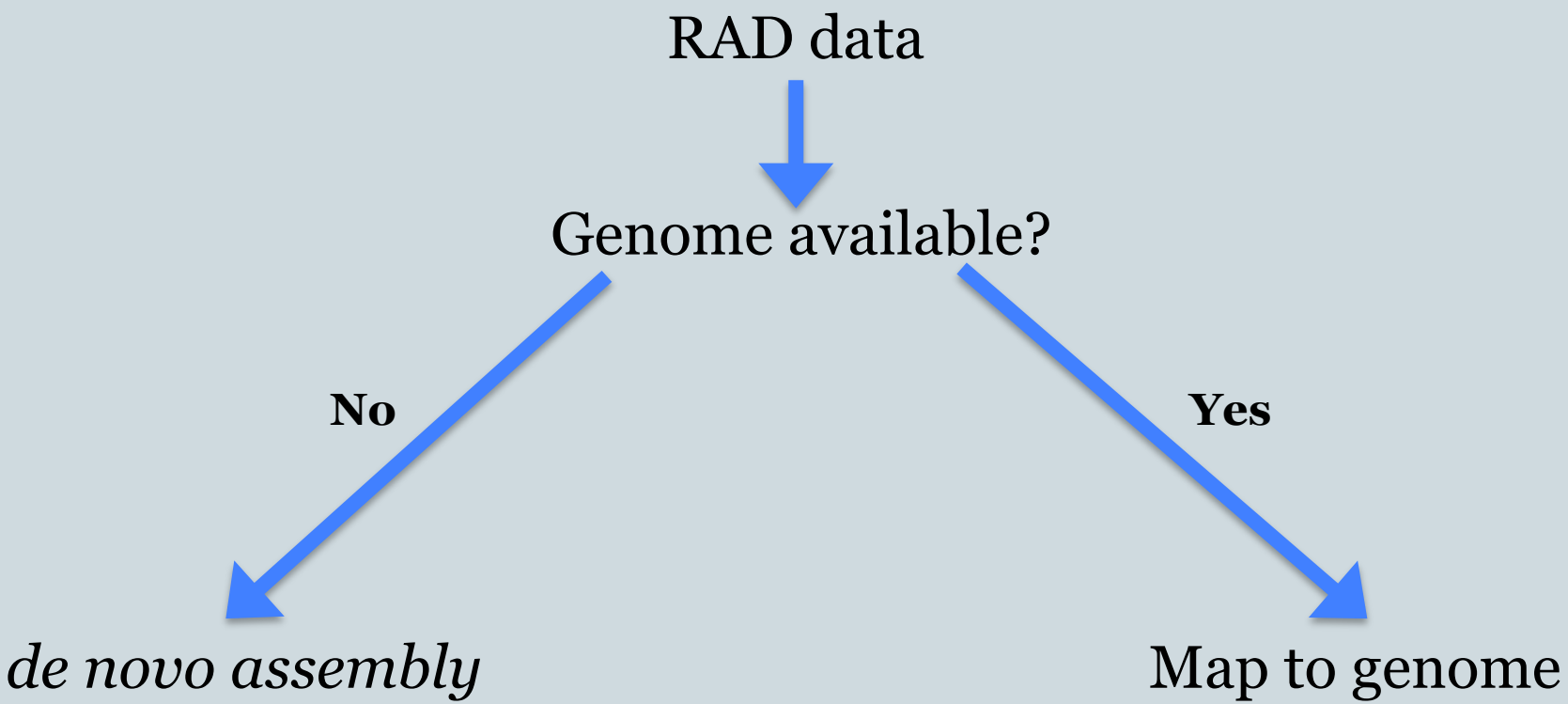
- Barcode OR Barcode + cutsite
- Check your data

CTATTG TGC

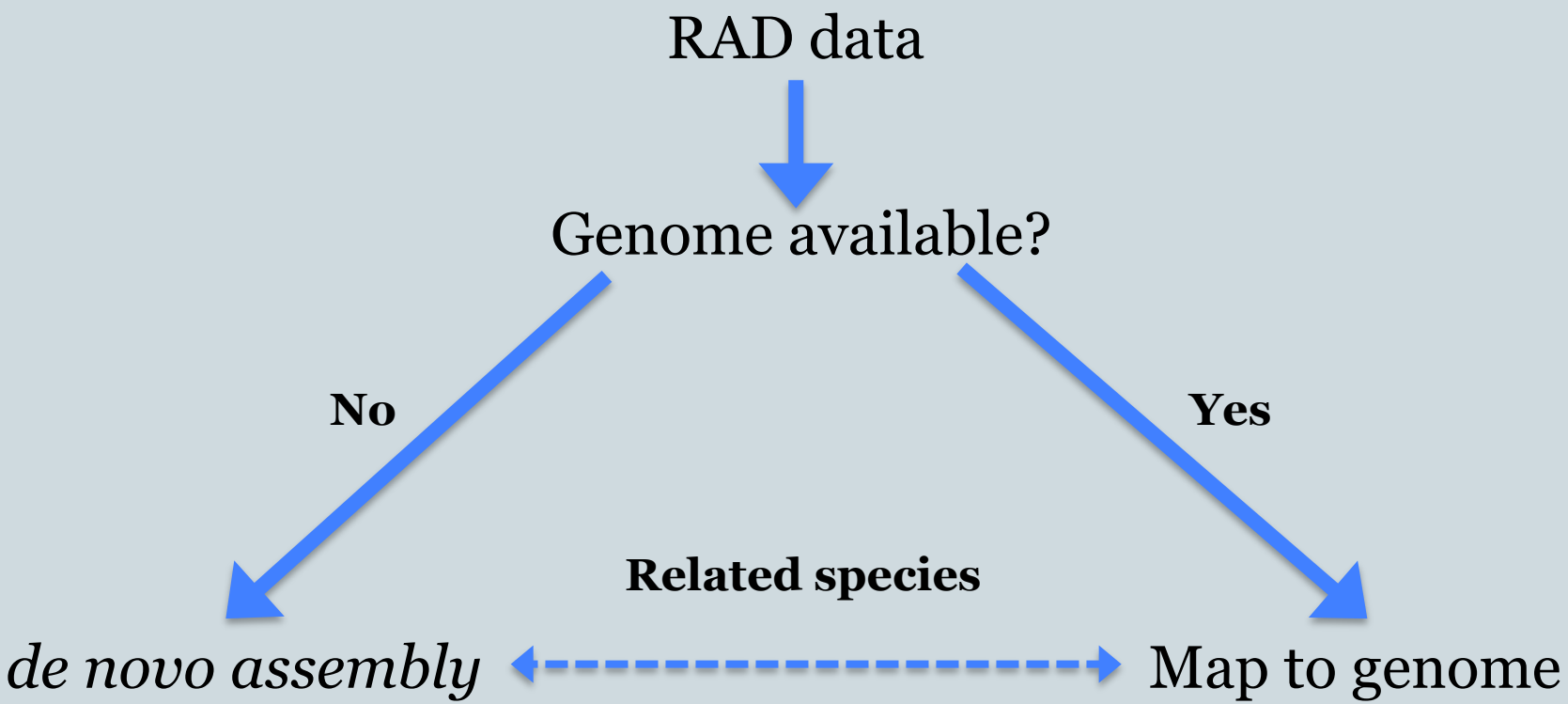
TCTATTGTGCAGGAACCCAGTGTGGACCGGCAGGCAGACCCG
CACGACTGCTTACTGCTAATGGCAGCTCCTGCCCACAGCTCAG
CCCCCGACACAGGCCTTACCAGCTTGTCTCTGAAAGCCCCG

CCTATTGTGCAGGTGGATTATTTACCACTGAGCCACGTGGGA
AGTCCTCTTACACAACAGGCACTCAATAAACATCATCCCTTCA
GCAGACATTTATCAAGCTTTTACTGGGAACCCAACCTGCAT

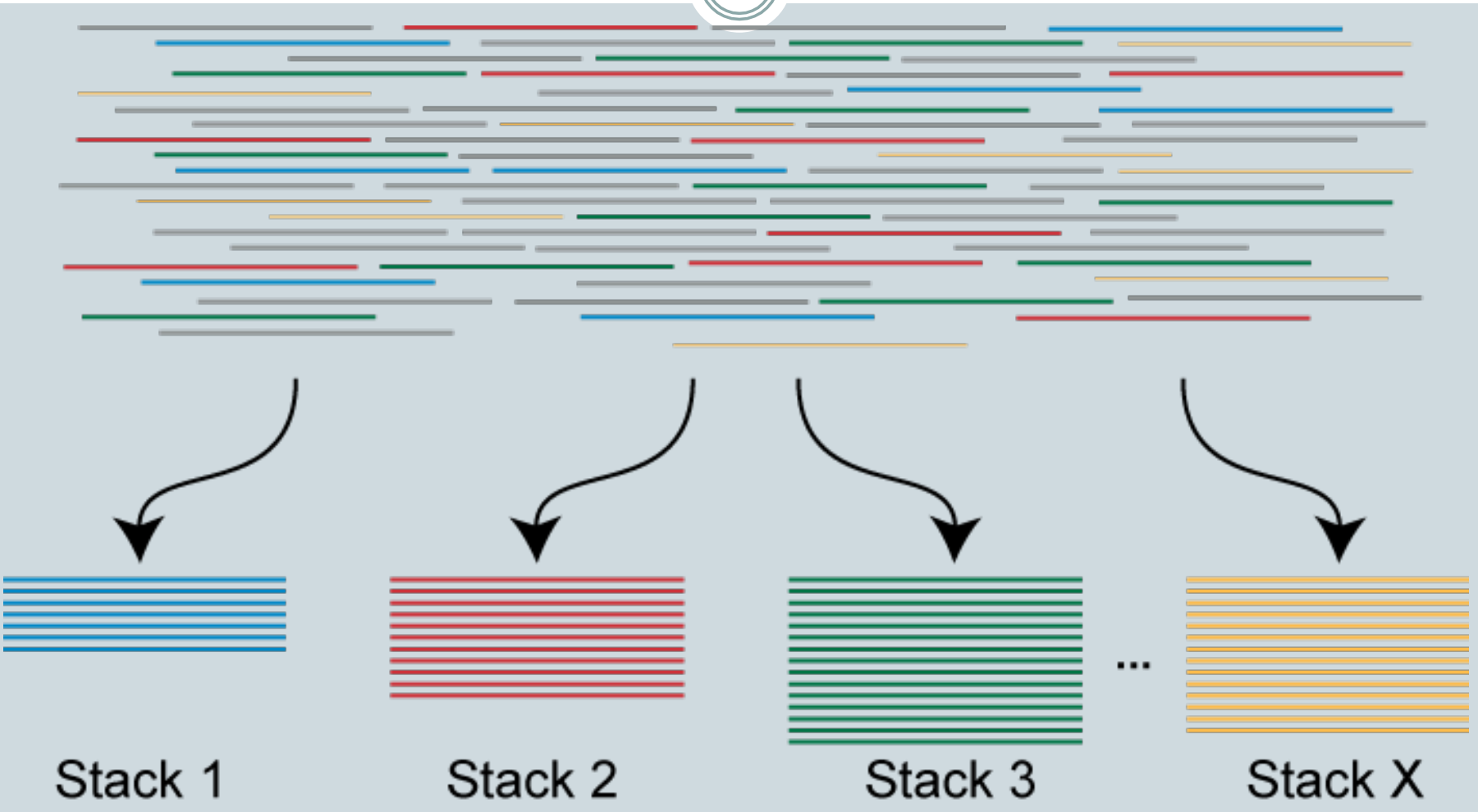
# 2. Find loci


RAD data
Genome available?
No
Yes
*de novo assembly*
Map to genome

# 2. Find loci

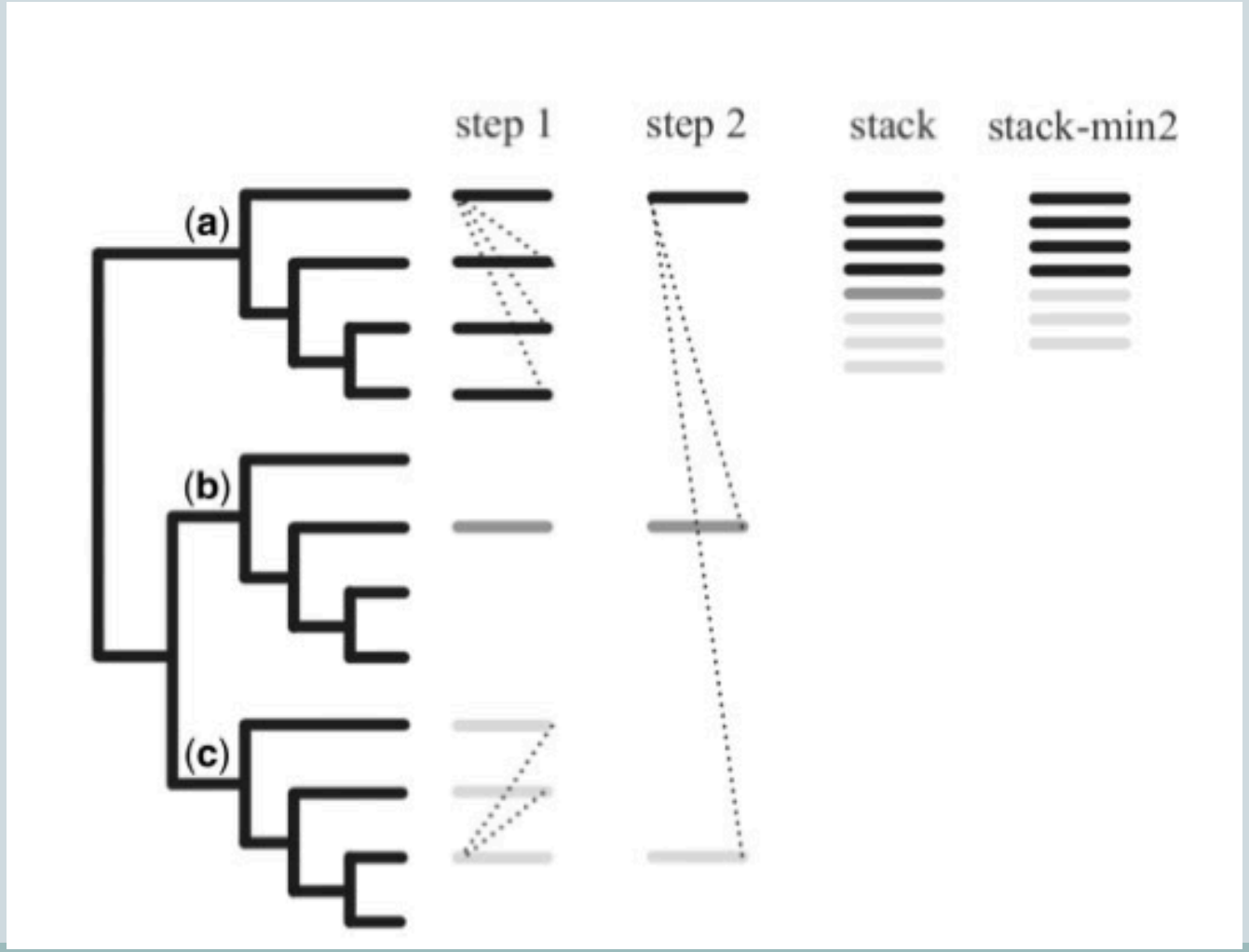

RAD data
Genome available?
No
Yes
Related species
*de novo assembly*
Map to genome

# 2. Find loci: *De Novo* Assembly



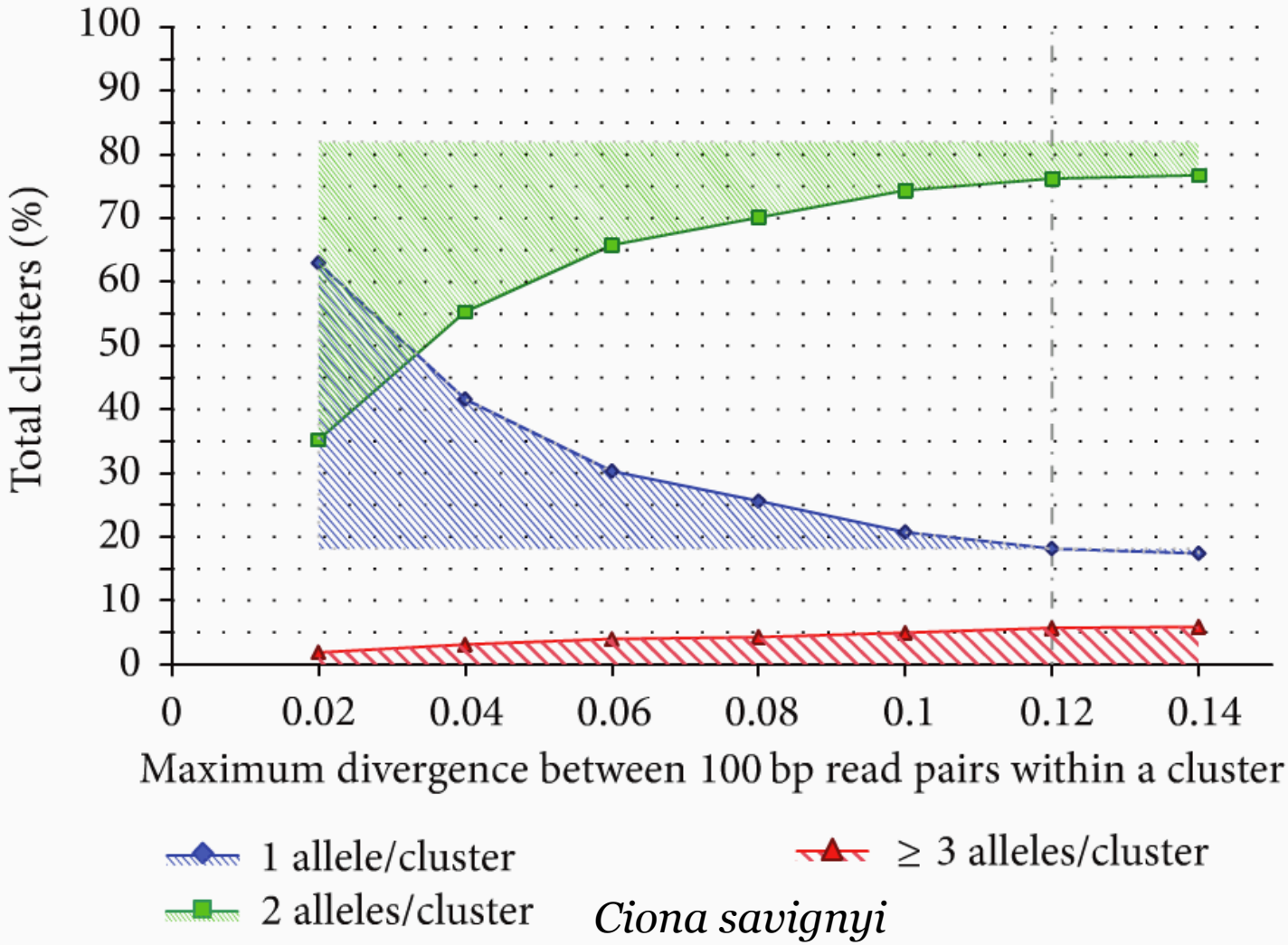

Catchen *et al.* 2011

# 2. Find loci: *De Novo* Assembly




Eaton *et al.* 2014

# 2. Find loci: *De Novo* Assembly




*Ciona savignyi*

Ilut *et al.* 2014

Mastretta_Yanes *et al.* 2015

# 2. Find loci: Map to genome

- Educated guess of read origin

```
CCTGCAGGGATTCC
|||||| || | | ||
CCTGCAGG–ATACC
```

- Global alignment- uses all characters in a read
- Local alignment- maximizes alignment scores through clipping

# 2. Find loci: Call SNPs

- Not all SNP callers are created equal:
  - INDEL sensitivity
  - Base quality awareness
  - Speed
  - Probability of false positives and negatives
  - Biases against homozygotes/heterozygote calls

# 3. SNP filtering

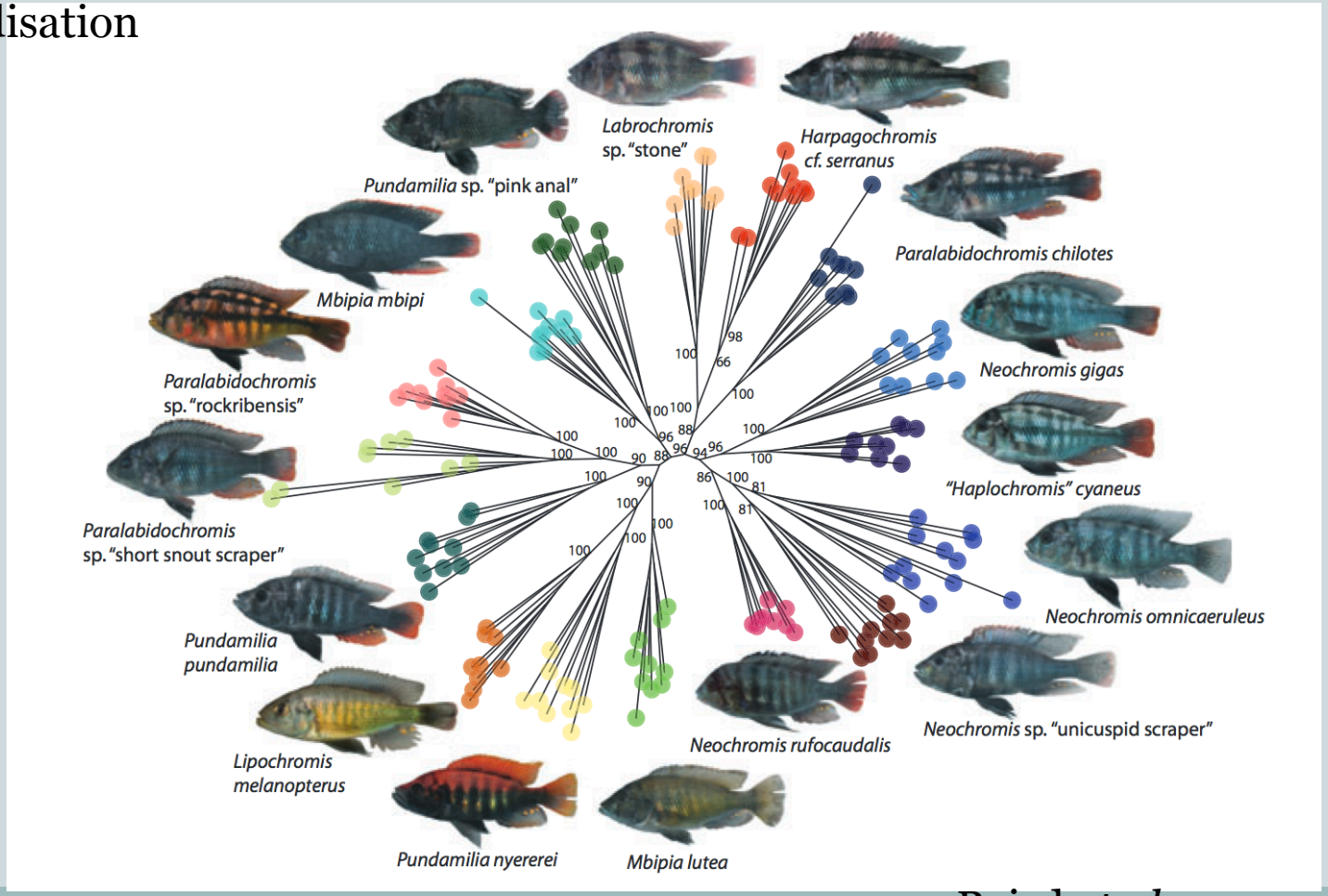
- SNP callers are over sensitive
- Final SNP output: false alleles and allelic dropout
- Filtering these out is essential…
  but the filters have to make sense!
- Filters have to keep in mind
  - Errors from sequencing/ library prep
  - Biological expectations
  - Downstream application of the data

# RAD: Tissue to data

- Thousands of loci
- Non model organism
- Relatively cheap

# Phylogenetics: Species boundaries of Lake Victoria Cichlids

- Radiation <15 000 ya
- Ongoing hybridisation




Baird *et al.* 2012

# RAD: Tissue to data

- Thousands of loci
- Non model organism
- Relatively cheap

- Caveat: Keep sources of error in mind
  - Experimental design
  - Wet Lab
  - Illumina Sequencing
  - SNP calling & filtering

# Thanks for listening!