



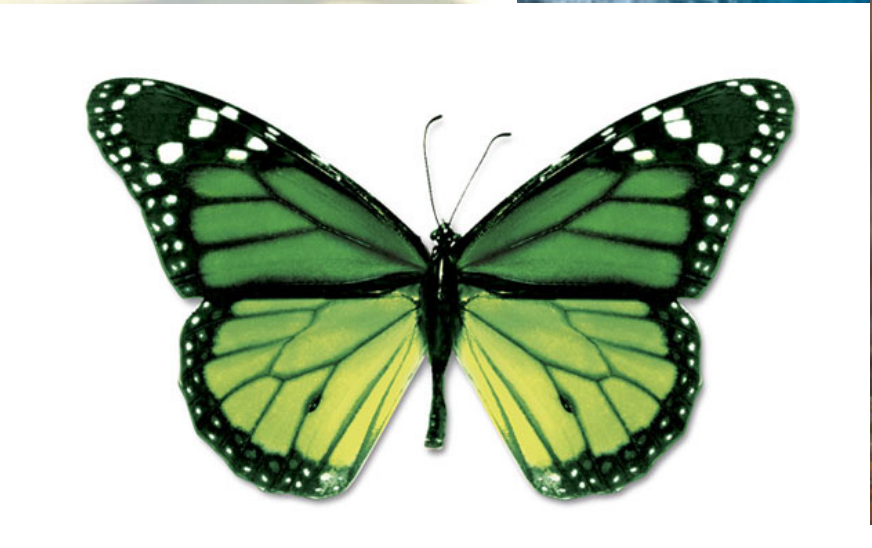
# Reduced representation libraries/RAD Introduction

Niklaus Zemp  
24 June 2020

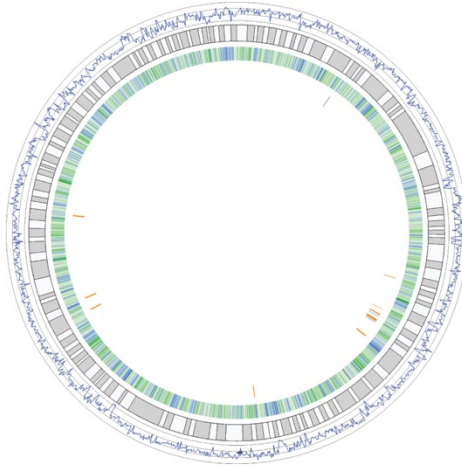
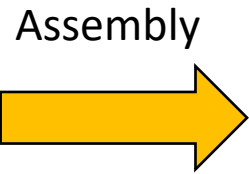
Genetic Diversity Centre (GDC)  
Bioinformatics  
ETH Zurich



# Non-model organisms



# Genomics of large and unexplored genomes



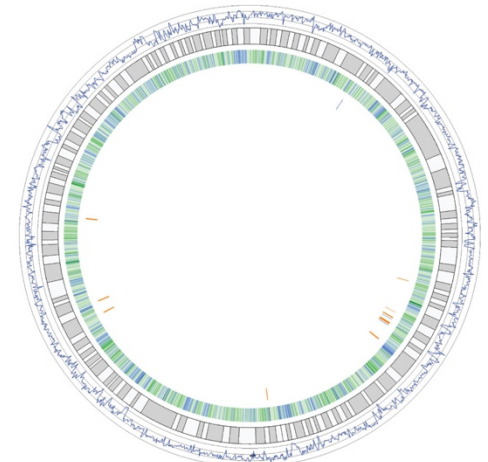
draft genome



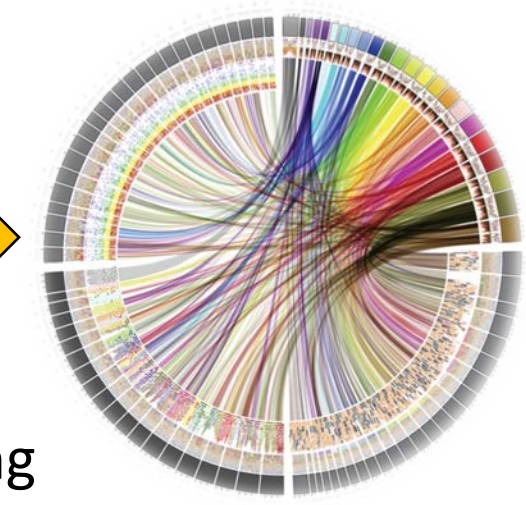
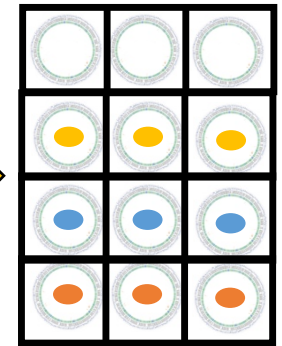
# Genomics of large and unexplored genomes



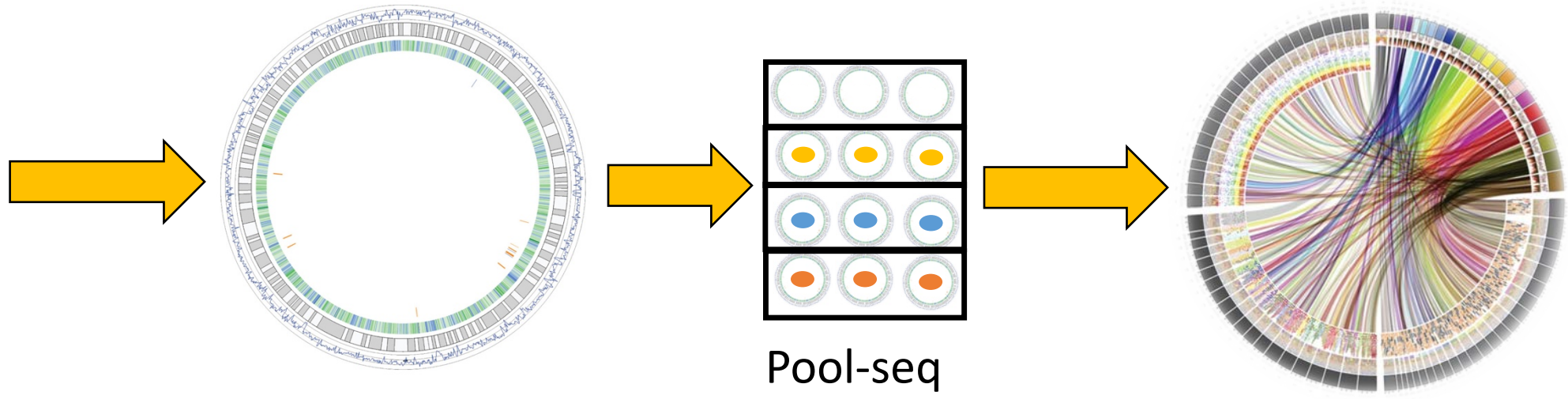
Assembly



(low coverage) resequencing

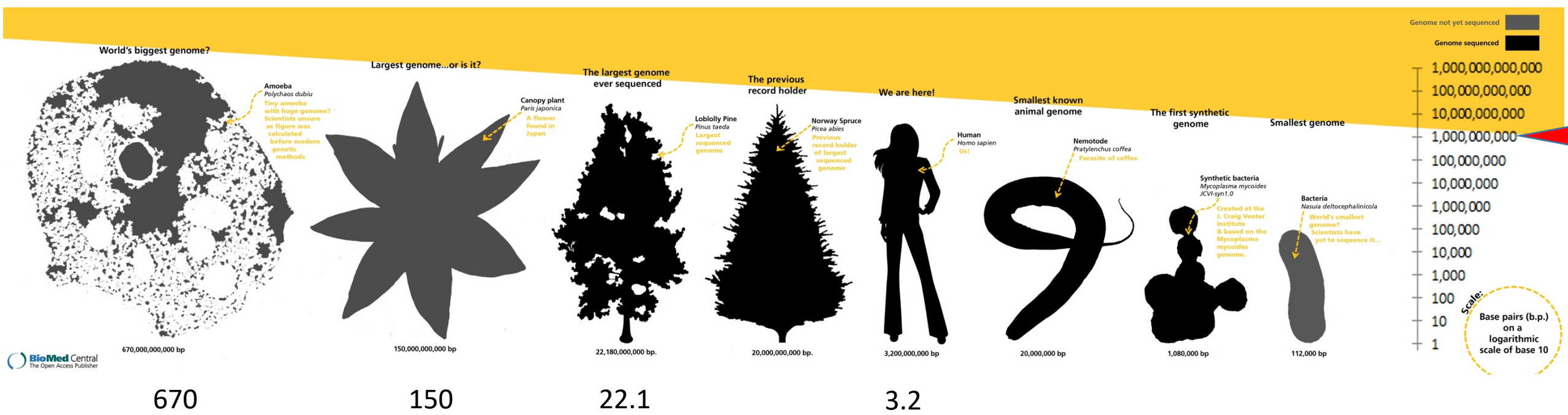


# Genomics of large and unexplored genomes





# Genomes can be large



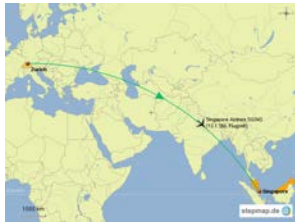
# Genomes can be large



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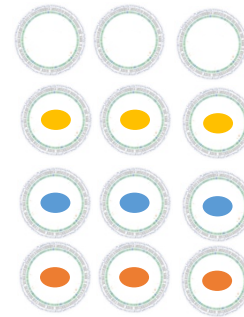
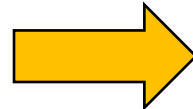
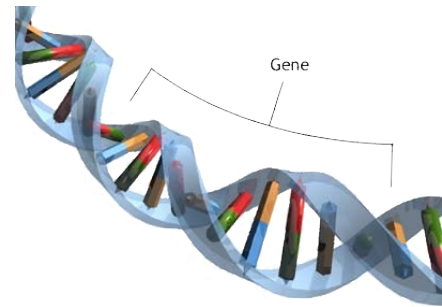
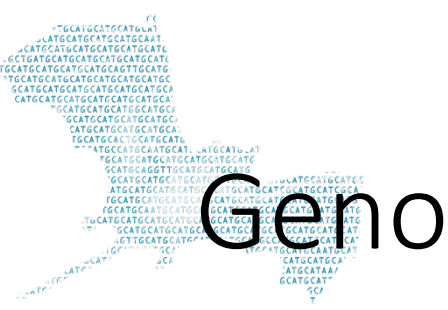
>1 year

2 weeks

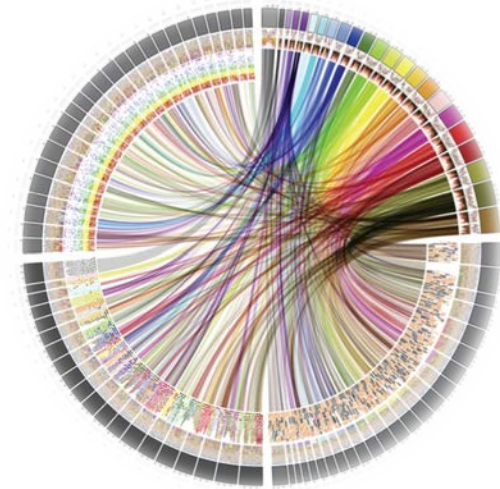


1 million bases is 1 minute

# Genomics of large and unexplored genomes

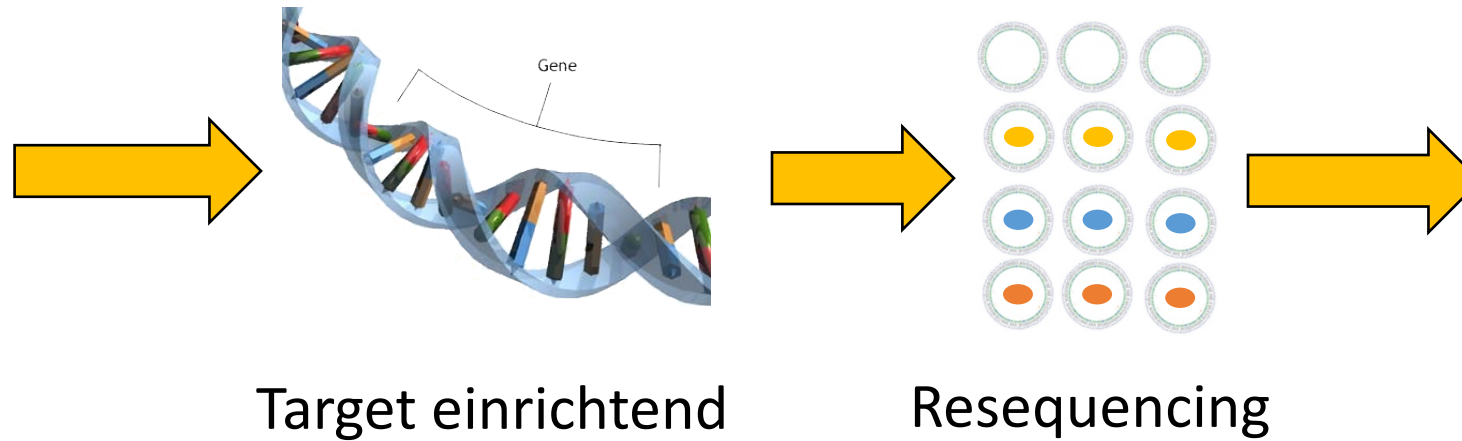


RNA-seq

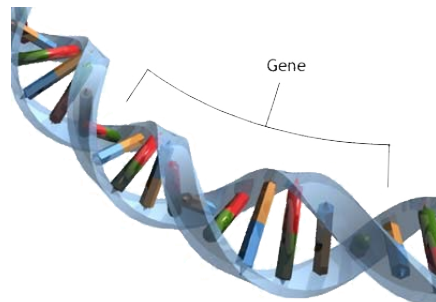
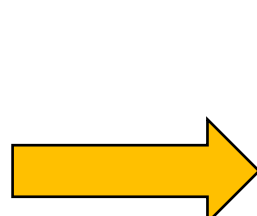




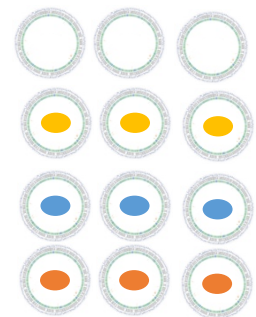
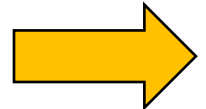
# Genomics of large and unexplored genomes



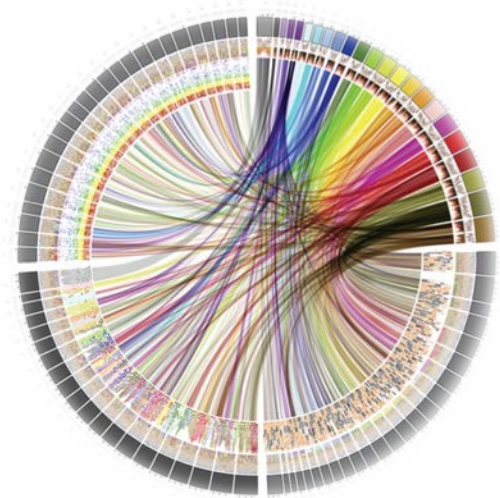
# Genomics of large and unexplored genomes



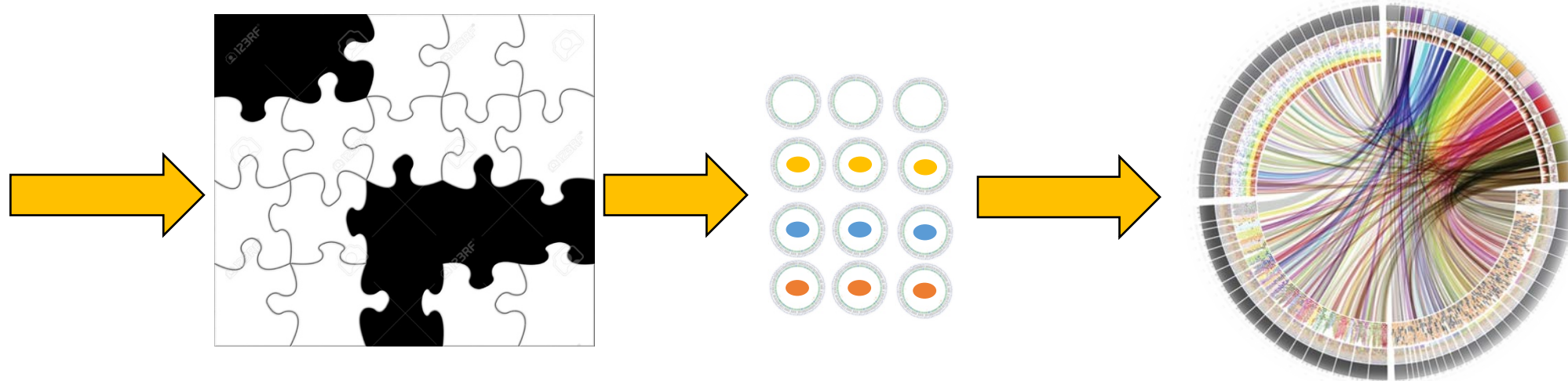
Target einrichtend



Resequencing

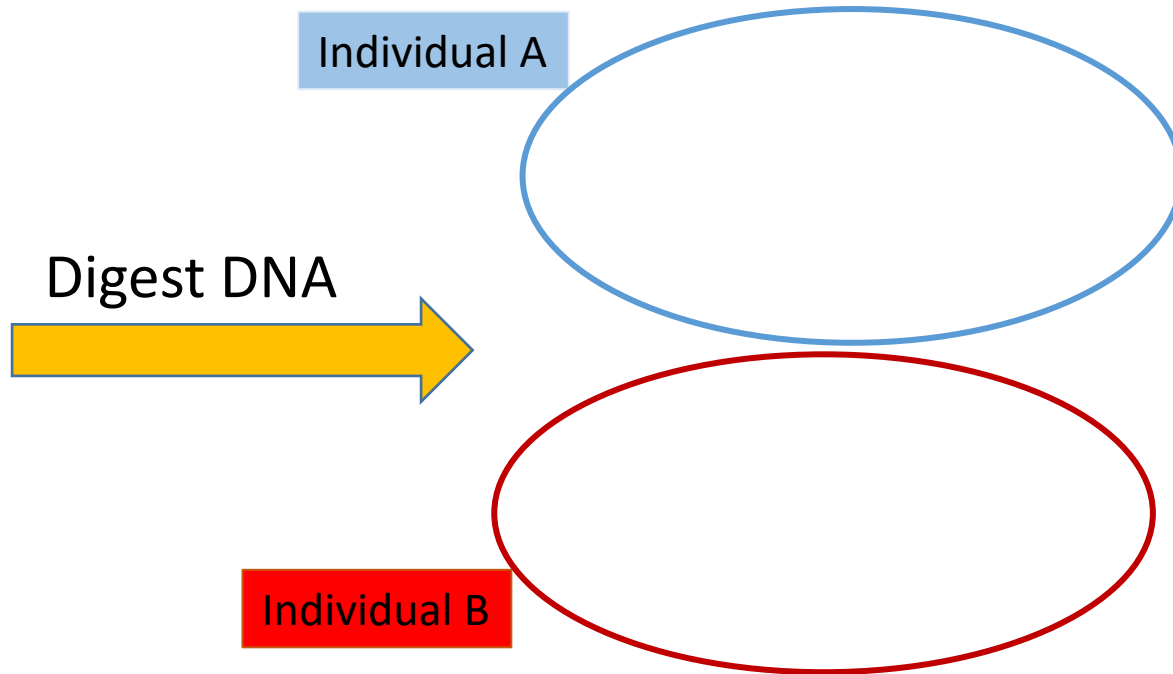


# Genomics of large and unexplored genomes

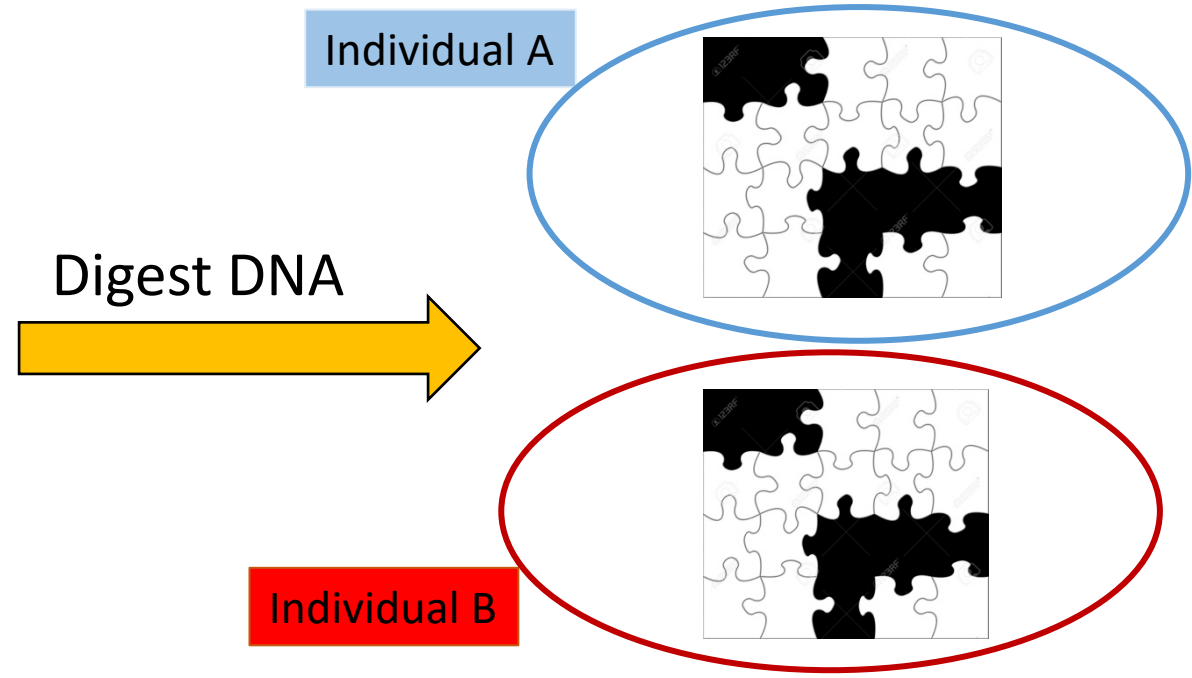


Reduced representation libraries/  
Restriction site associated **DNA** (RAD)

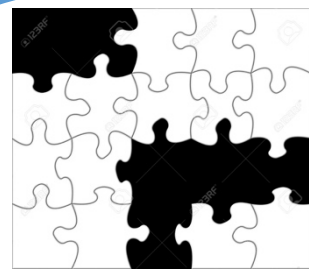
# Reduced representation libraries (RAD)



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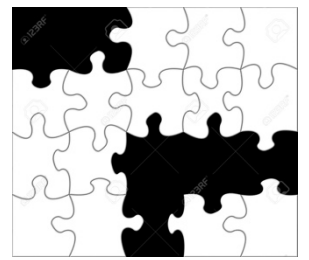
Individual A



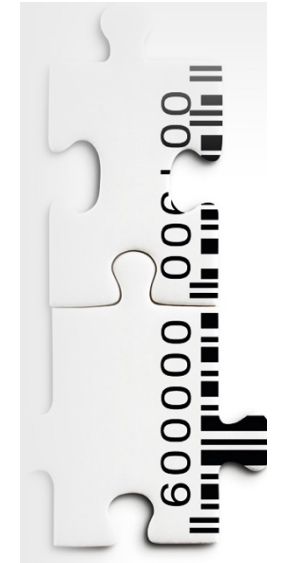
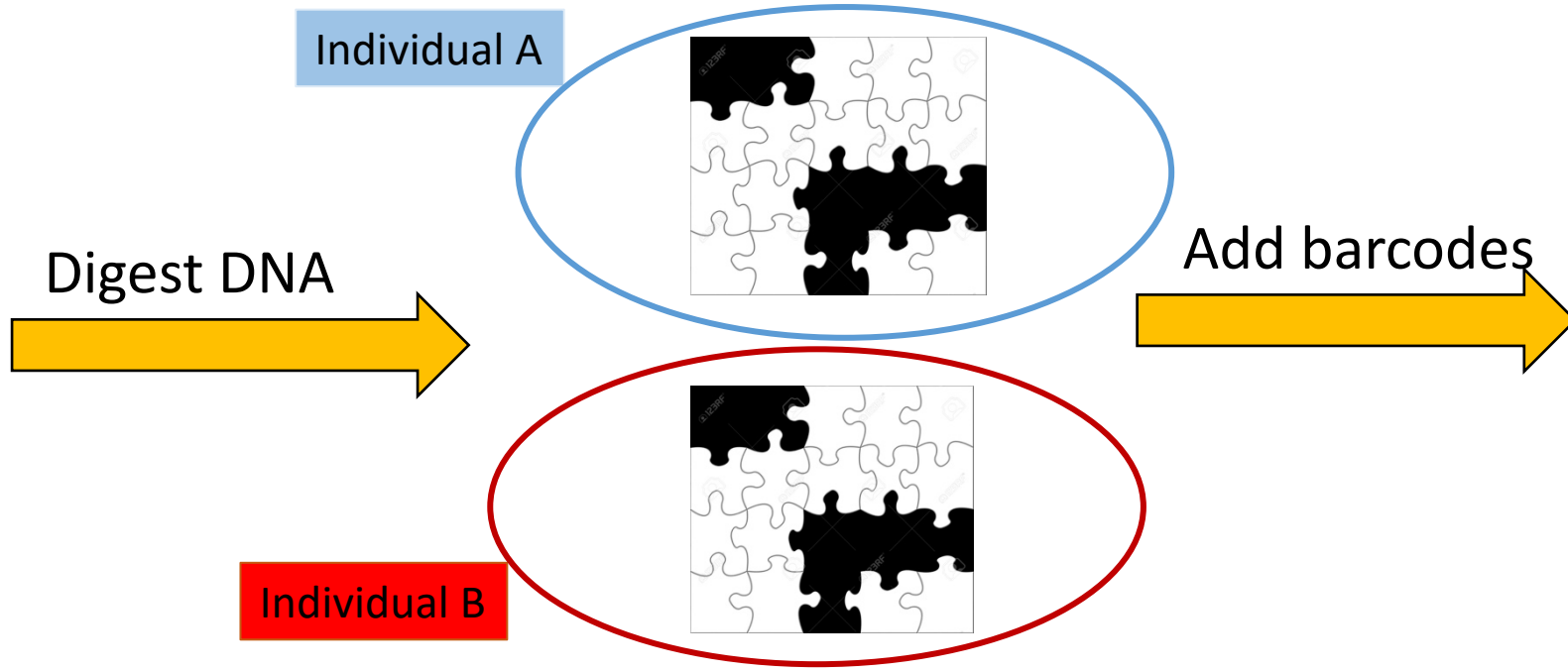
Digest DNA



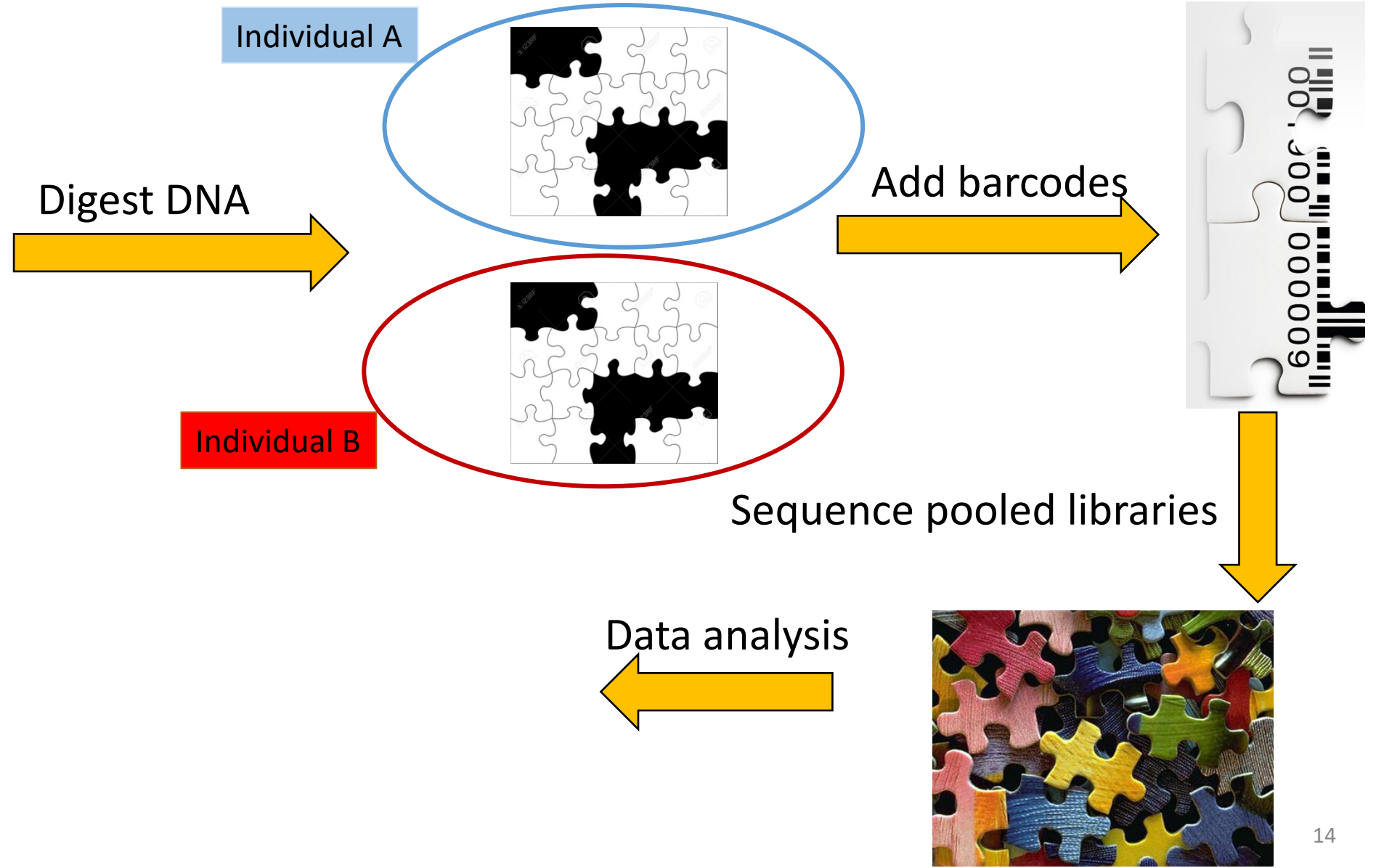
Individual B



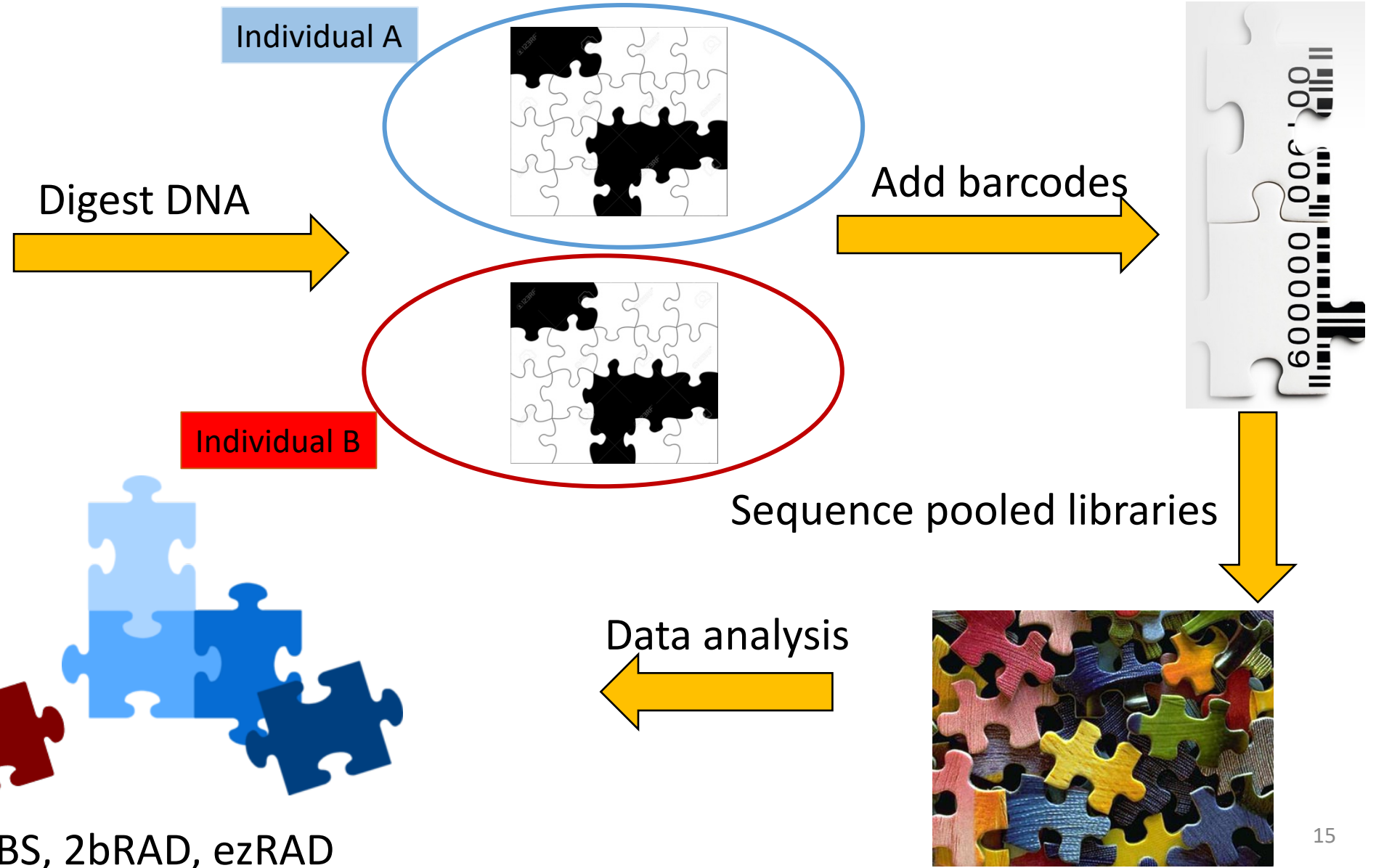
# Reduced representation libraries (RAD)



# Reduced representation libraries (RAD)

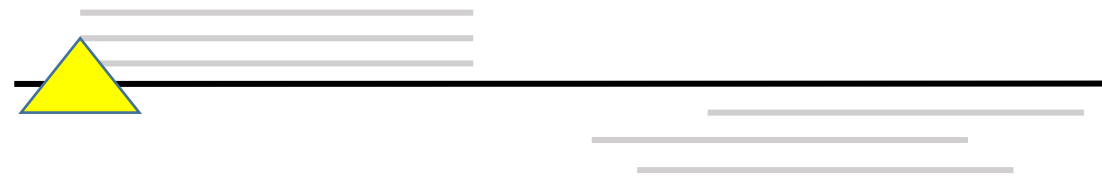
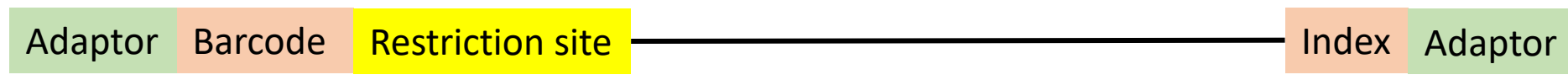


# Reduced representation libraries (RAD)

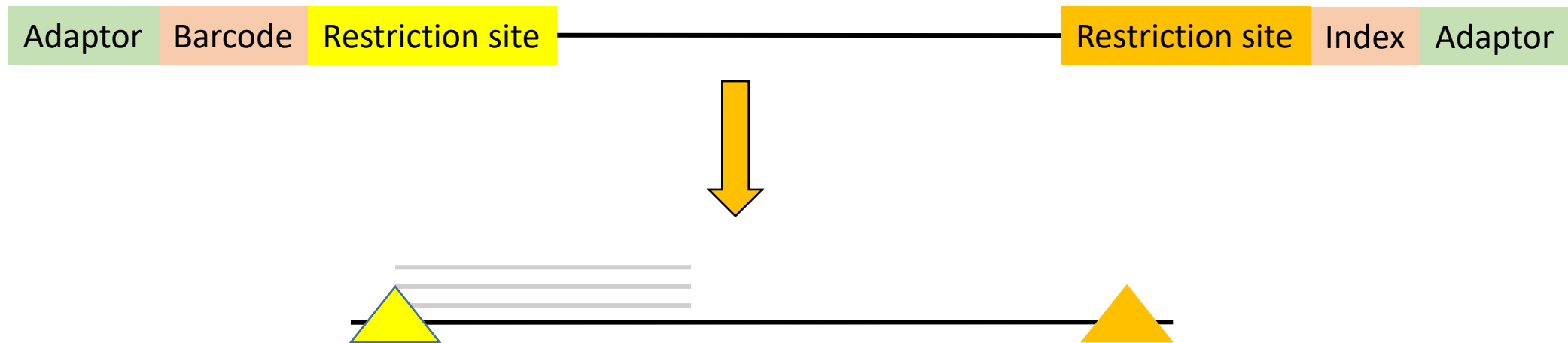


mbRAD, ddRAD, GBS, 2bRAD, ezRAD





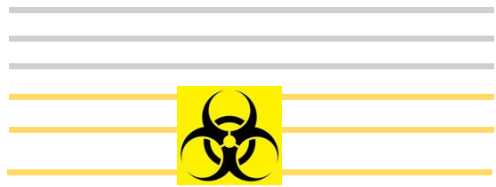
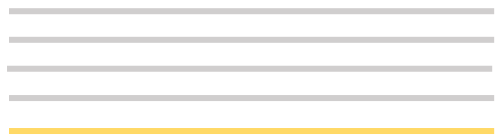
- Digestion with one rare cutting enzyme
- Barcoded adapters ligated to fragments
- Ligated fragments are then sonicated
- Size selection is used to reduce sampled genome
- Paired-end reads



- Digestion with one rare and one common cutter
- Barcoded adapters ligated to fragments
- Size selection is used to reduce sampled genome
- Often single-end reads

# mbRAD versus ddRAD

	<b>mbRAD</b>	<b>ddRAD</b>
<b>Costs</b>	higher	lower
<b>PCR duplicates</b>	can be detected	not possible
<b>Allele dropouts</b>	reduced	increased





# How many fragments do I get?

- Restriction enzyme
- $\propto$  Genome size
- $\propto$  Sequencing depth

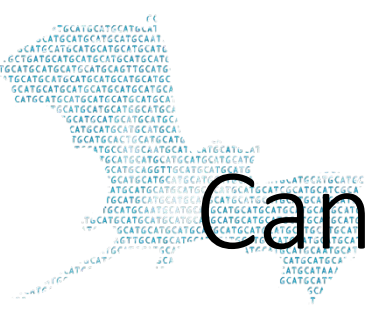
## Genome available:

*In silico* digest (simRAD)

## *de novo*:

Predictions are possible based on the concentration but a test run is often needed

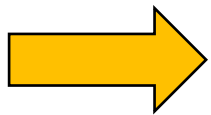




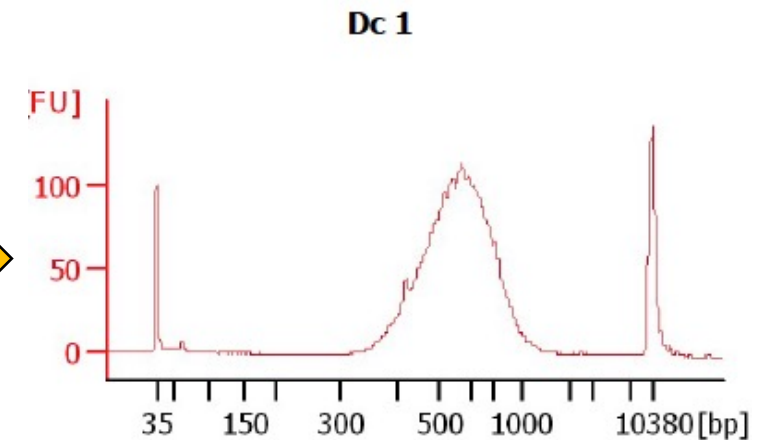
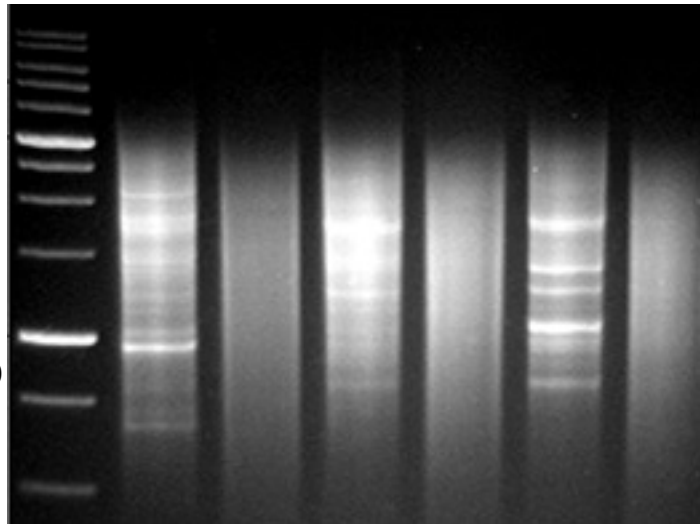
# Can I produced these libraries at the GDC?

Primers sets and protocols for producing RAD and ddRAD libraries are available

200-500 ng high quality DNA



500 bp





# Take home message

- Useful and inexpensive approach for non model organisms
- For many species applicable

