H3ABioNet

Pan African Bioinformatics Network for H3Africa

16SrRNA Intermediate Bioinformatics Online Course: Int_BT_2019

Module 3:

Sample collection, extraction and library preparation for 16S NGS analyses

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Learning Outcomes

Describing different aspects of planning for 16S experiments; for example study design, DNA extraction methods and laboratory workflows.

- Understand what is meant by the term “16S rRNA gene” and why we are interested in this gene.
- Understand the concepts behind Sanger sequencing and high-throughput sequencing
- Know why it is important to clearly plan an experiment and how different components to the study and experimental design may influence data generated and downstream analyses.
- Know that techniques other than 16S rRNA sequencing are also available to study microbial profiles.

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Module 3:

Sample collection, extraction and library prep for 16S NGS analyses

Part 3.1

16S rRNA high throughput sequencing: how it works

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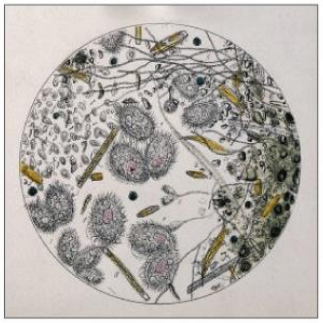
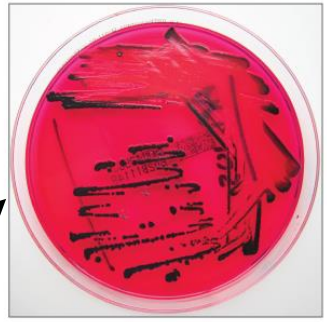
16S rRNA high throughput sequencing: how it works

circa 1600: Microscope invented

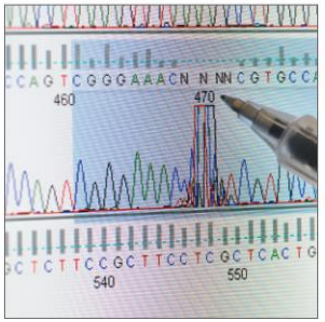


mid-1600s: First microbes described

1800s: Connection made between microbes and disease



1800s – Present: Culture, staining, and microscopy used to study microbes that can be cultured



1990s: DNA sequencing becomes available, allowing study of microbes that cannot be cultured

Advantages: Study viable organisms
Disadvantages: Thought that less than 1% of all bacterial species are cultivatable

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16S rRNA high throughput sequencing: how it works

Culture-independent molecular methods:

Marker gene-dependent

↓

Fingerprinting techniques

Sanger sequencing

High-throughput sequencing

↓

16S rRNA gene

All organisms need ribosomes to make protein

Any gene that makes up ribosome may be a good marker gene

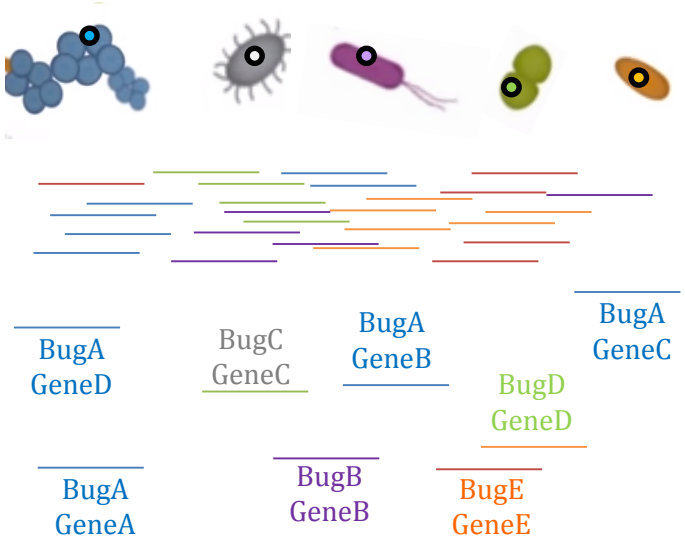
Ribosomal RNA (rRNA) never gets transcribed to protein

Gene focussed on: **16SrRNA gene** (~1500bp)

Marker gene-independent

↓

High-throughput sequencing (shotgun sequencing)



https://www.youtube.com/watch?v=8Aa_mnyXm70

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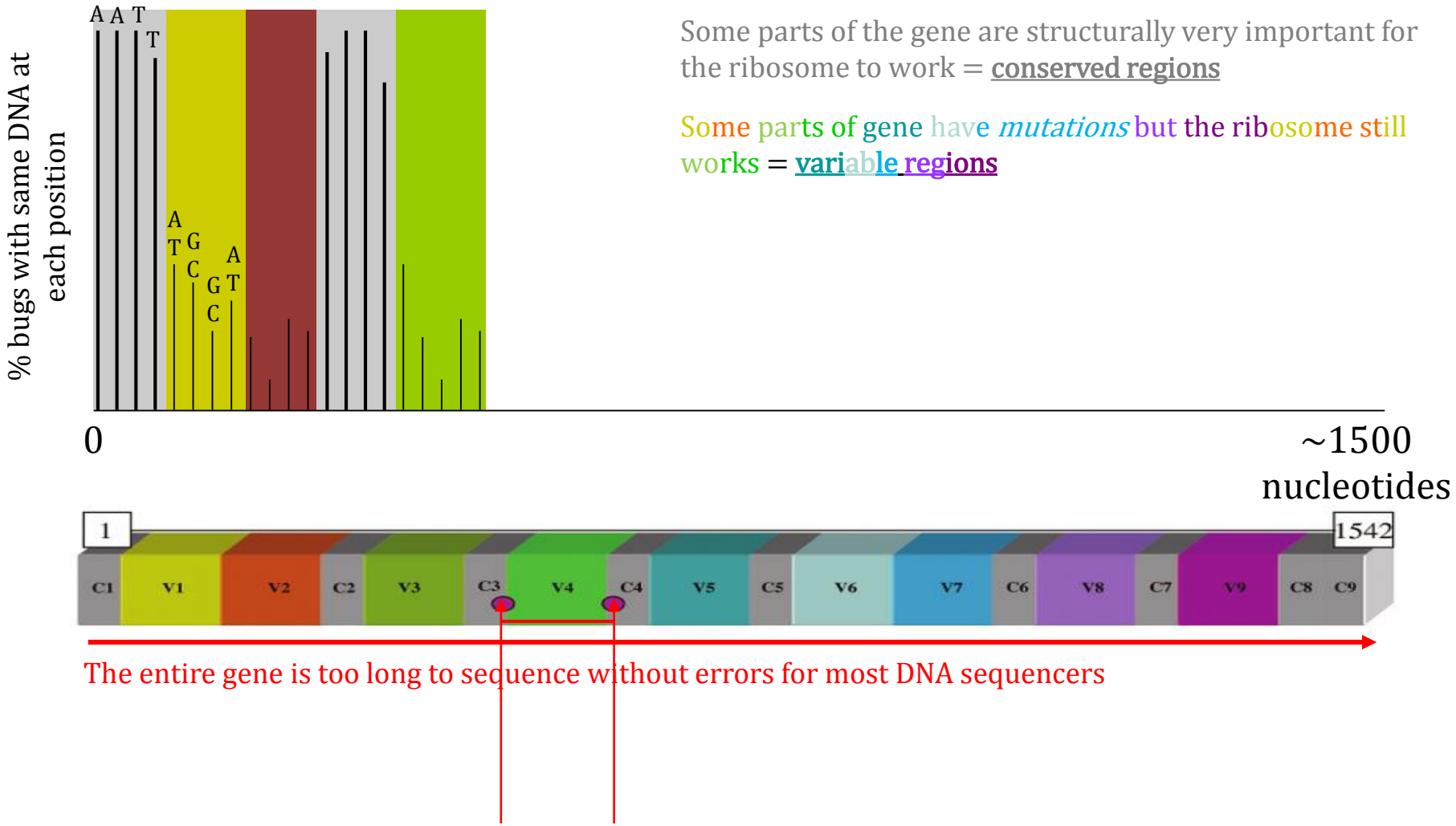
16S rRNA high throughput sequencing: how it works

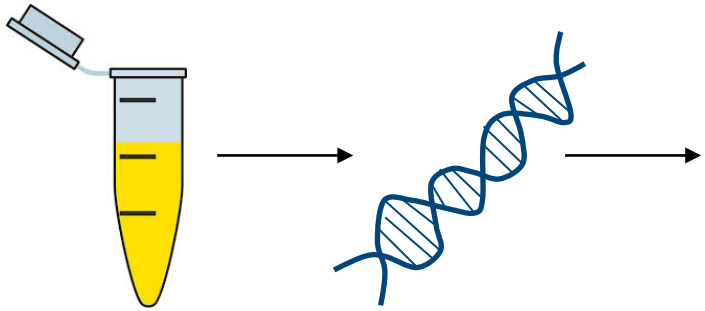


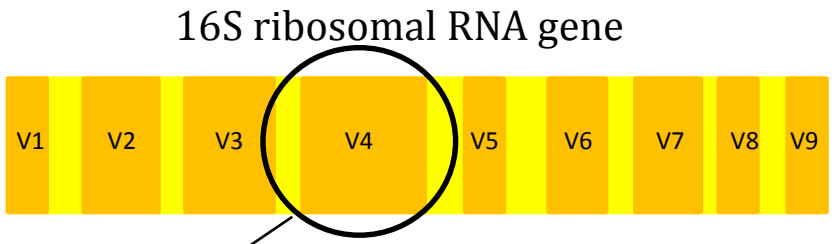
Some parts of the gene are structurally very important for the ribosome to work = conserved regions

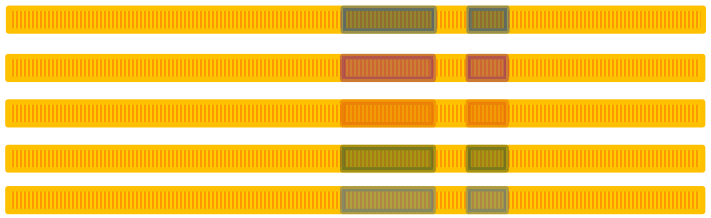
Some parts of gene have *mutations* but the ribosome still works = variable regions

https://www.youtube.com/watch?v=8Aa_mnyXm70

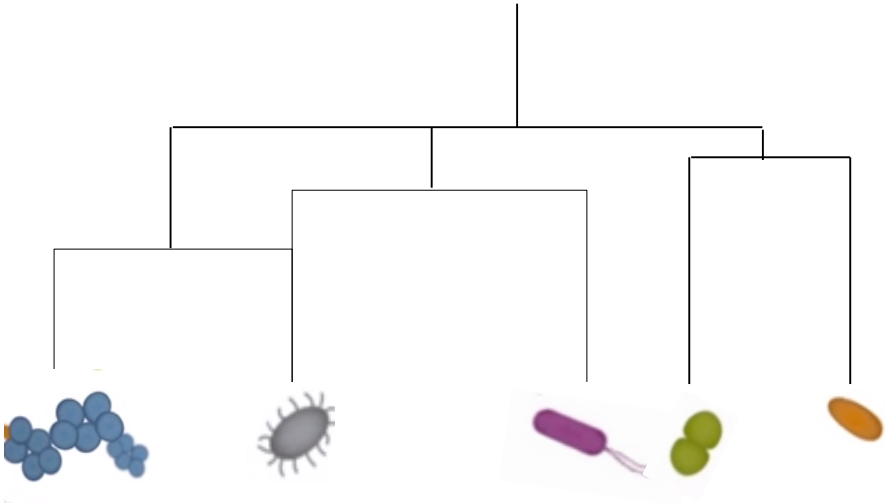
16S rRNA high throughput sequencing: how it works







DNA amplicons



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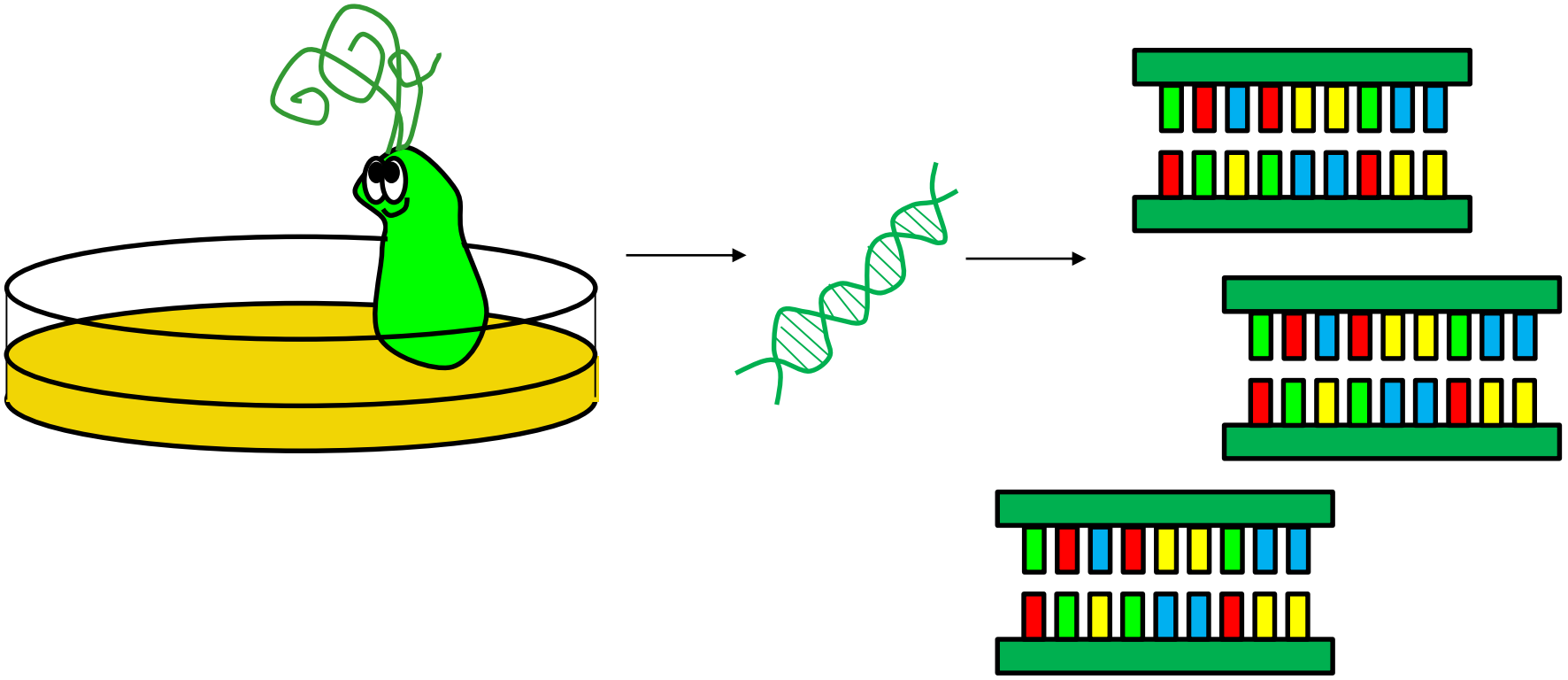
16S rRNA high throughput sequencing: how it works

Sanger method only sequences a single DNA fragment at a time



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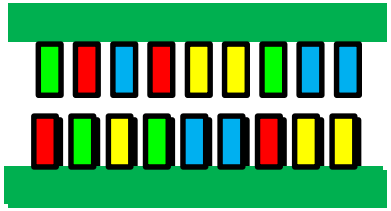
16S rRNA high throughput sequencing: how it works

Sanger method sequences a single DNA fragment at a time



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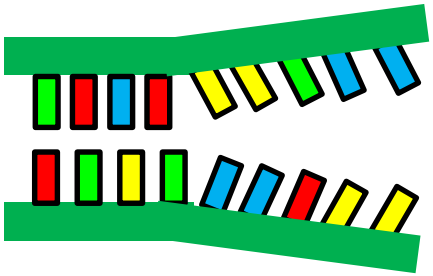
16S rRNA high throughput sequencing: how it works

Sanger method sequences a single DNA fragment at a time



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16S rRNA high throughput sequencing: how it works

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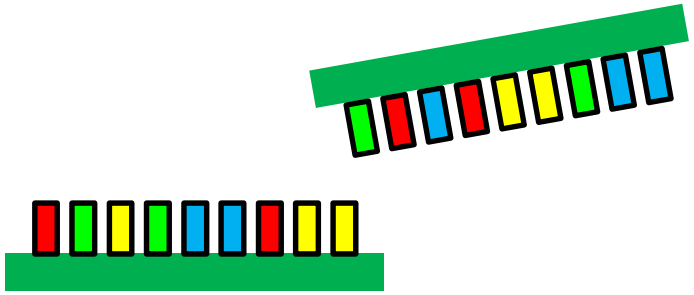

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16S rRNA high throughput sequencing: how it works

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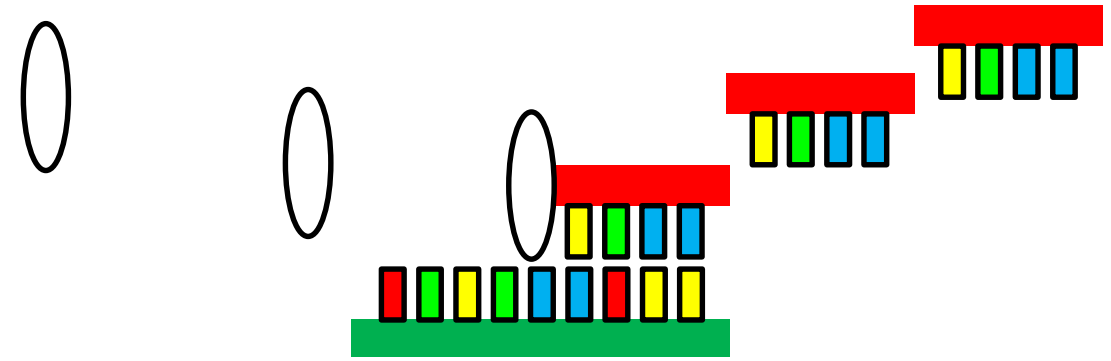

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16S rRNA high throughput sequencing: how it works

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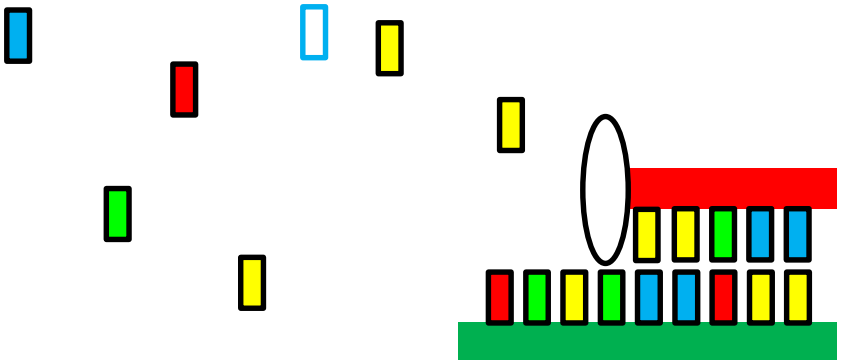

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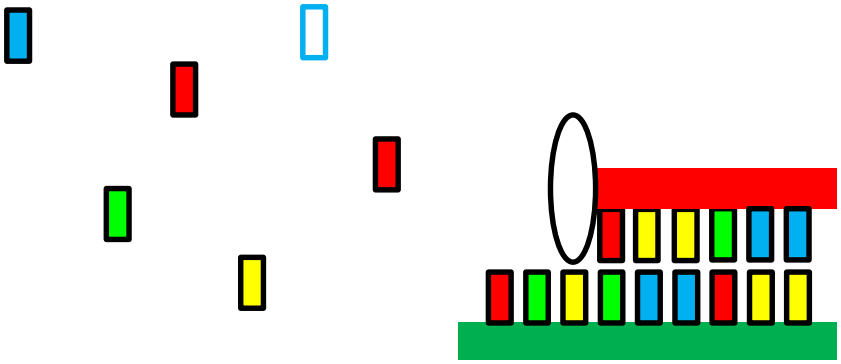

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16S rRNA high throughput sequencing: how it works

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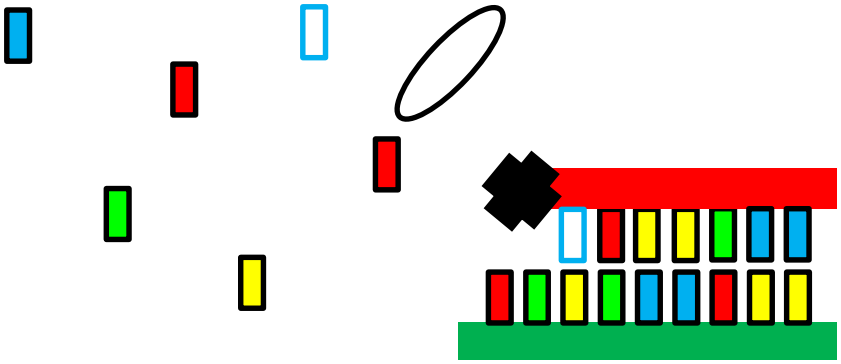

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16S rRNA high throughput sequencing: how it works

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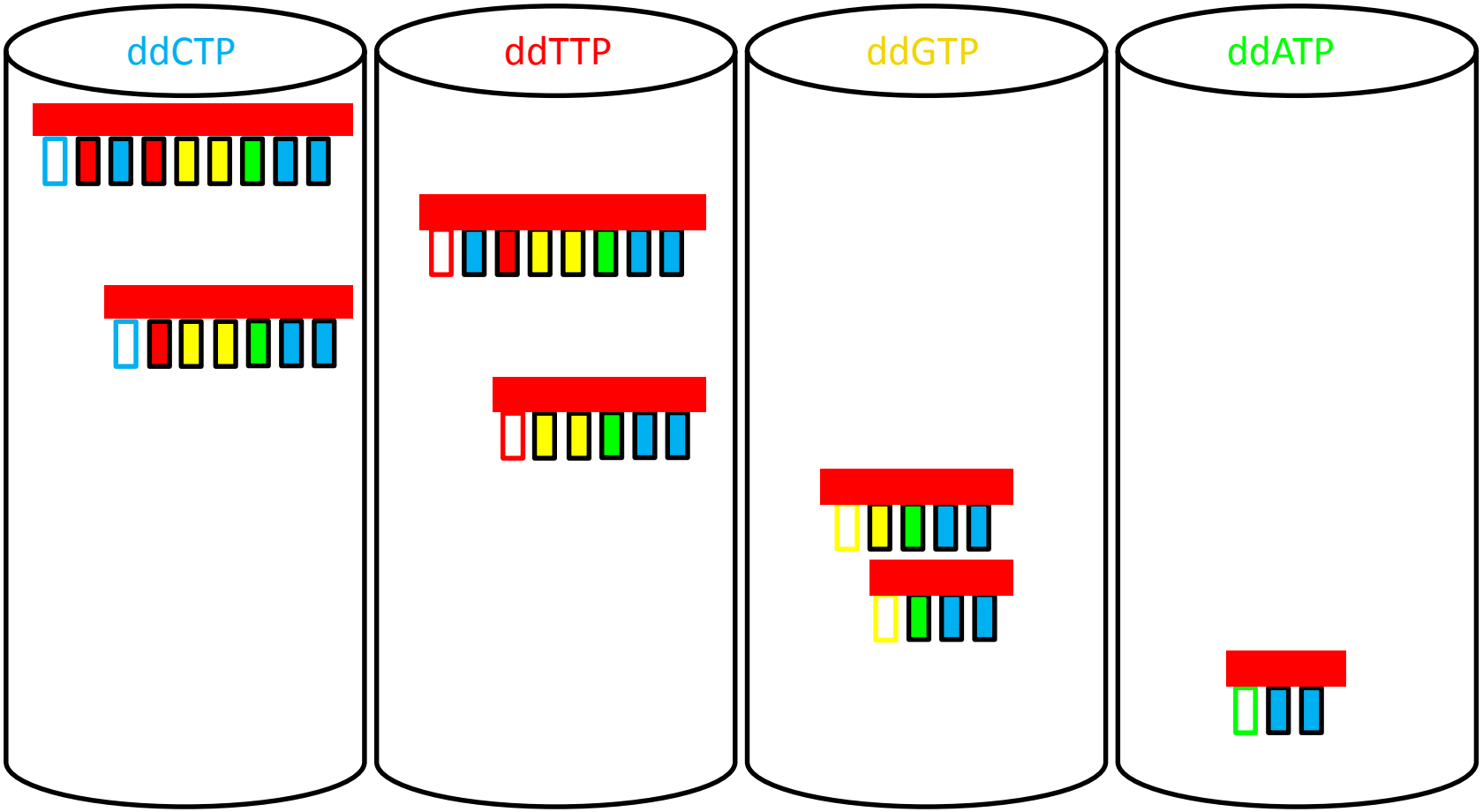

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16S rRNA high throughput sequencing: how it works

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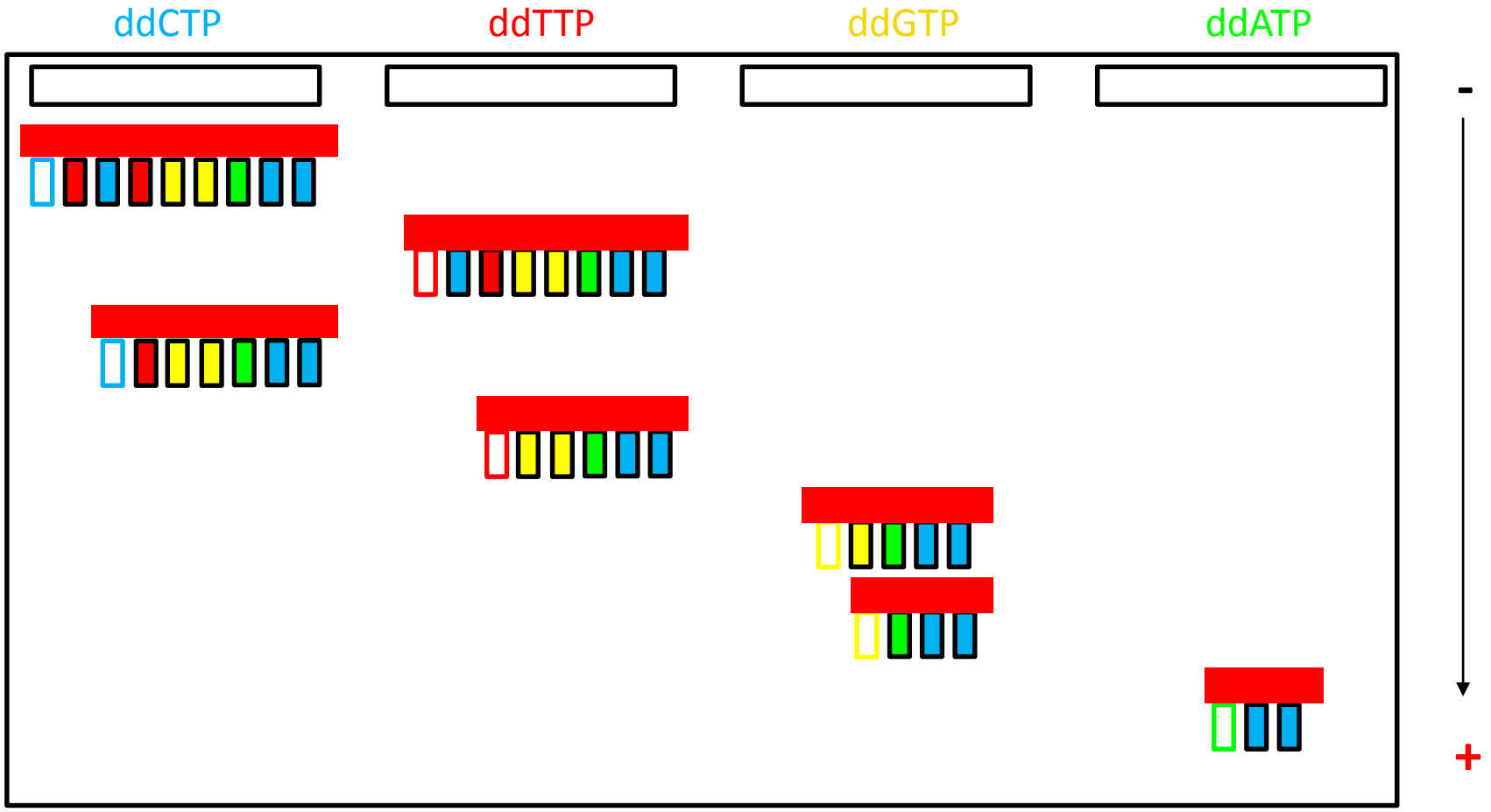

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16S rRNA high throughput sequencing: how it works

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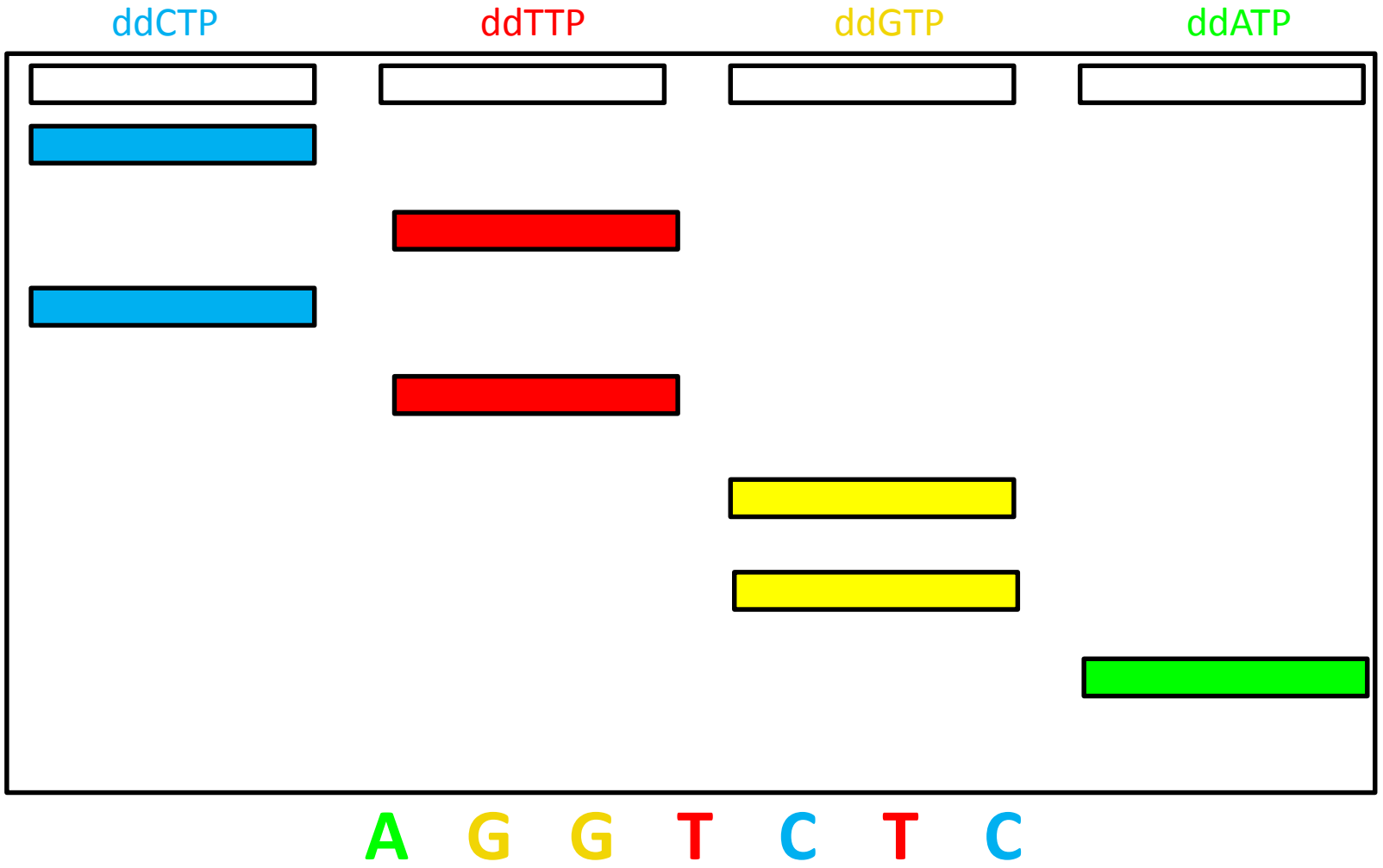

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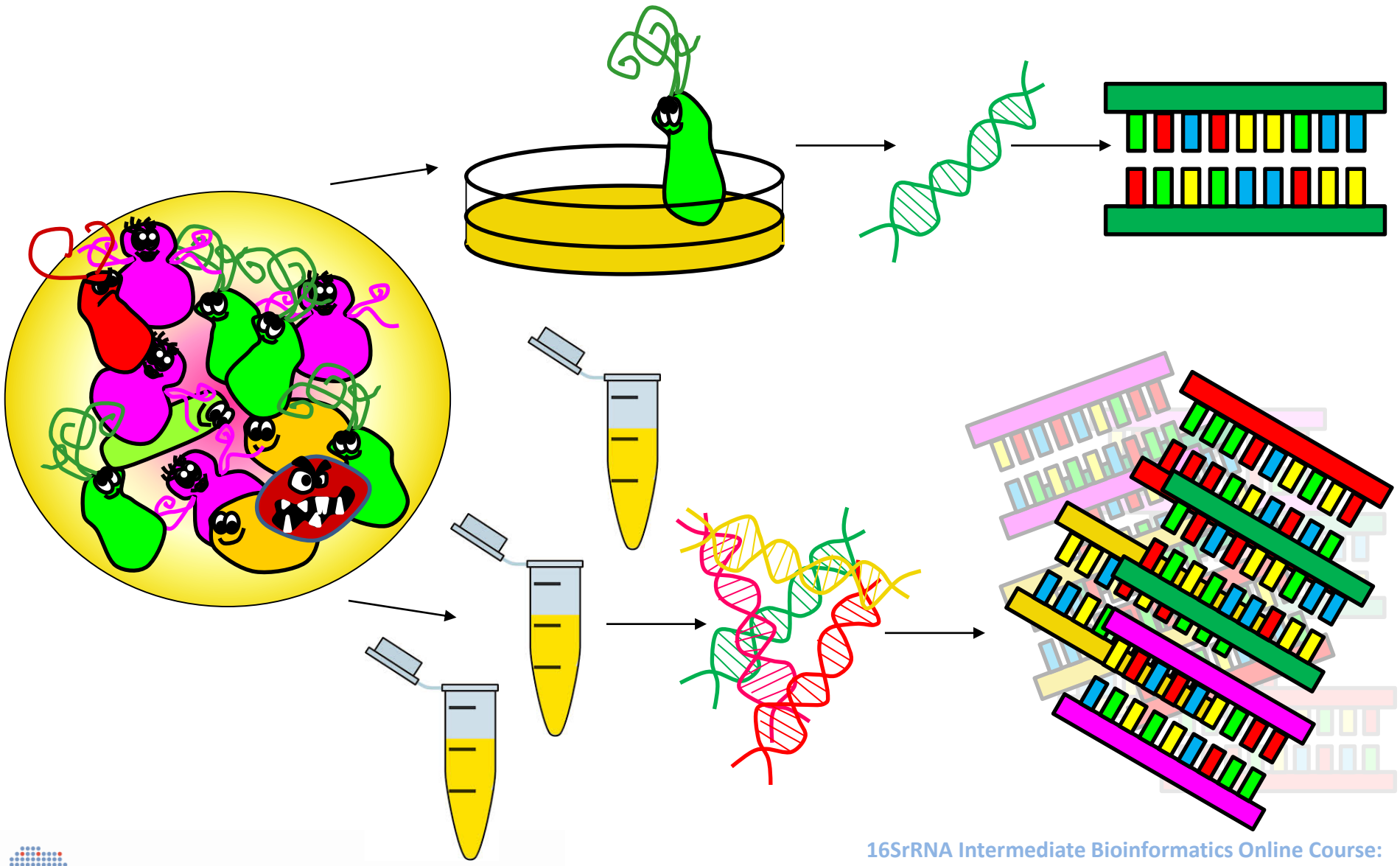
16S rRNA high throughput sequencing: how it works



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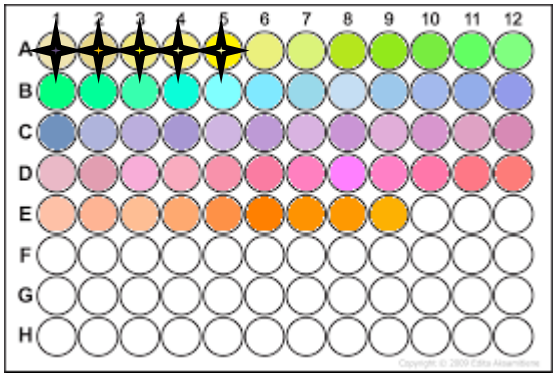
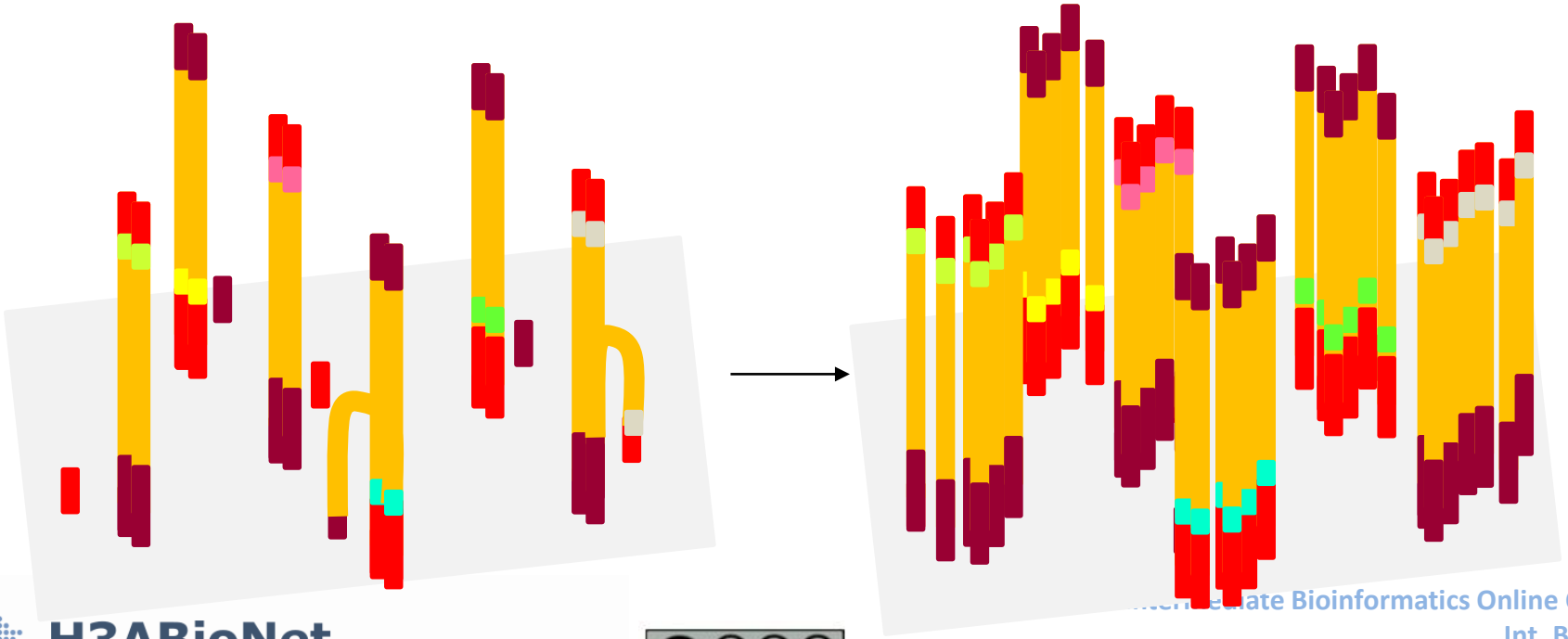
16S rRNA high throughput sequencing: how it works

DNA from a number of samples



Undergo amplification using a multiplex approach



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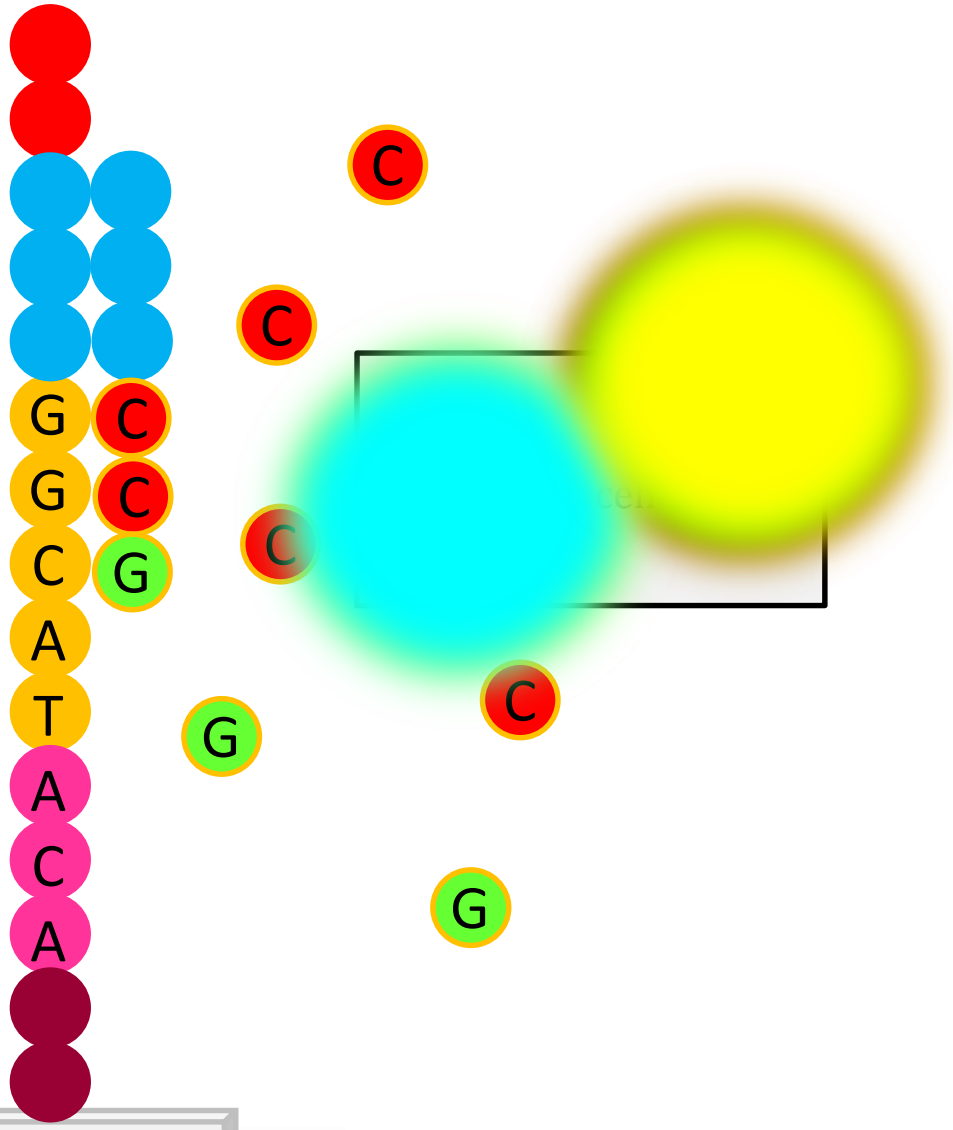
16S rRNA high throughput sequencing: how it works



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16S rRNA high throughput sequencing: how it works

In summary:

- 16S rRNA gene is by far the most common housekeeping gene targeted to study bacterial phylogeny and classification.
- High throughput sequencing targeting the 16S rRNA gene allows for large quantities of DNA to be sequenced much more quickly and cheaply compared to Sanger sequencing.
- High throughput sequencing of the 16S rRNA gene allows for identification as well as relative abundance quantification of all bacteria present in a sample.
- 16S rRNA high-throughput sequencing allows for processing multiple samples together via the use of barcoded (indexed) primers.

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