

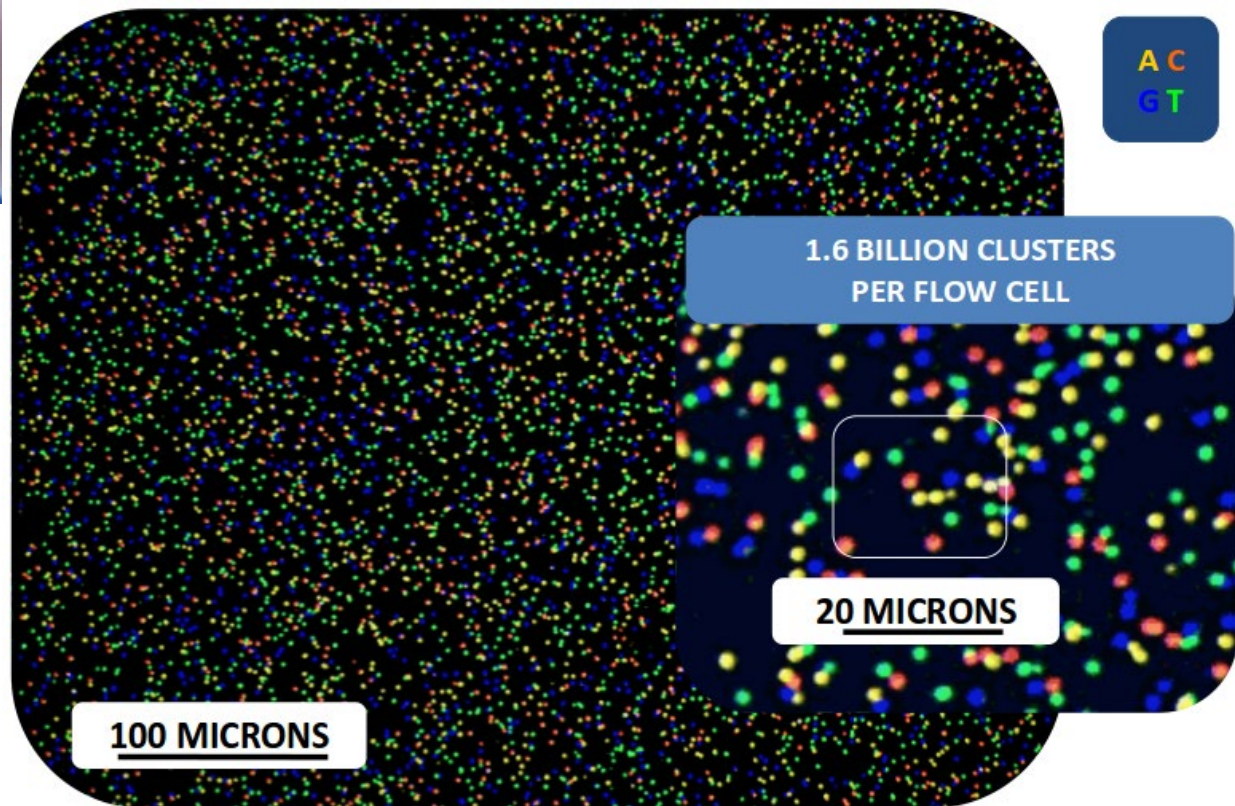
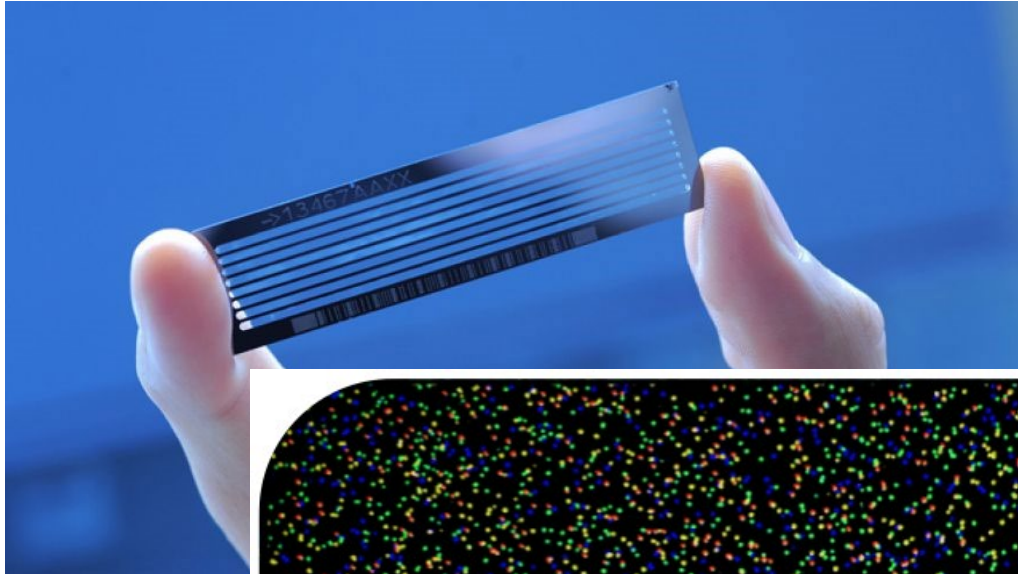
Biol 432

Transcriptome sequencing

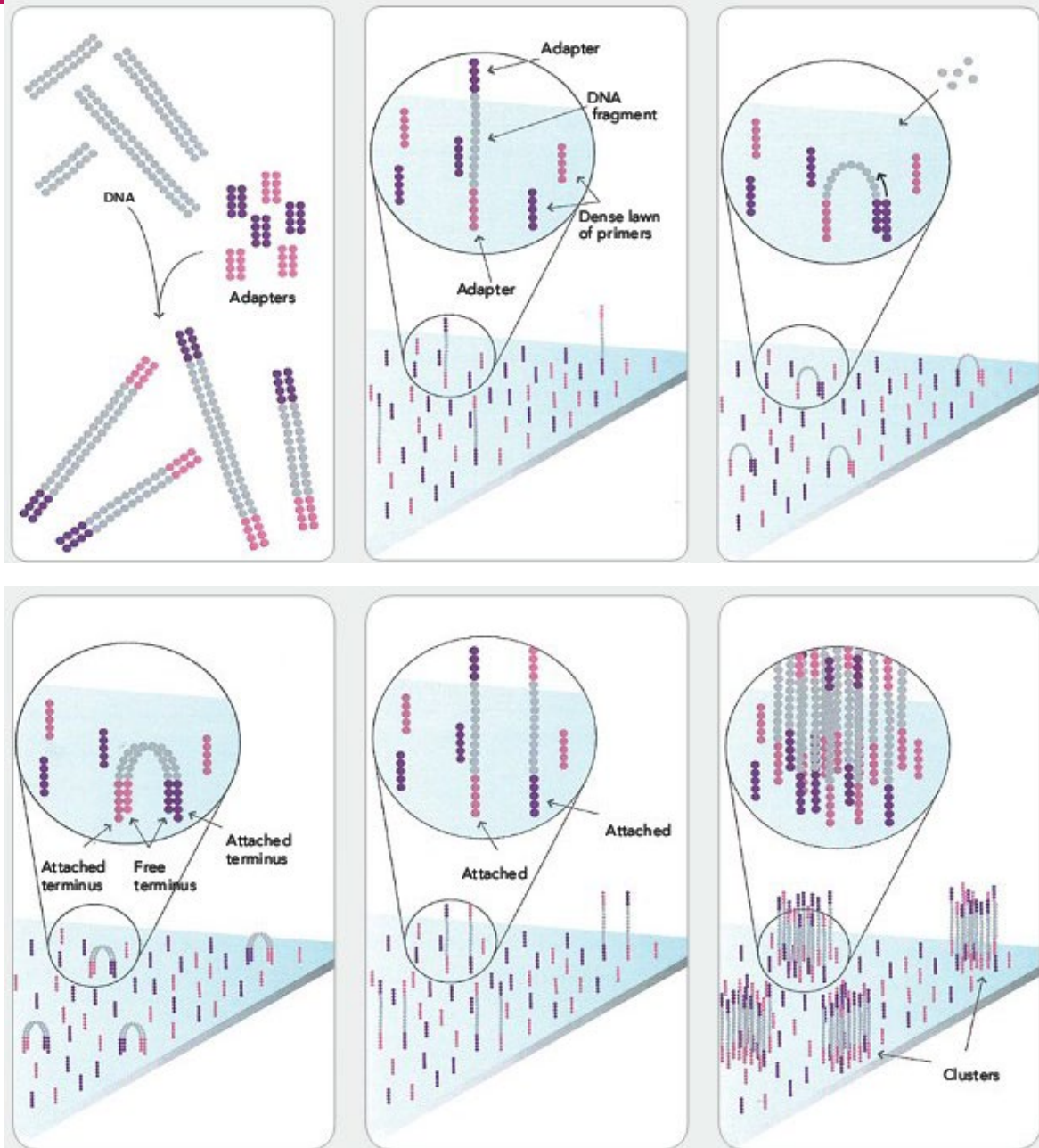
Sequencing Review

High-throughput sequencing (HTS)

Illumina flow cells

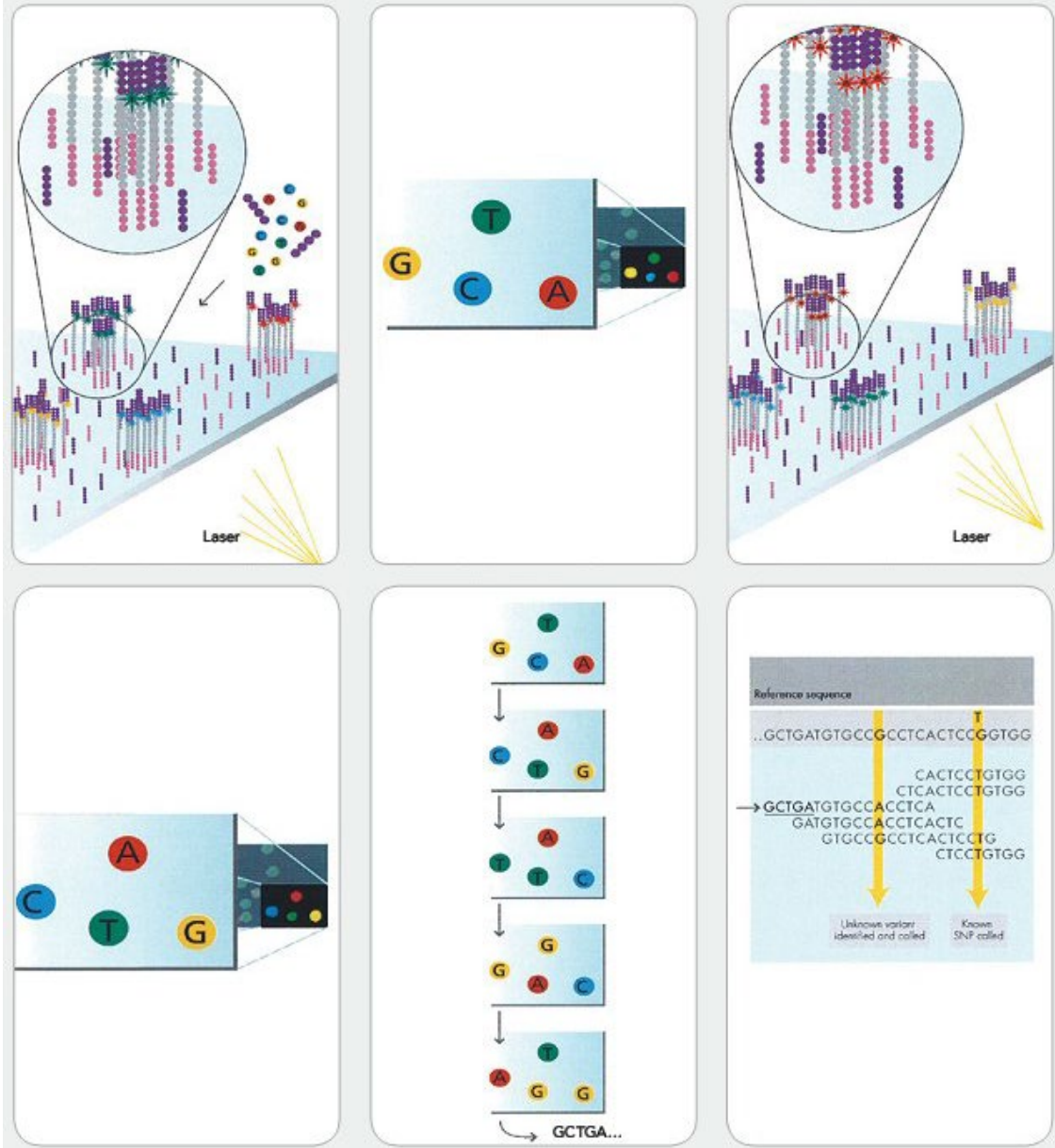


Illumina sequencing (formerly Solexa)



1. Prepare genomic DNA
2. Attach DNA to surface
3. Bridge amplification
4. Fragment become double stranded
5. Denature the double stranded molecules
6. Complete amplification

Illumina sequencing



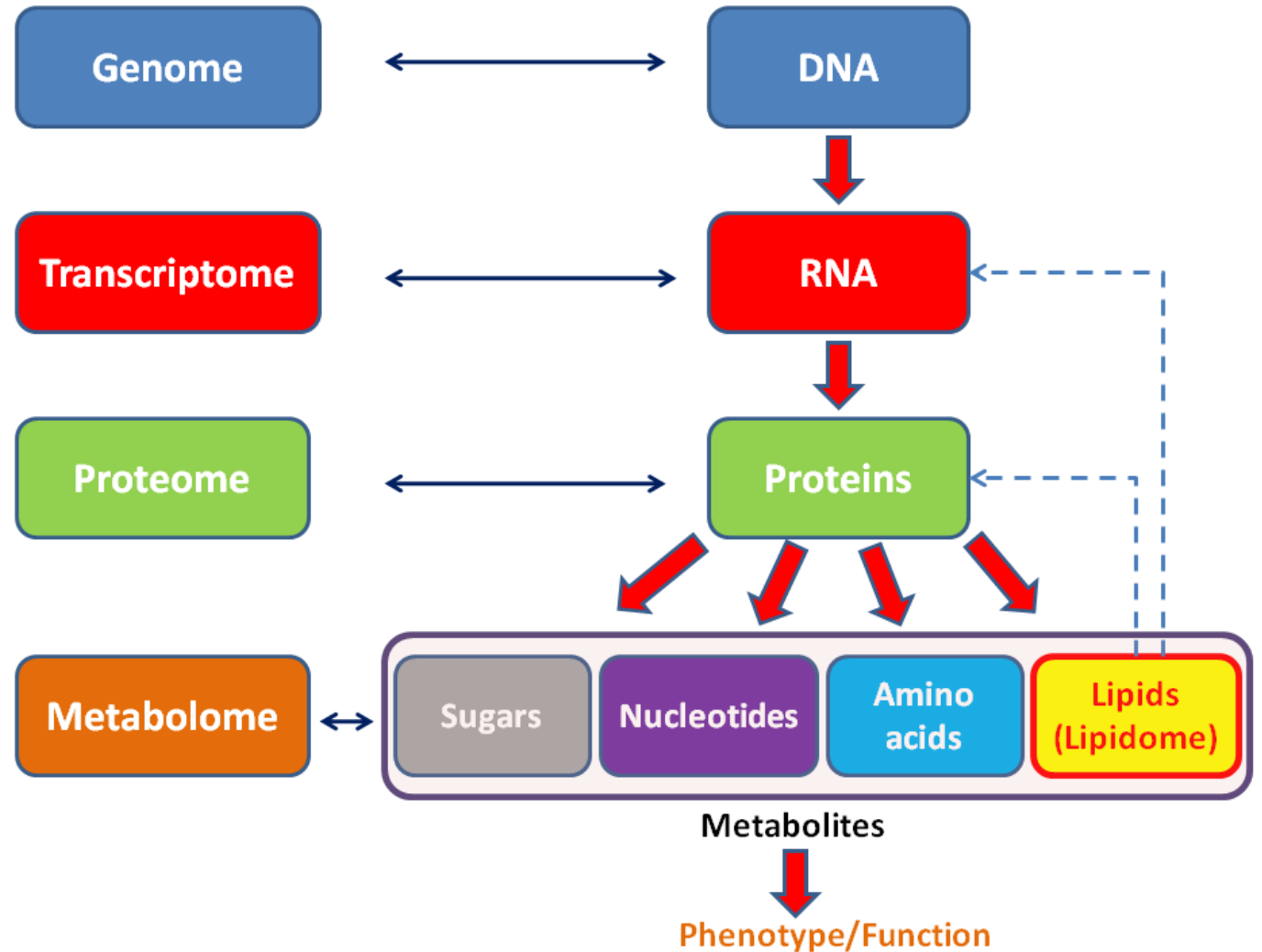
7. Determine first base
8. Image first base
9. Determine second base
10. Image second base
11. Sequence reads over multiple cycles
12. Align data

Review of 'omics'

Genome – DNA content
of an organism

Exome – all coding genes
in a genome

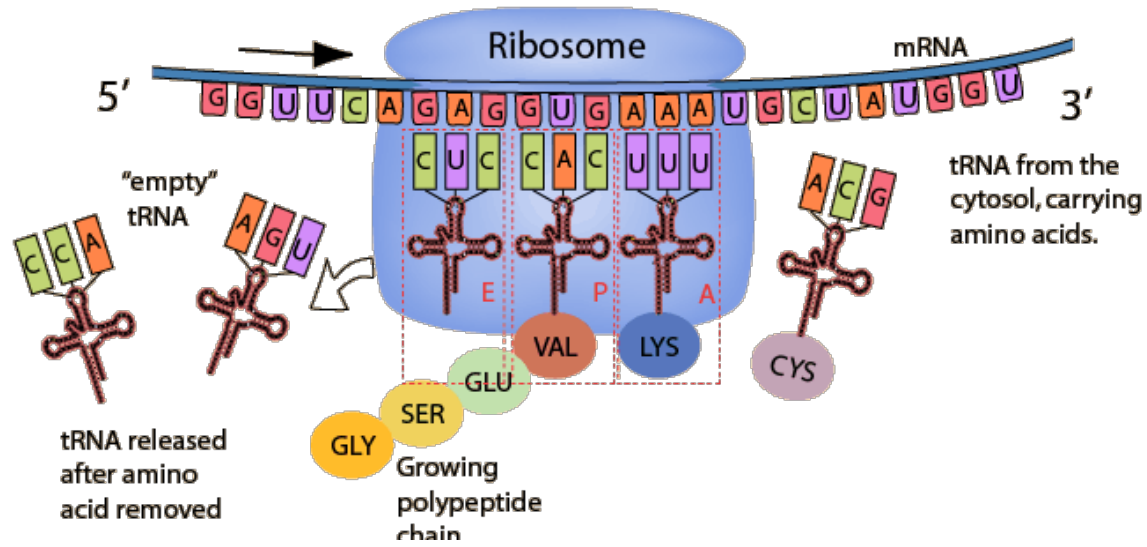
Transcriptome –
expressed RNA



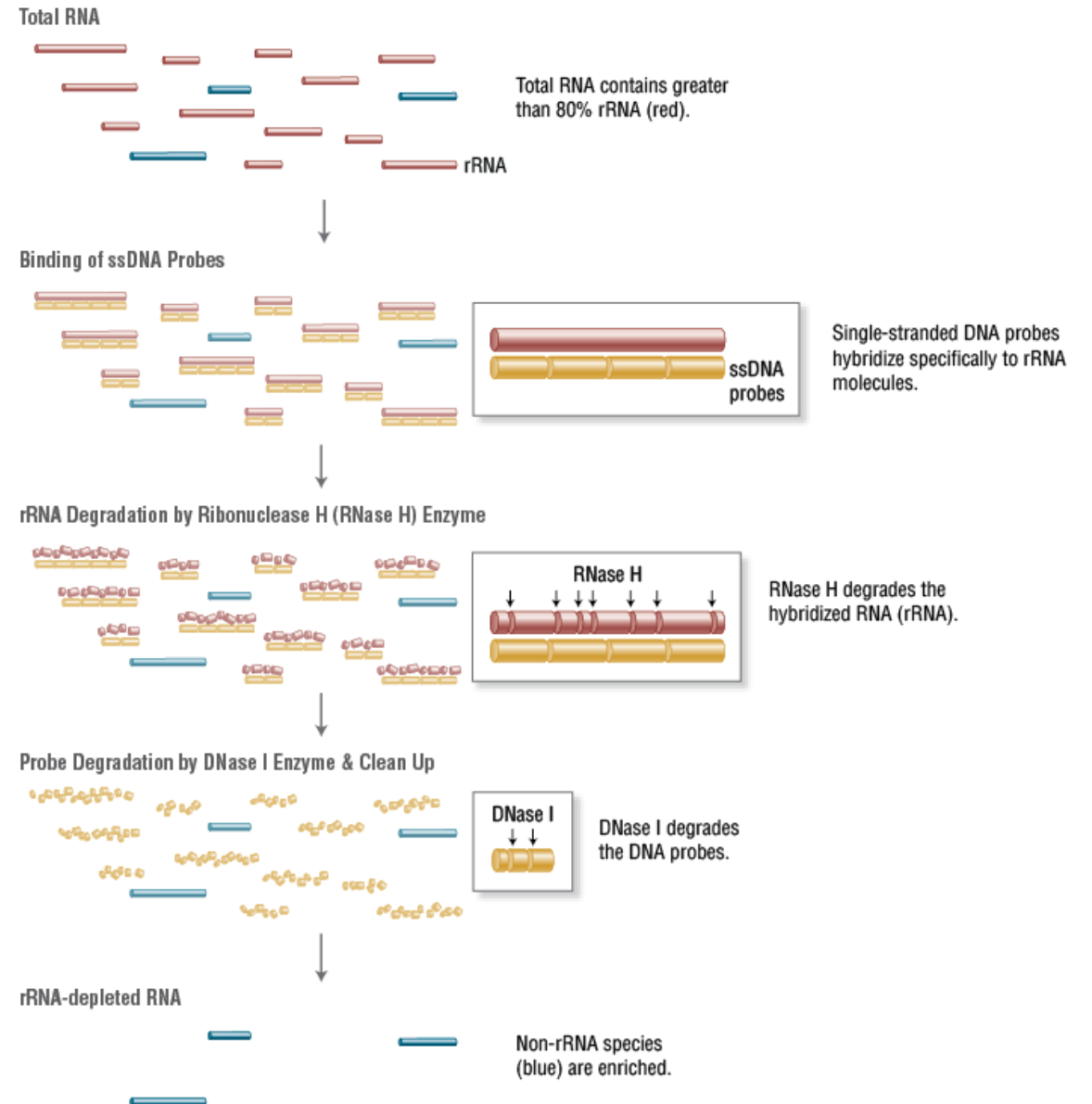
RNA sequencing



rRNA-Depleted whole RNA sequencing

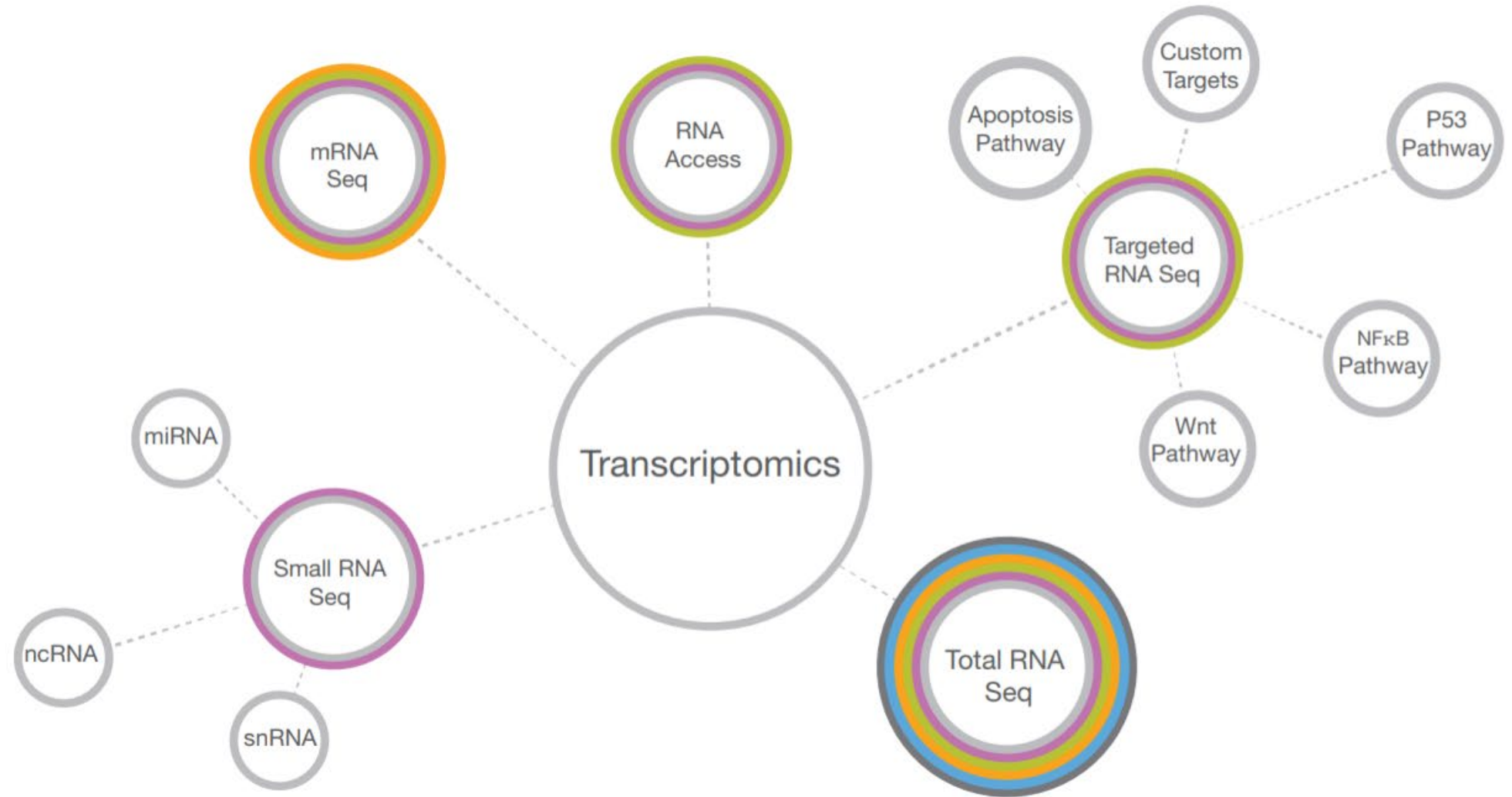


(Most intracellular RNA
Is ribosomal RNA)

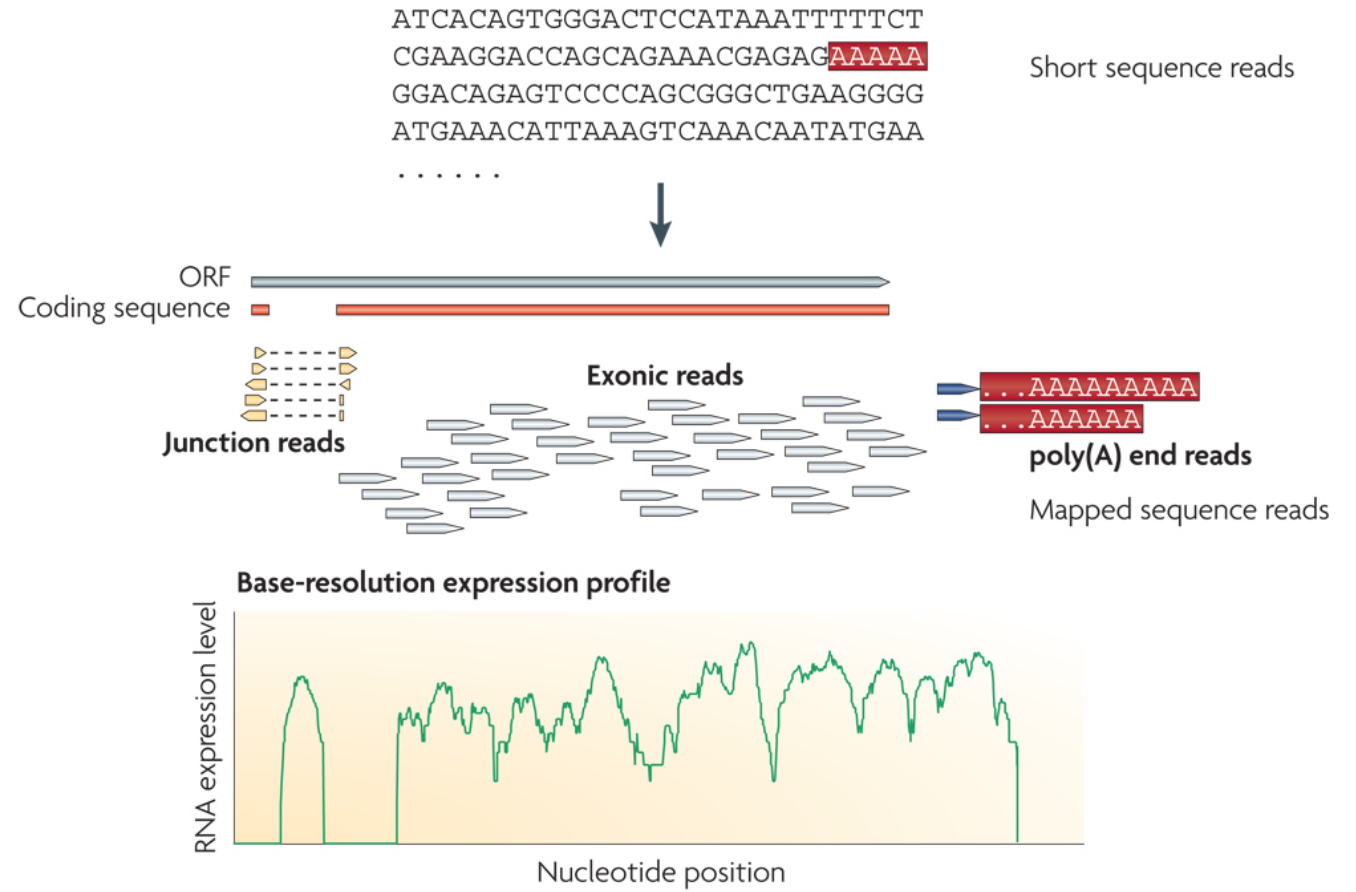


rRNA-Depleted transcriptomics

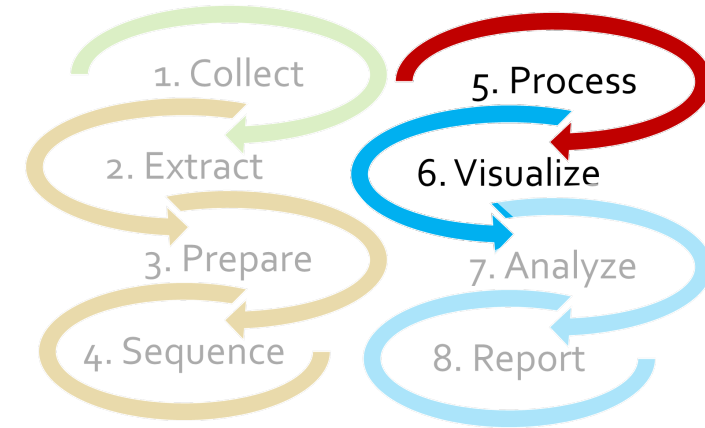
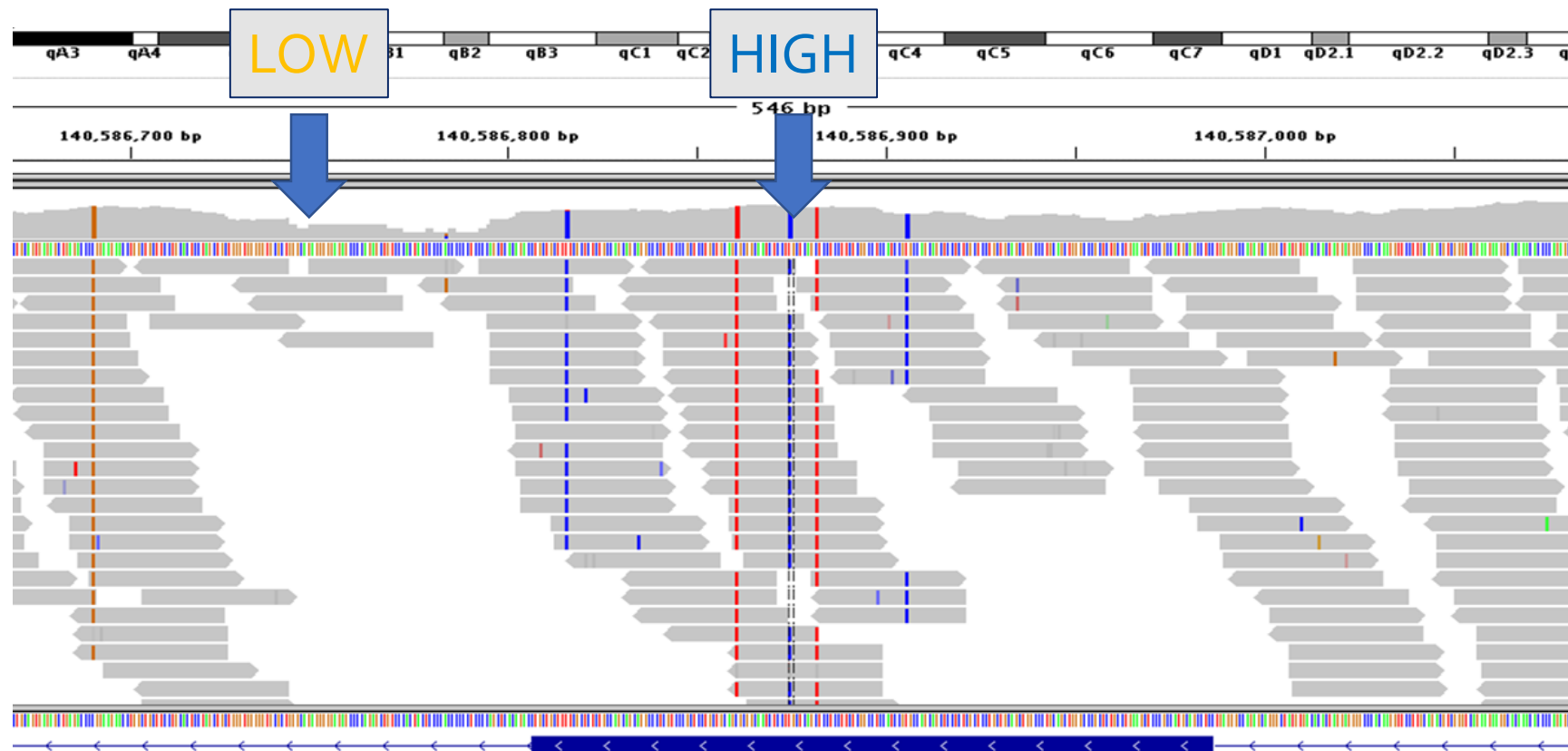
Illumina
Sequencing
Kits:



rRNA-Depleted transcriptomics



Alignment coverage (e.g. RNA-Sequencing)



Gene expression – number of sequences (or normalized/standardize/relative number of sequences)

Upregulated – genes that increase in expression

Downregulated – genes that decrease in expression

Differential expression analysis – looking for genes with changes in expression (i.e. upregulated or downregulated)

Brainstorm three different biological applications where transcriptome analysis could be used.

e.g. Test which genes of a crop plant are differentially expressed (i.e. upregulated/downregulated) following exposure to a herbivore.