



Module 2: Measuring gene expression

Introduction to DLD-1 and RNA-seq

03/05/20

DNA is the hereditary material in all known organisms

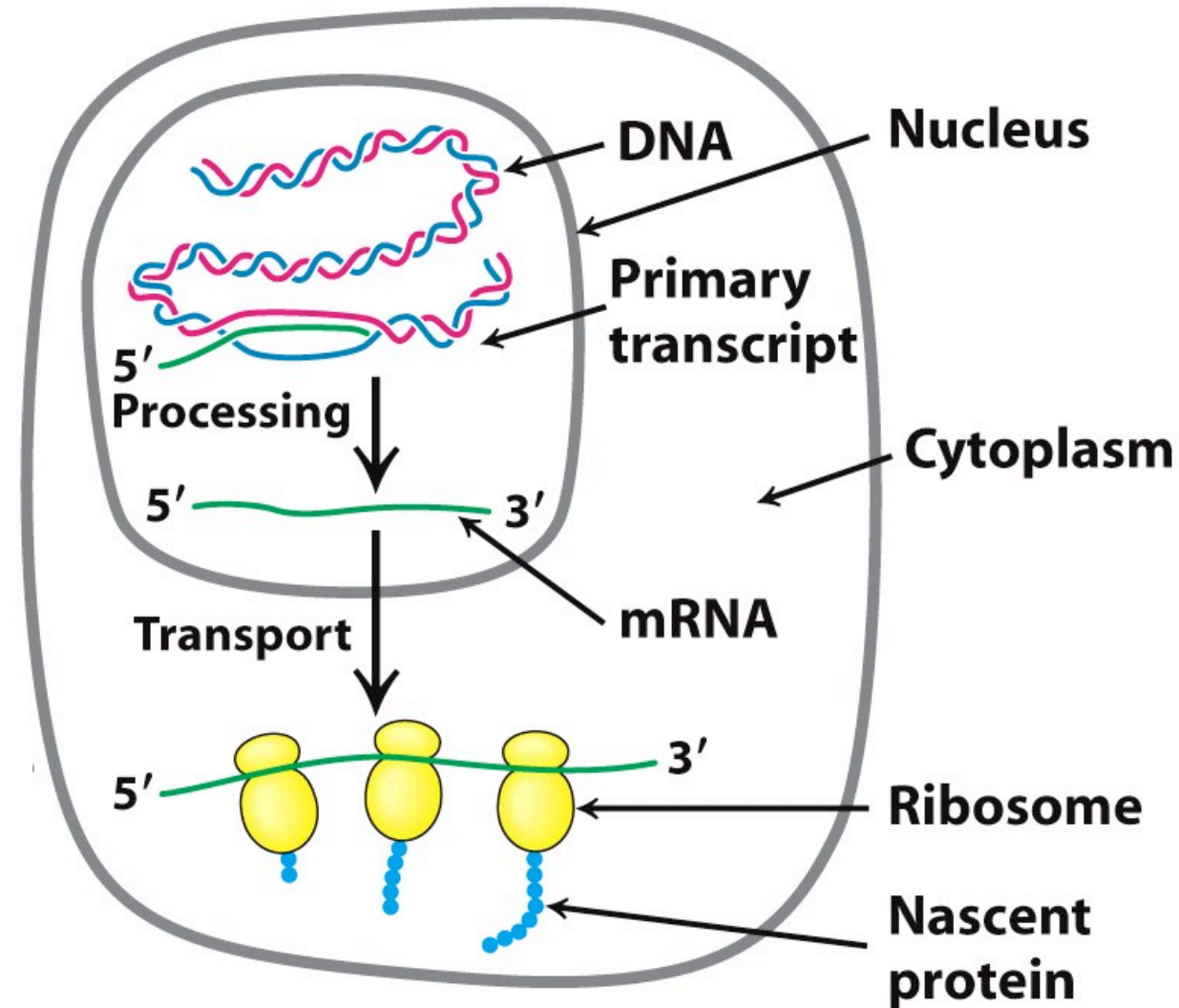


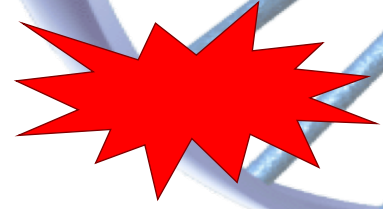
Figure 29.21
Biochemistry, Seventh Edition
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In the time it takes you to read this sentence,
your cells will accumulate ~10 trillion DNA
lesions throughout your body!

Assumptions:

20,000 lesions / cell / day, 10^{13} cells in body, 4 seconds to read

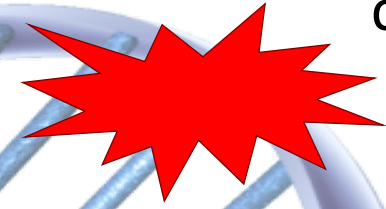
How is DNA damaged?



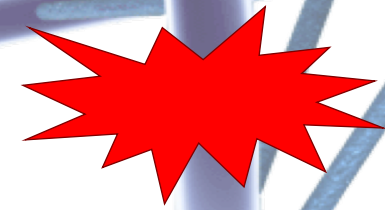
sunlight



reactive oxygen species



chemicals



metabolites



radiation

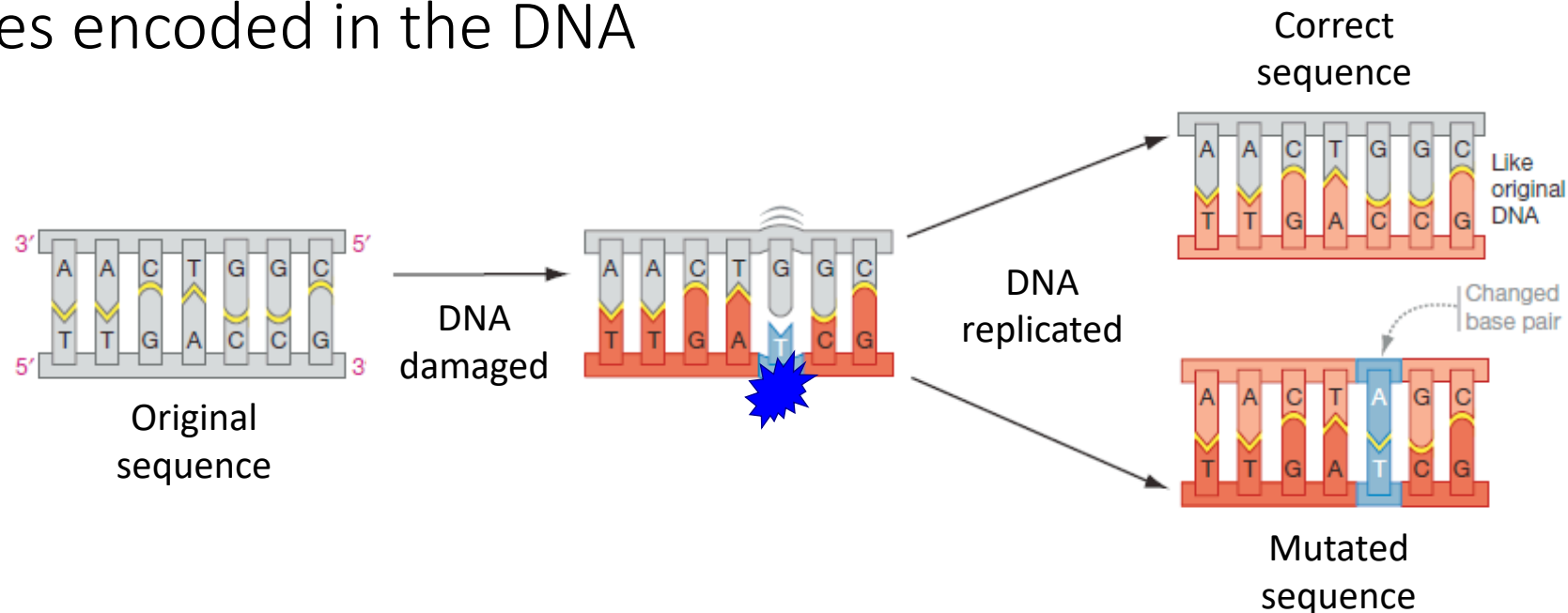


inflammation

and breathing!!

DNA damage \neq mutation

- Damage is the creation of a DNA lesion
 - Basepair 'decorations' and strand breaks
 - Results in cell death or mutation in genetic code
- Mutations occur when the damage is 'copied' during replication and becomes encoded in the DNA

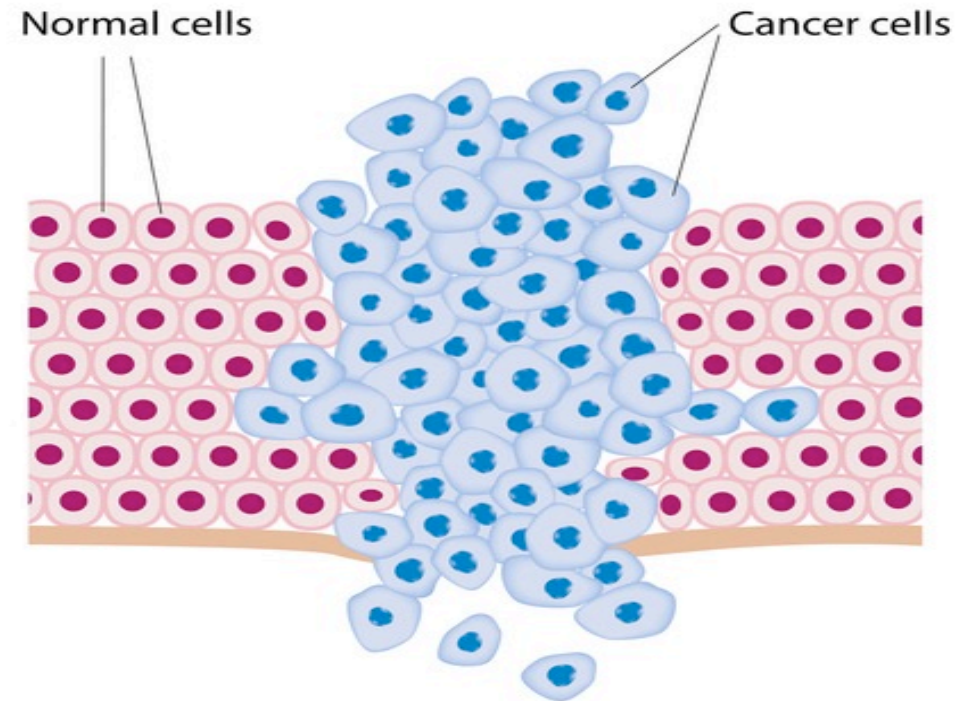


How does DNA damage promote cancer?

- Lesions / adducts are important first step in carcinogenesis
- May result in gene mutations and give rise to altered proteins
 - Mutation may occur in oncogene (tumor suppressor gene)
 - Mutation may occur in gene that encodes DNA repair enzyme
 - Mutation may occur in gene that regulates cell cycle
- Divided into three stages: initiation, promotion, and progression

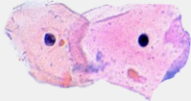
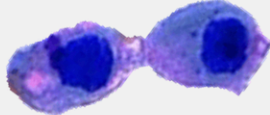
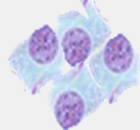
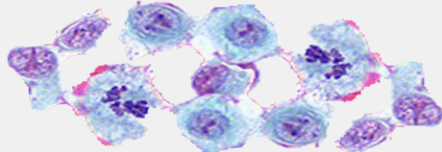

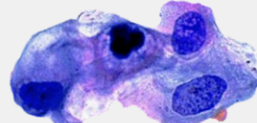
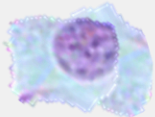
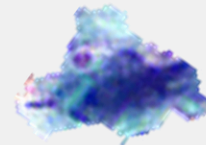
Last year, over 600K cancer deaths in US

- Group of more than 100 diseases
- Able to start in / effect any location of the body
- Defined by abnormal cell growth / features



What are the 'hallmarks' of cancer cells?

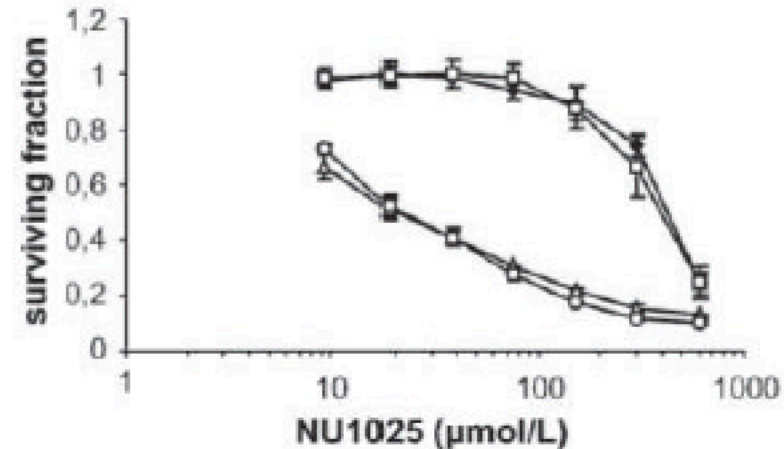
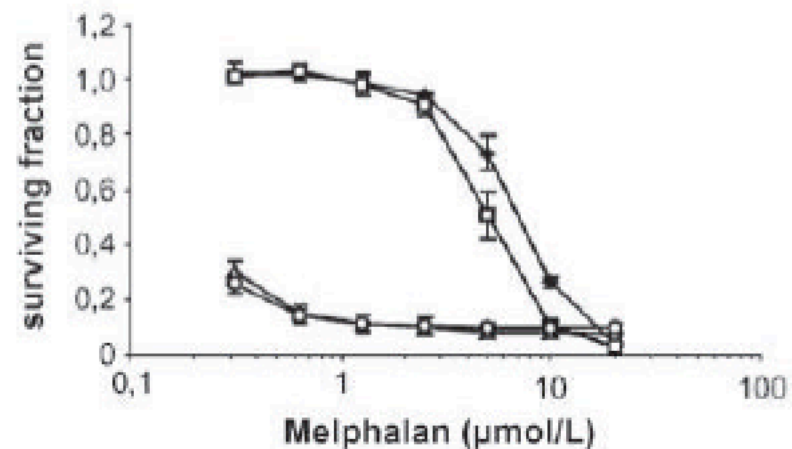
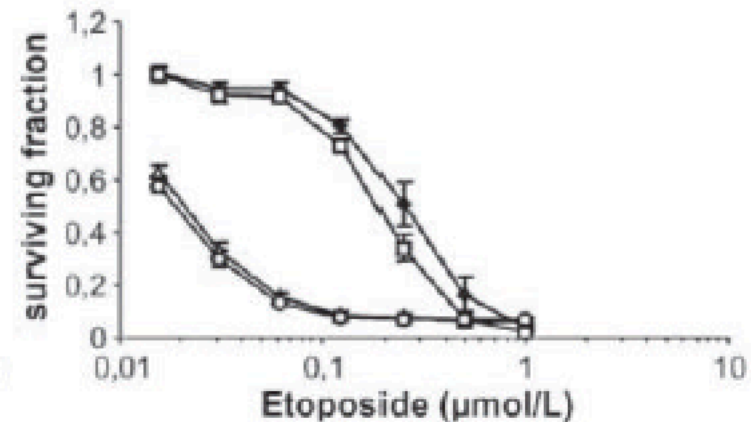
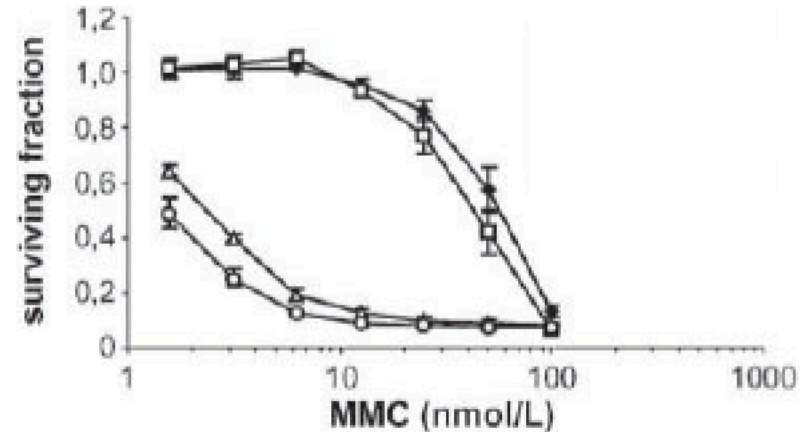
- Undifferentiated
- Unresponsive to cell signaling
 - Autonomous proliferation
 - Inactivate tumor suppressor genes
 - Evade programmed cell death
- Sustained angiogenesis
- Invasion and metastasis

Normal	Cancer	
		Large, variably shaped nuclei
		Many dividing cells; Disorganized arrangement
		Variation in size and shape
		Loss of normal features

How can DNA damaging agents be leveraged against cancer cells?

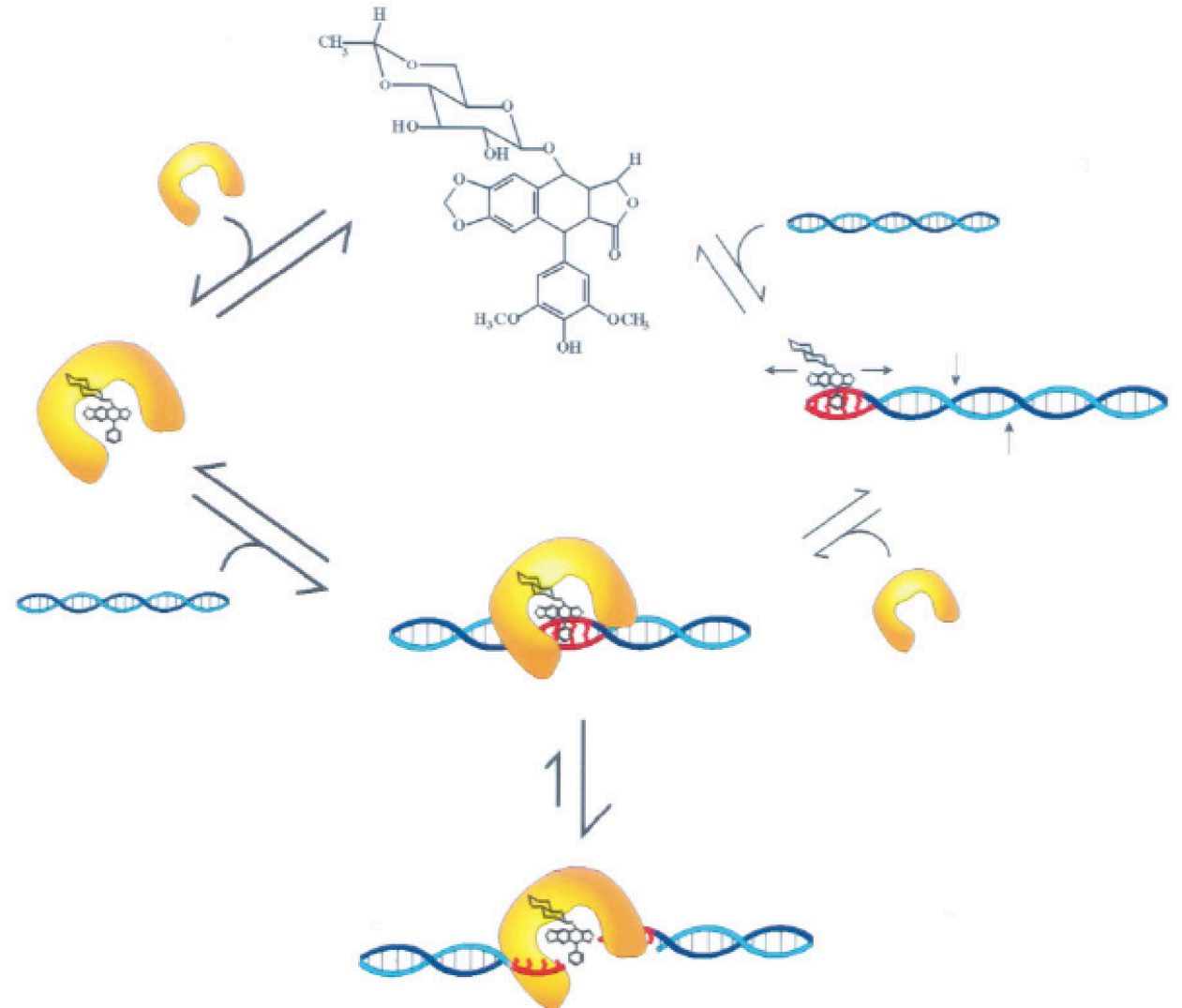


Cancer cells deficient in DNA repair are more sensitive to DNA damaging agents



Etoposide causes DNA cleavage complexes

- Alters the structure of DNA
 - Able to bind DNA and topoisomerase II directly
 - DNA-etoposide-topoisomerase II complex results in cleavage
- Cleavage due to inhibition of DNA religation by topoisomerase II



Research question: What is the effect of etoposide treatment on the transcriptome?

Experimental approach:

- 1) Use RNA-seq data set to examine up- / down-regulated genes in response to etoposide treatment.
- 2) Use quantitative PCR to confirm results of RNA-seq.
- 3) Compare RNA-seq results to publicly available data set.

Cell lines used for RNA-seq data sets

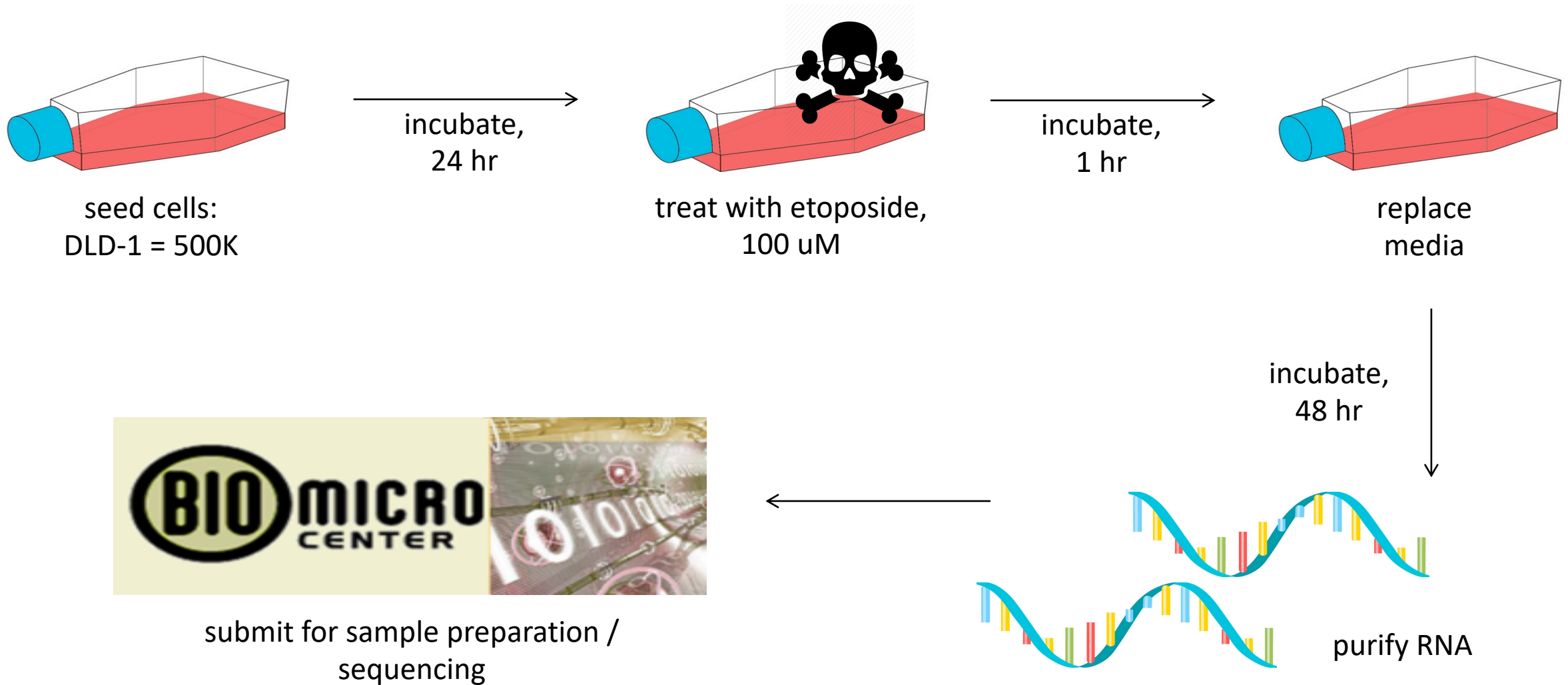
DLD-1 (20.109 data set)

Organism: human
Tissue: colon
Cell type: epithelial
Disease: colorectal
adenocarcinoma
Donor: M/adult

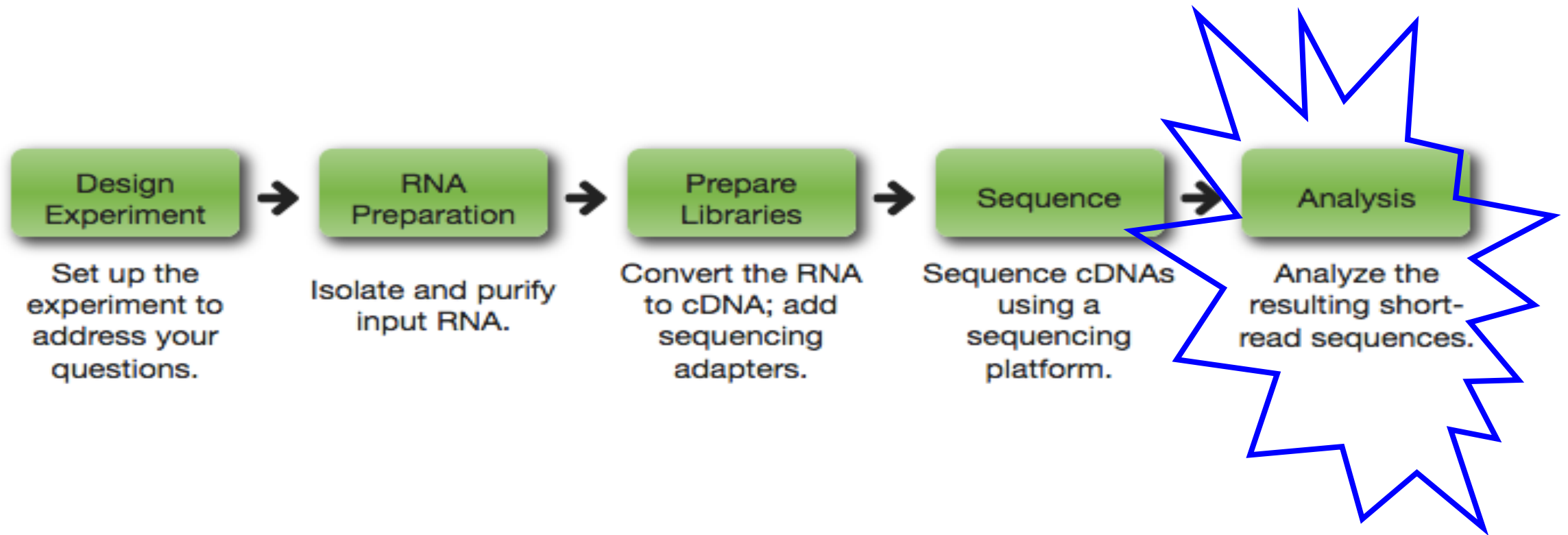
A549 (published data set)

Organism: human
Tissue: lung
Cell type: epithelial
Disease: carcinoma
Donor: M/58/Caucasion

Workflow for RNA-seq library prep



RNA-seq measures transcript levels

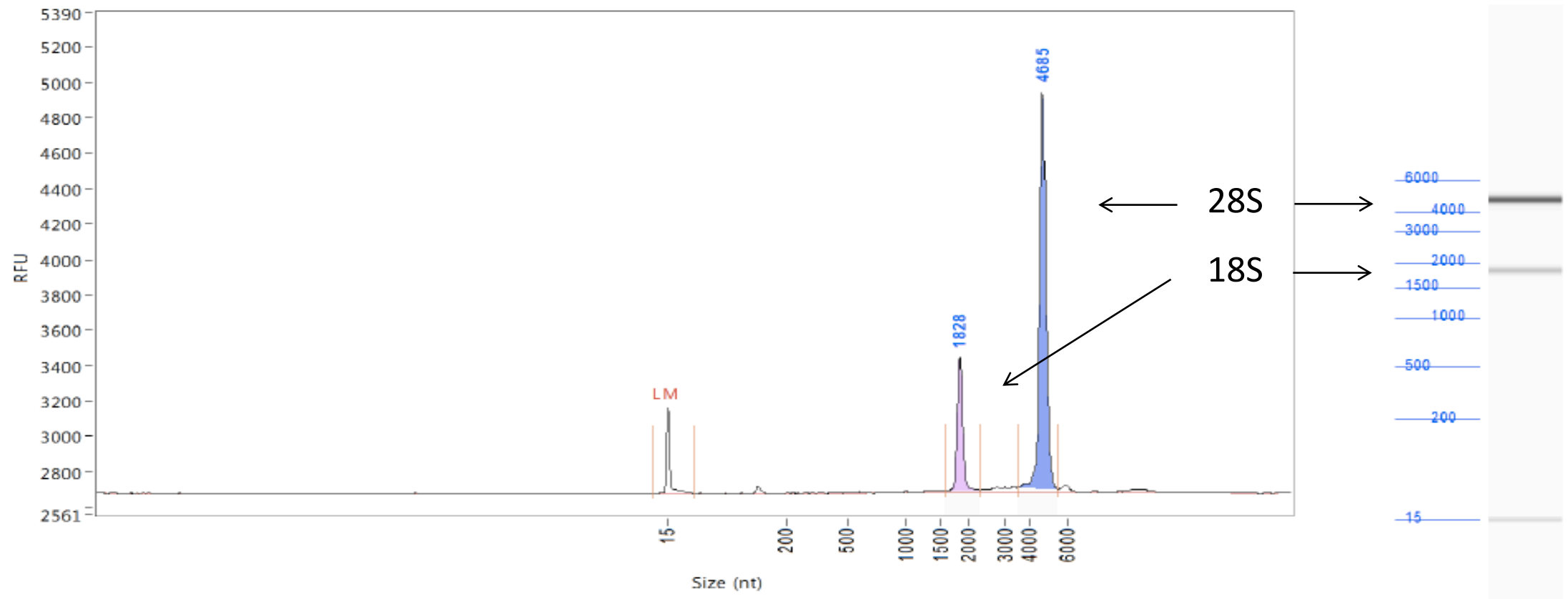


RNA-seq: design experiment

- Type / number of replicates
 - Biological
 - Technical
- Library sequencing method
 - Single-end
 - Paired-end
- Read length
- Coverage and sequencing depth

RNA-seq: RNA preparation

28S and 18S rRNA (ribosomal subunits) should be distinct peaks / bands at 2:1 ratio

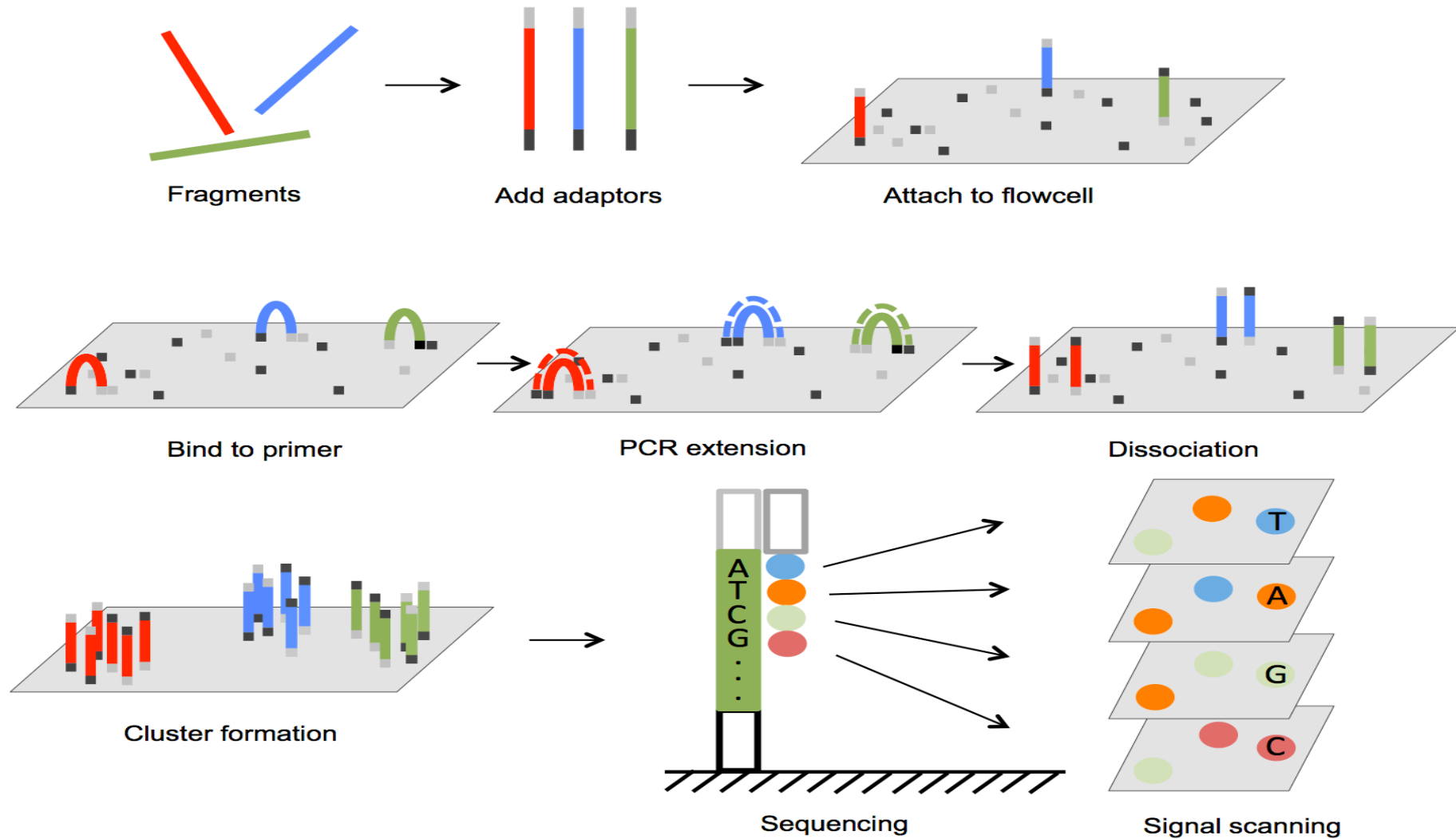


RNA-seq: prepare libraries

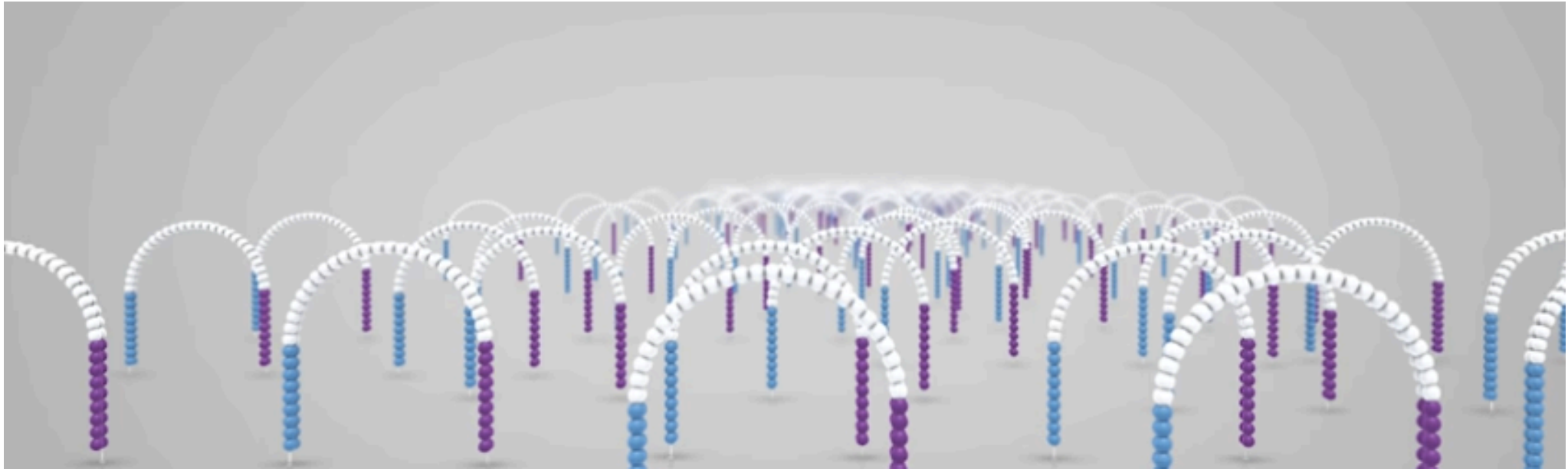
- Amplification element: required for clonal amplification of the cDNA or 'insert'
- SP (sequencing priming site): initiates sequencing reaction
- Barcode: allows for samples to be pooled



RNA-seq: sequence



A closer look at Illumina sequencing method



<https://www.illumina.com/science/education/sequencing-workflow-accuracy.html>

RNA-seq: analysis

- Align the reads



- Annotate the transcripts
- Count the reads

How will you assess transcript data?

- R available through through interface RStudio.cloud
 - Programming language used for statistical computing
 - Useful for clustering analysis



In the laboratory...

- Prepare cells for etoposide drug treatment
- Introduction to R
- Paper discussion

Next time (and for the majority of Mod2)...

- Prof. Fraenkel will deliver lectures focused on data analysis concepts and methods