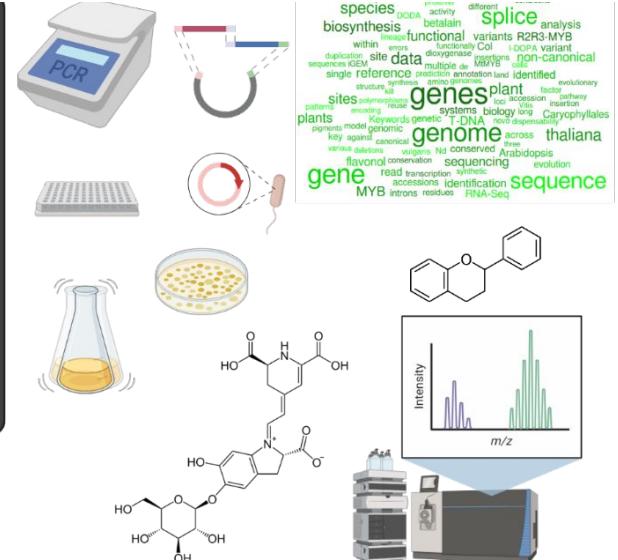
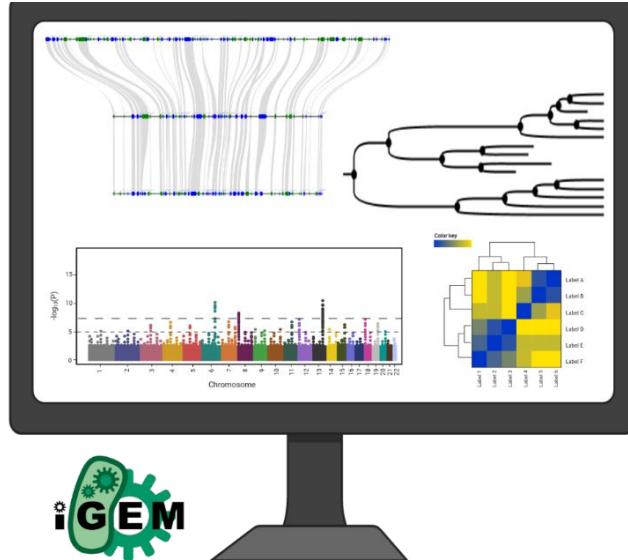
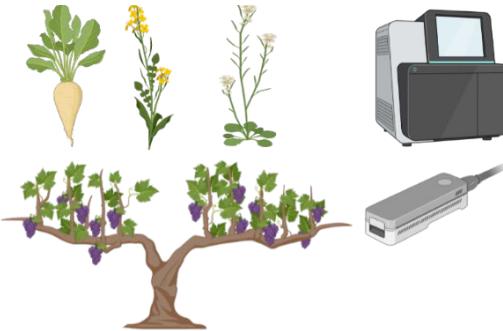




Technische  
Universität  
Braunschweig



# Introduction to plant transcriptomics

Prof. Dr. Boas Pucker  
(Plant Biotechnology and Bioinformatics)

# Availability of slides

- All materials are freely available (CC BY) - after the lectures:
  - StudIP: **Applied Plant Transcriptomics**
  - GitHub: <https://github.com/bpucker/teaching>
- Questions: Feel free to ask at any time
- Feedback, comments, or questions: b.pucker[a]tu-bs.de

My figures and content can be re-used in accordance with CC BY 4.0, but this might not apply to all images/logos. Some figure were constructed using bioRender.com.

# What causes these pigmentation patterns?

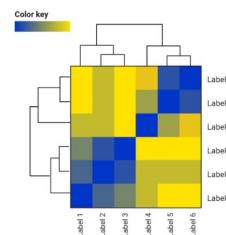
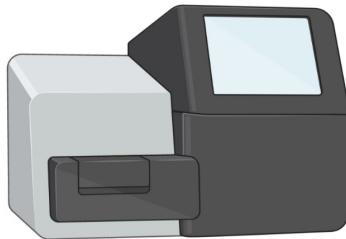


# What is transcriptomics?



# What is transcriptomics?

- Study of the transcriptome with high-throughput methods
- Characterized by technological progress and big data analyses



# What is the transcriptome?



# What is the transcriptome?

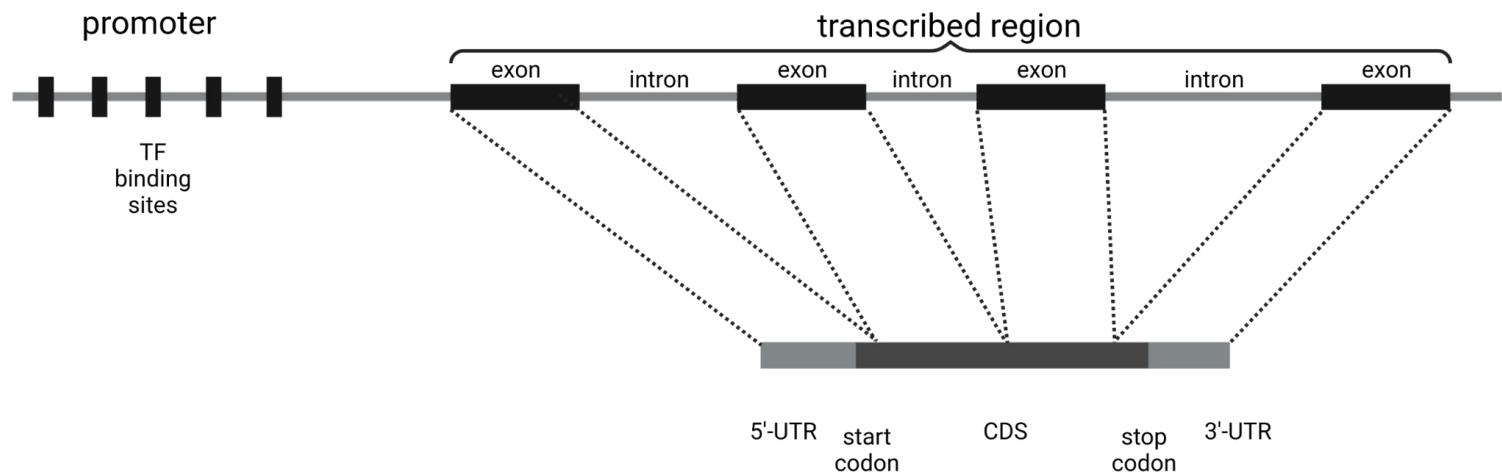
- “The set of all transcripts and their abundances in a defined cell/tissue/organism under defined conditions at a certain time point.”
- Highly variable over time
- Responding to various stimuli

# What is a gene?

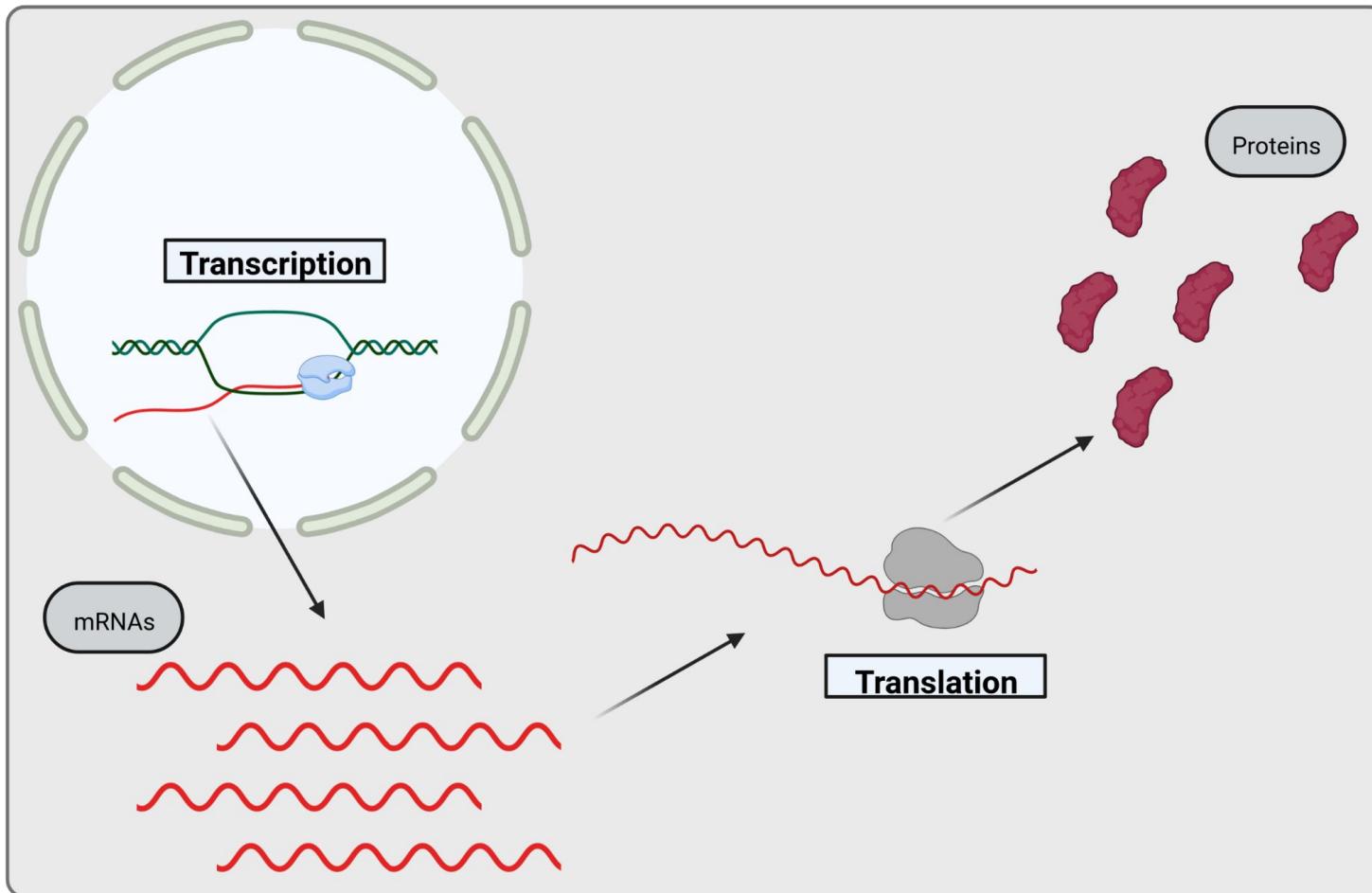


# What is a gene?

- No perfect and universal definition
- Restrict to protein coding gene in plants:
  - promoter region
  - UTRs
  - coding sequence
  - introns



# Protein biosynthesis (overview)

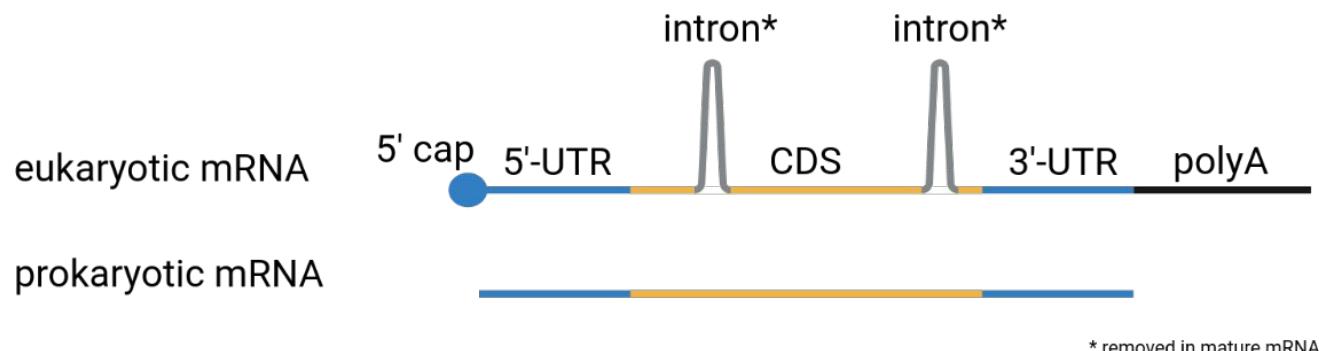


# Types of RNA

- rRNA
- tRNA
- mRNA
- Various regulatory RNAs

# Prokaryotes vs. eukaryotes - transcripts

- Eukaryotic genes can harbour introns
- Eukaryotic genes have a 5'-cap
- Eukaryotic genes have a poly-A tail



# RNA extraction

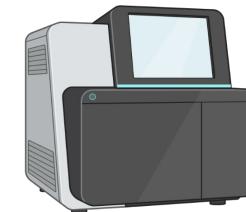
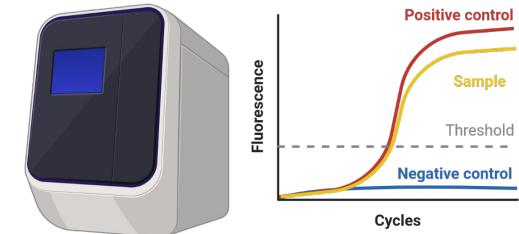
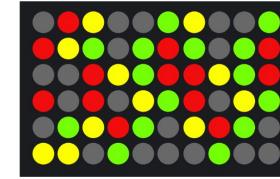
- Classical RNA extraction approaches:
  - Phenol-based methods: helpful to remove high polysaccharide contaminants
  - Trizol-based methods: commercial reagent combining phenol and guanidine isothiocyanate
  - CTAB-based methods: successive removal of polysaccharides, proteins, and specialized metabolites
- Kit-based RNA extraction methods

# How to measure gene expression?



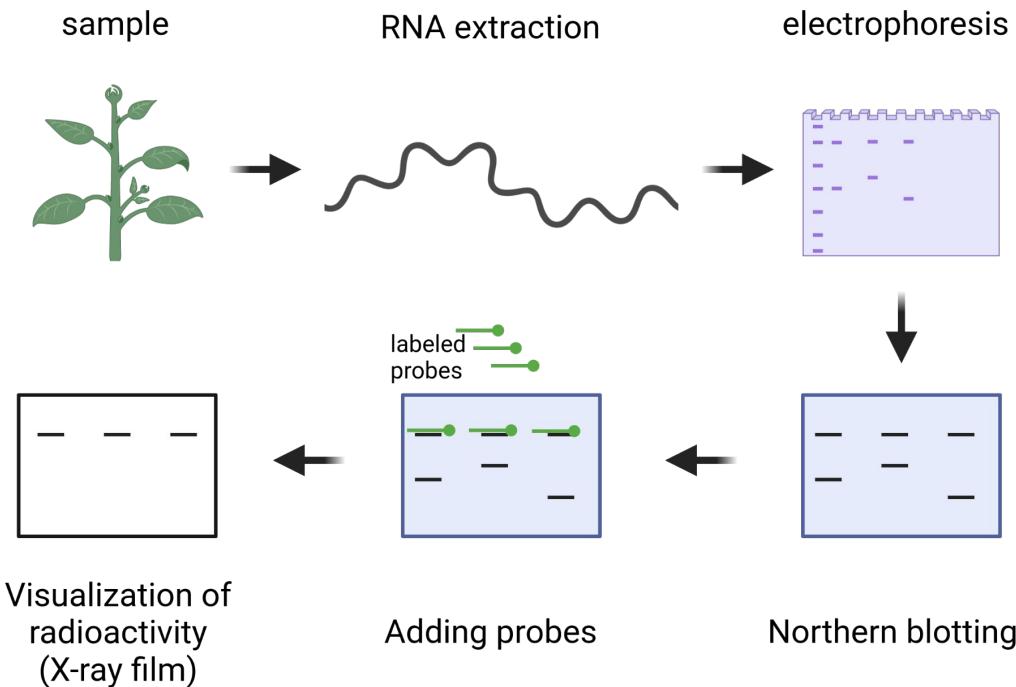
# How to measure gene expression?

- Northern blot
- Reverse transcription quantitative PCR (RT-qPCR)
- Microarray
- Expressed Sequence Tags (ESTs)
- Serial Analysis of Gene Expression (SAGE)
- RNA-seq (not RNA sequencing!!)
- Droplet digital PCR (ddPCR)
- direct RNA sequencing



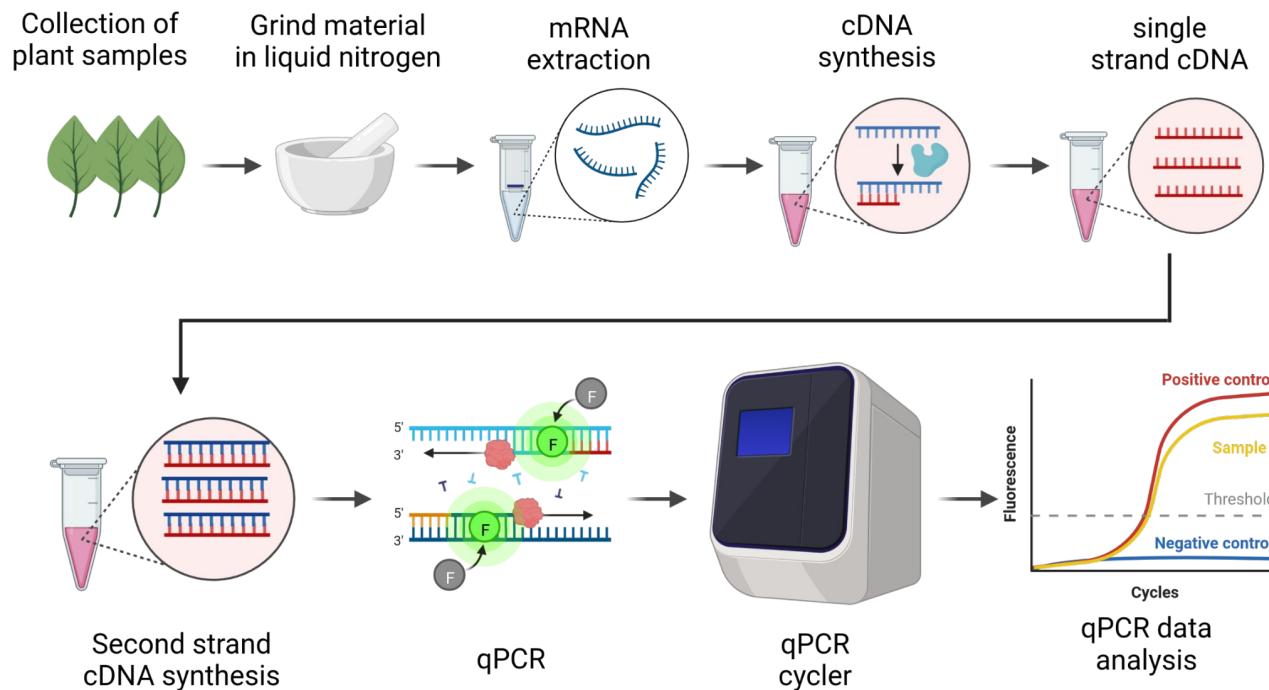
# Northern blot

- Electrophoresis to separate mRNAs on a gel
- Visualization of RNA with radioactively labeled probes

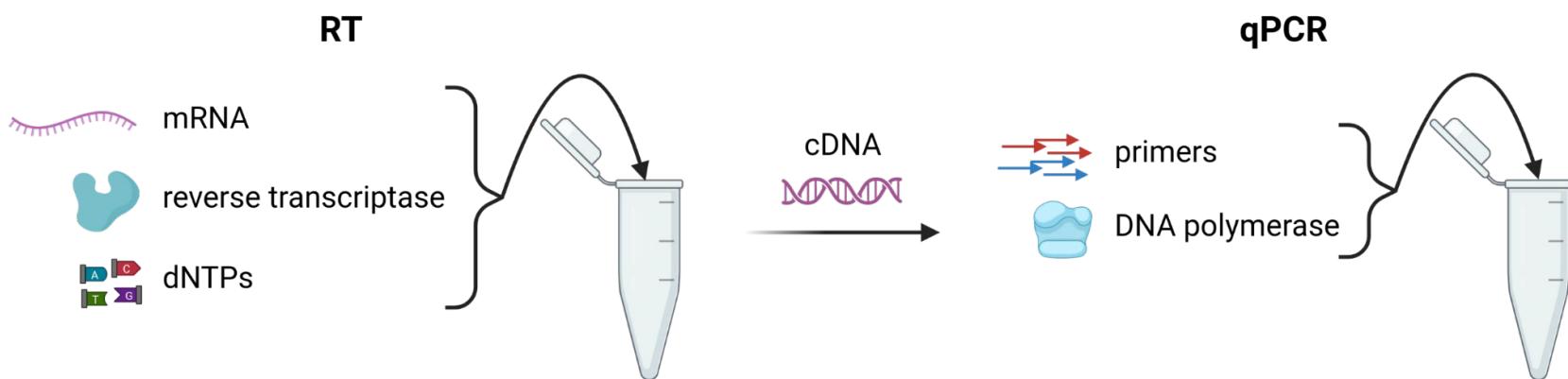
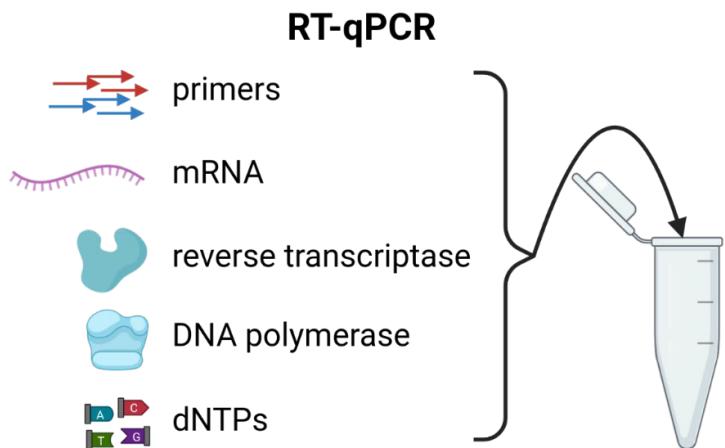


# Reverse transcription quantitative PCR (RT-qPCR)

- Reverse transcription = conversion of mRNA into cDNA
- qPCR = quantitative PCR, real time detection of product formation

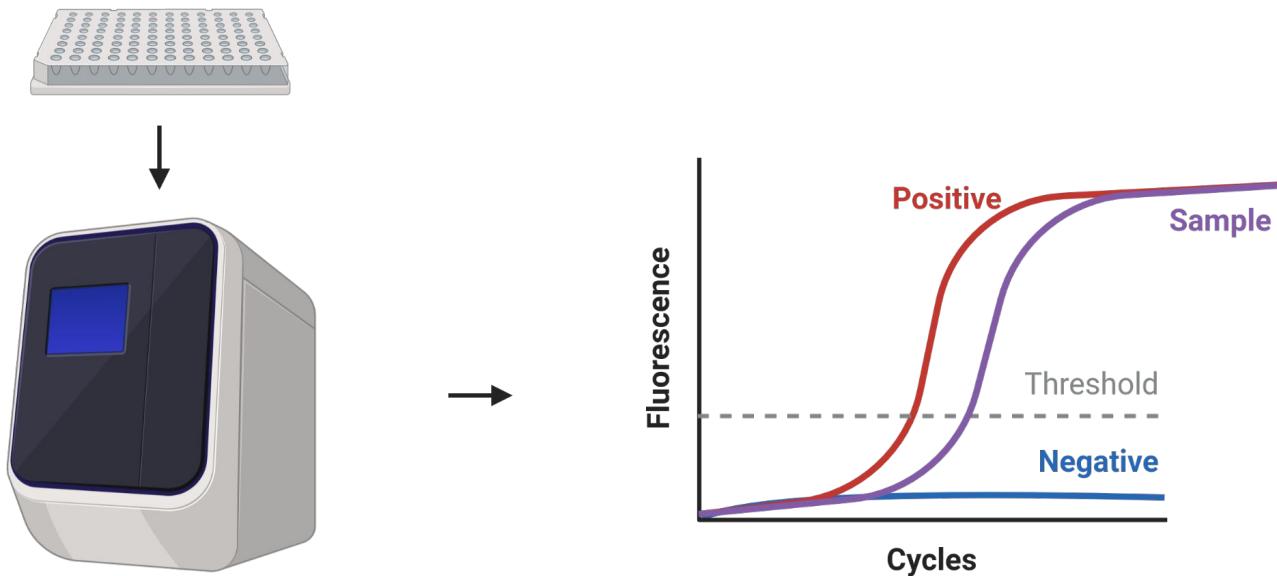


# One-step vs. two-steps

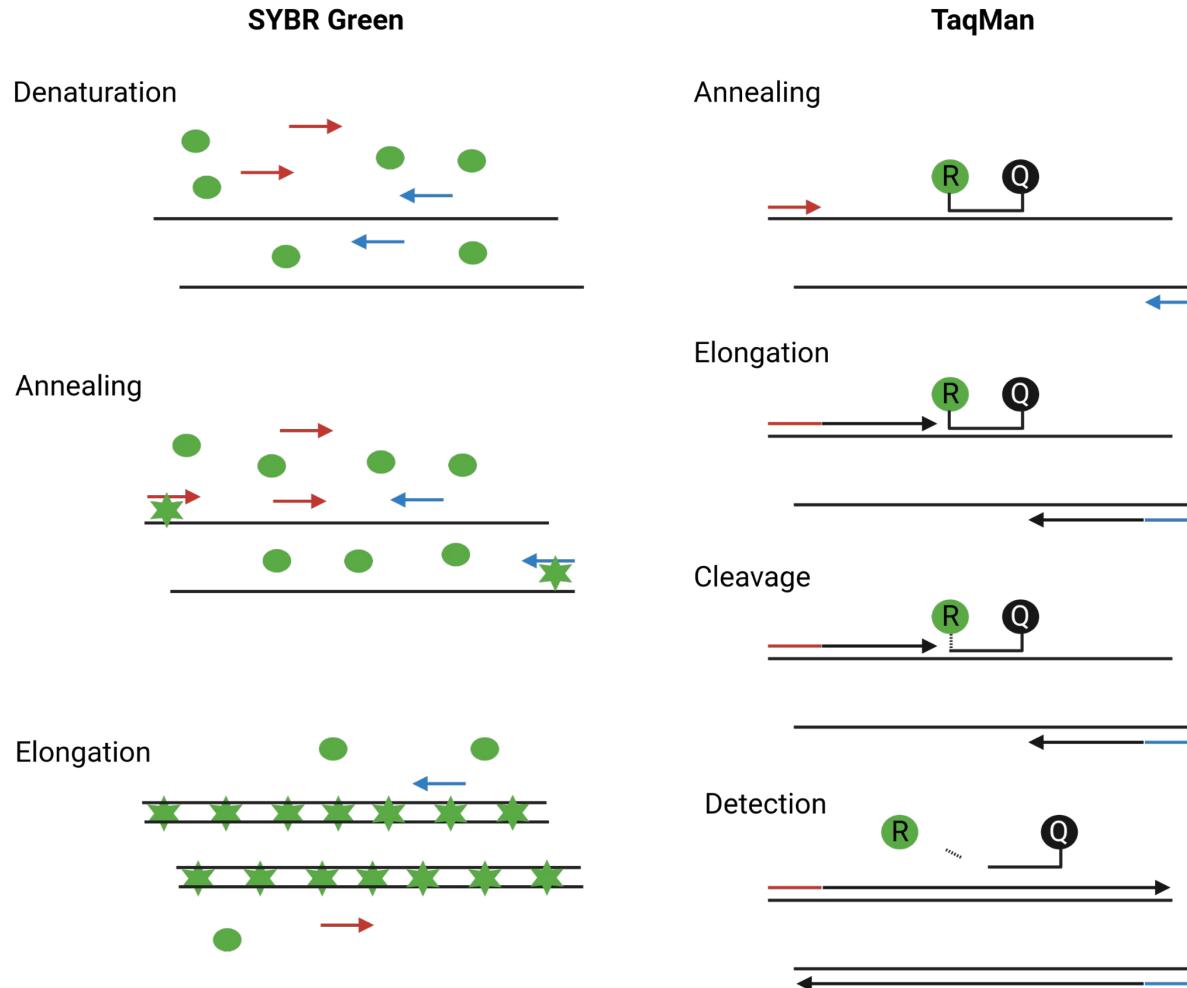


# qPCR concept

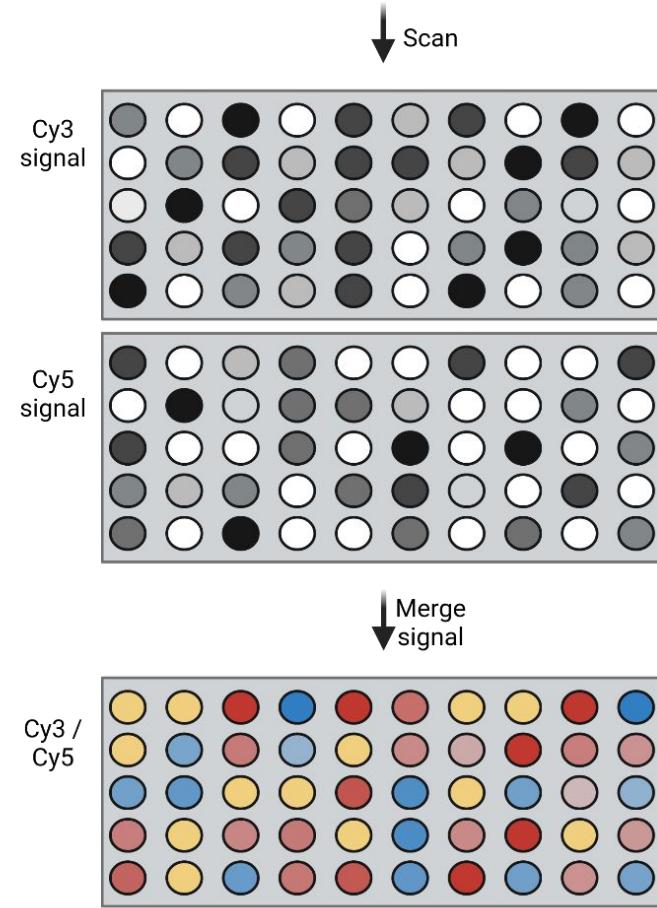
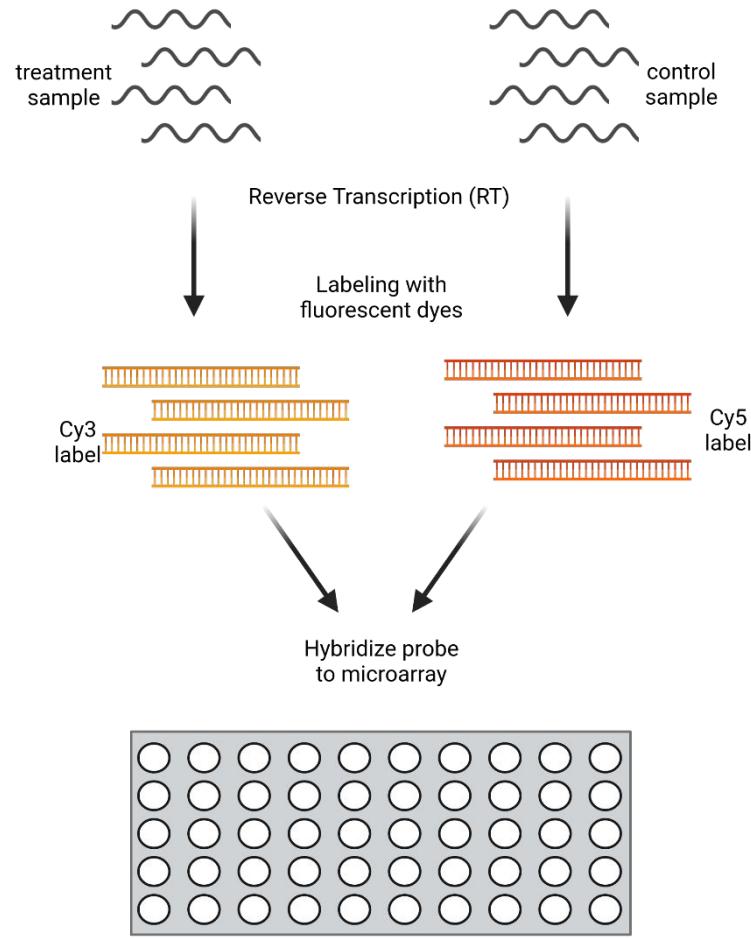
- Monitoring formation of PCR product in real time
- Comparison of sample against positive/negative controls
- Ct value = cycle in which the threshold is crossed



# qPCR: SYBR green vs. TaqMan

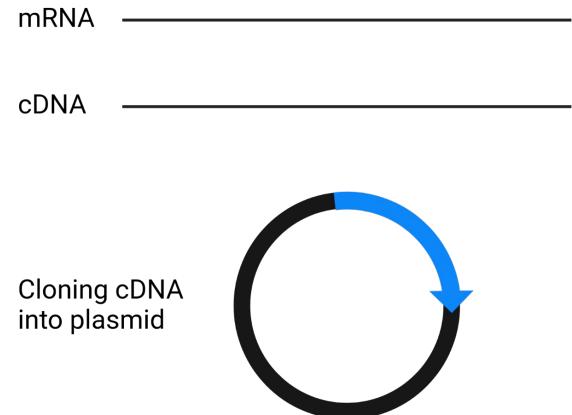


# Microarray



# Expressed Sequence Tags (ESTs)

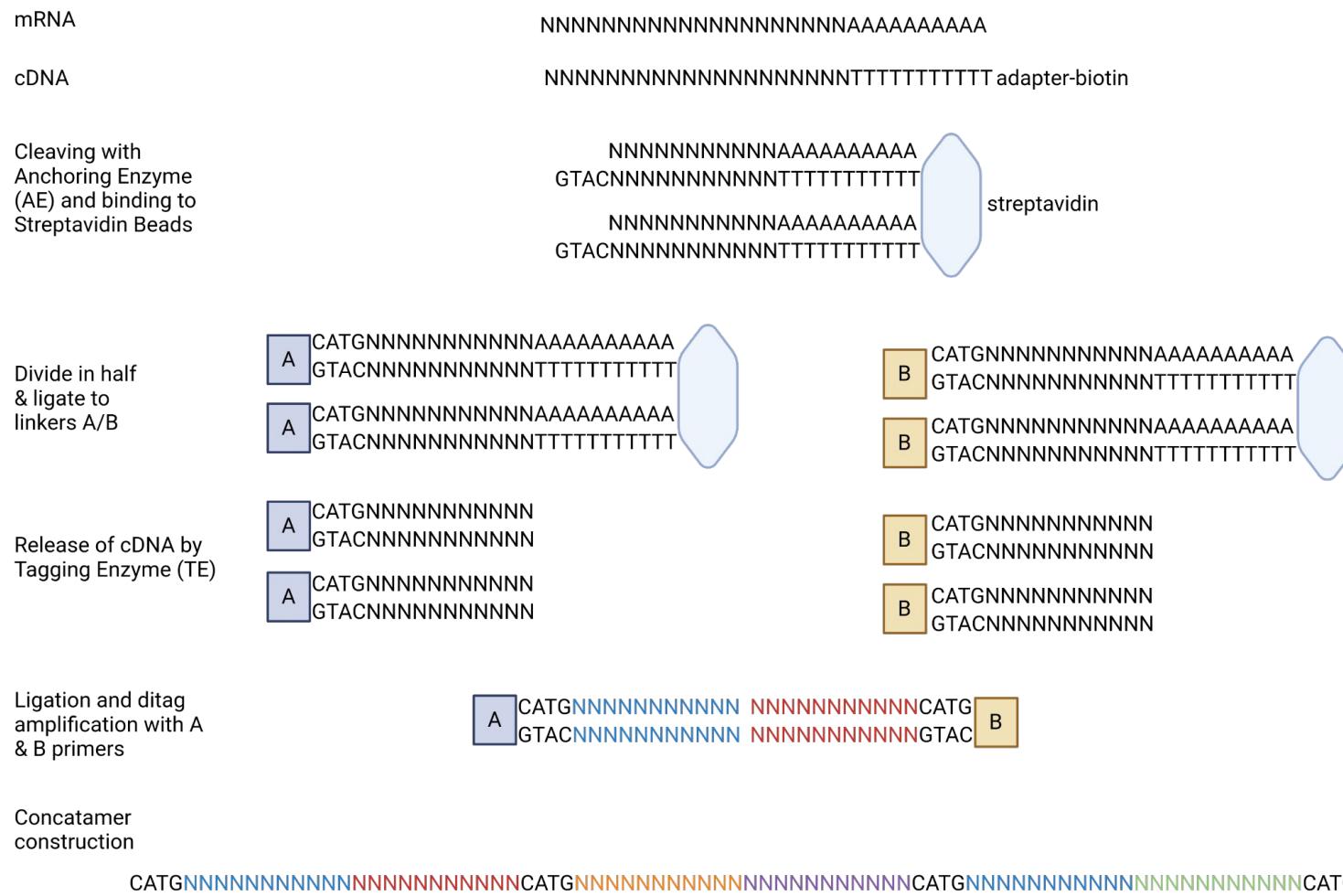
- Fragment of a cDNA
- Indicates transcription of certain genomic segments
- Restriction to full length mRNAs/cDNAs possible
- Number of ESTs correlates with transcript abundance



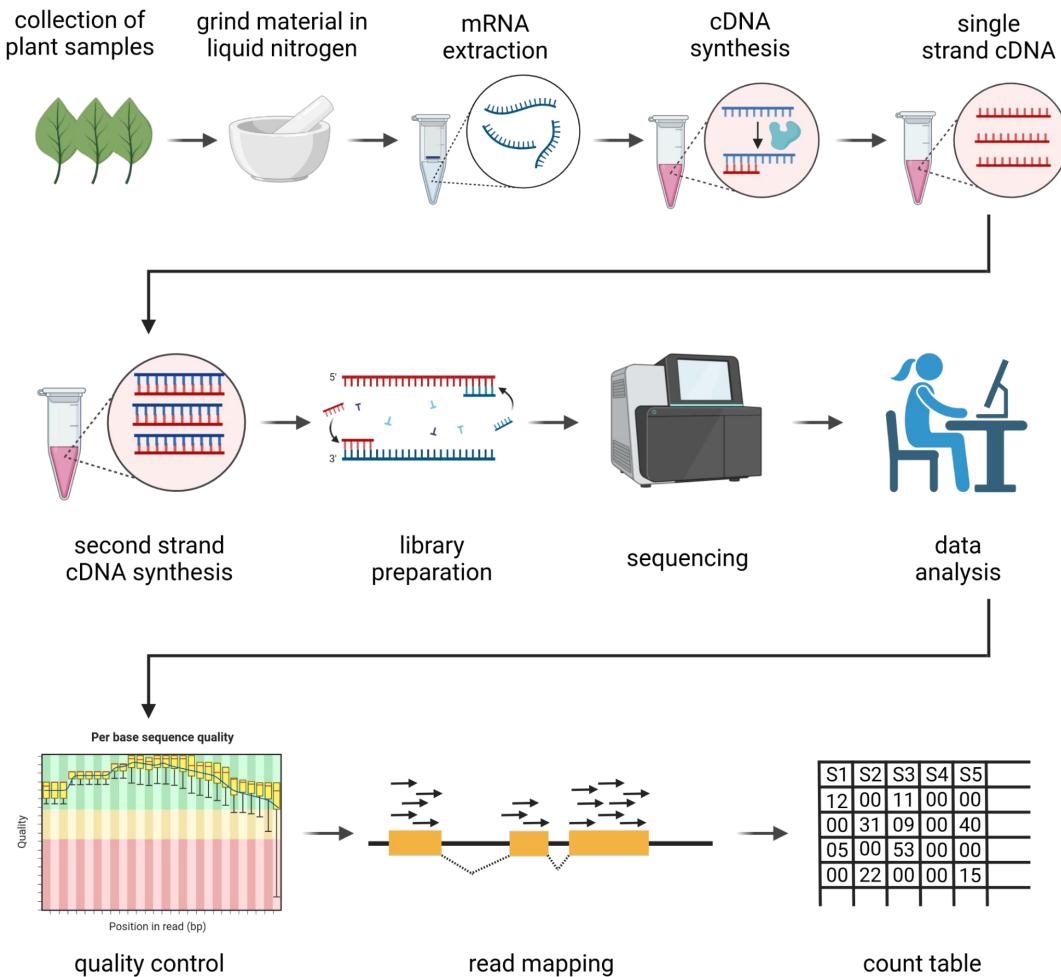
5' EST

3' EST

# **Serial Analysis of Gene Expression (SAGE)**

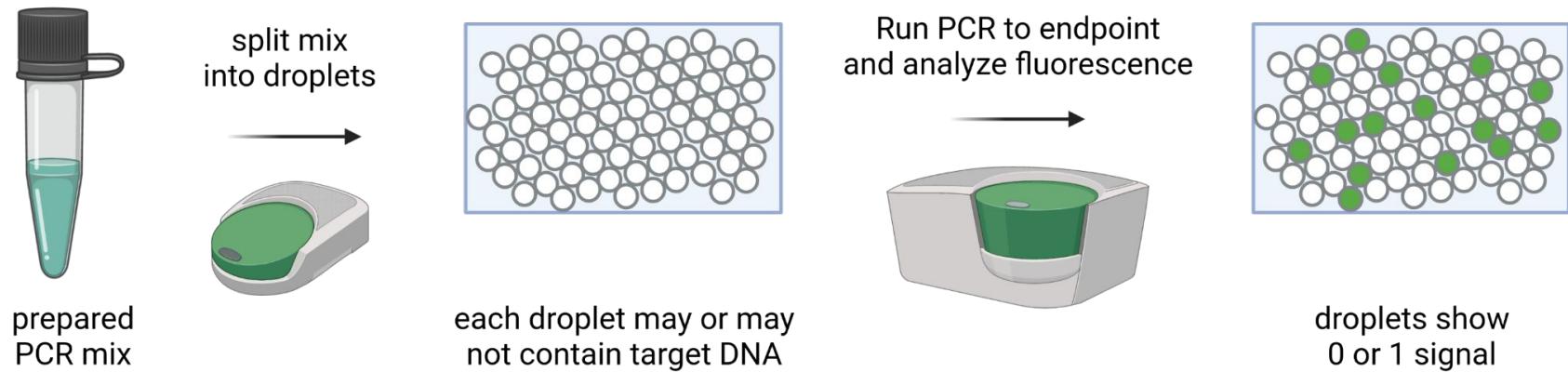


# RNA-seq (not RNA sequencing!)

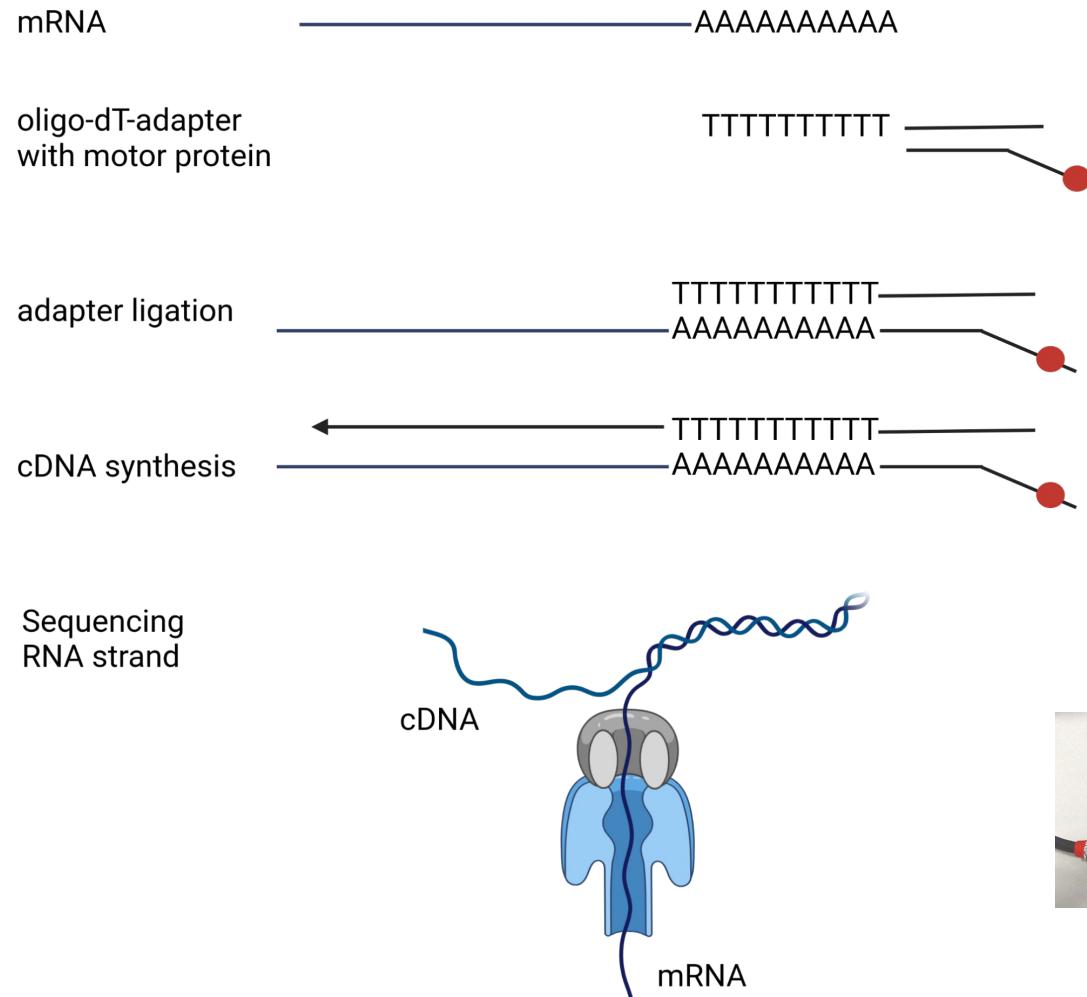


# Droplet digital PCR (ddPCR)

- Digital = individual droplets show 0 or 1 signal
- Superior to qPCR in certain applications
- Requires specific hardware (droplet generation and analysis)



# Direct RNA sequencing



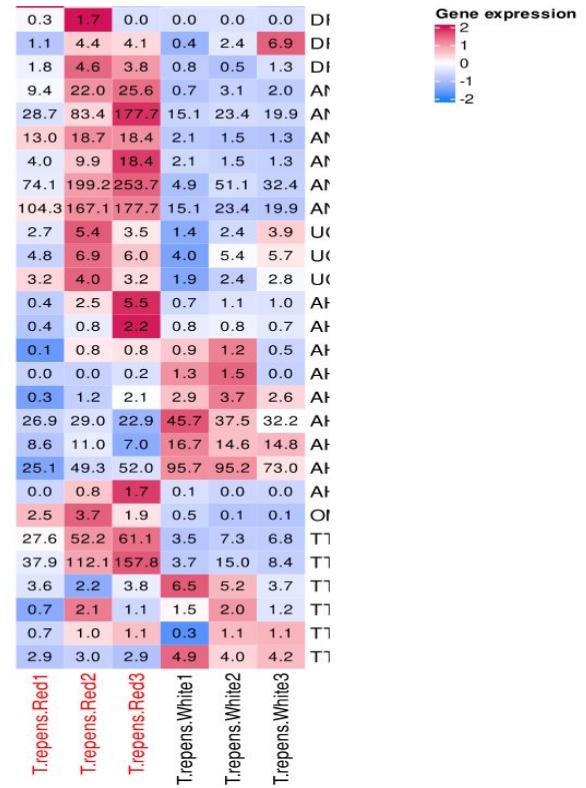
(photo credit: Melina Nowak)

# It is all about comparison

- Gene expression is usually performed in a comparative way
  - Between samples: fold change differences
  - Between transcript of same samples: transcript per million transcripts
- Absolute measurement of transcript numbers is extremely tricky
- Differentially expressed genes (DEGs)

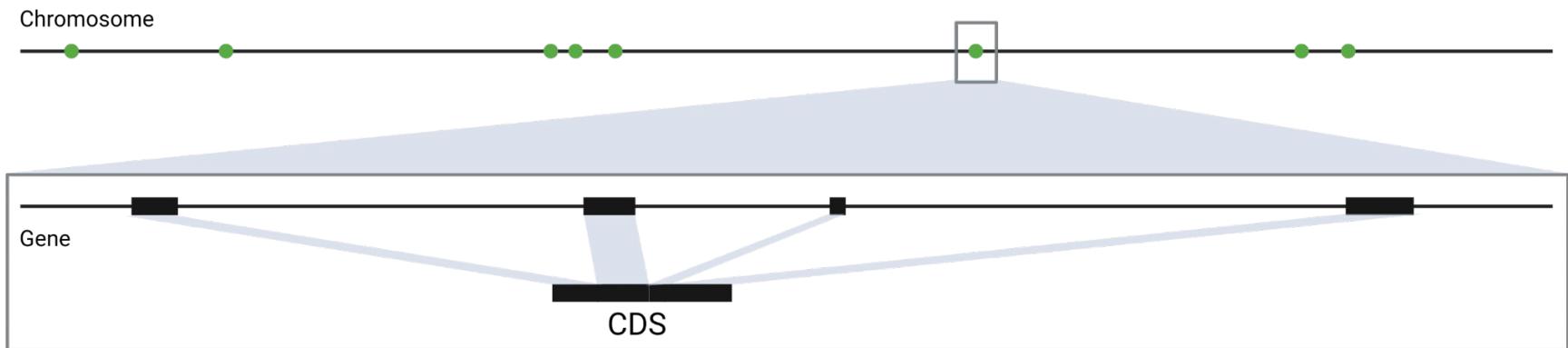
# Heatmaps and visualization of gene expression

- Transcriptomic data sets are complex
- Simplified illustration required for interpretation
- Colors help to make data intuitive



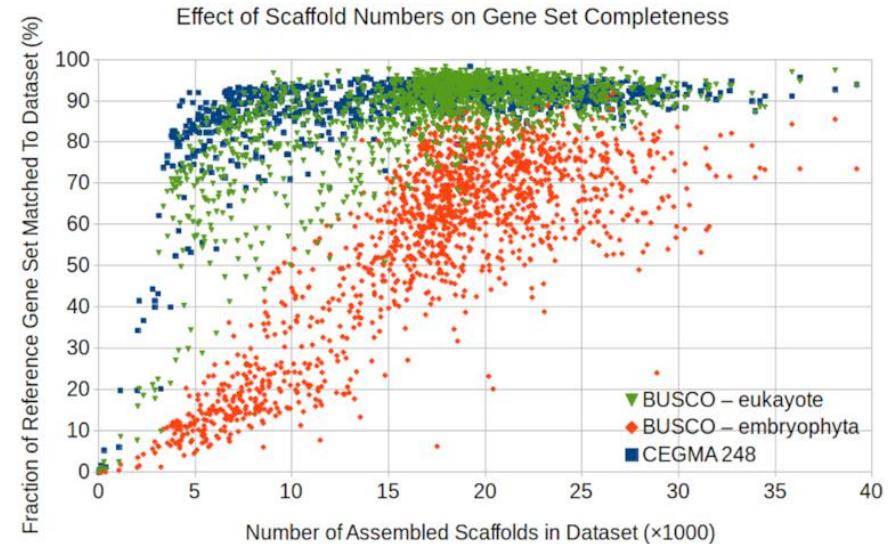
# *De novo* transcriptome assembly

- Coding sequences account for tiny fraction of plant genome
- Transcriptome analysis can be cost-effective compared to genome sequencing
- Reveals genes relevant under certain conditions/at certain time point
- Analysis can be faster and straight forward



# 1KP

- Phylogenetics project to cover taxonomic width of plants
- Transcriptome assemblies for 1000 (1k) plants
- Large, international consortium for sequencing projects



<http://www.onekp.com/samples/list.php>  
<https://doi.org/10.1093/gigascience/giz126>

# Data sharing

- Gene Expression Omnibus (GEO): <https://www.ncbi.nlm.nih.gov/geo/>

## Gene Expression Omnibus

GEO is a public functional genomics data repository supporting MIAME-compliant data submissions. Array- and sequence-based data are accepted. Tools are provided to help users query and download experiments and curated gene expression profiles.



Keyword or GEO Accession  Search

- Sequence Read Archive (SRA): <https://www.ncbi.nlm.nih.gov/sra>



**SRA - Now available on the cloud**

Sequence Read Archive (SRA) data, available through multiple cloud providers and NCBI servers, is the largest publicly available repository of high throughput sequencing data. The archive accepts data from all branches of life as well as metagenomic and environmental surveys. SRA stores raw sequencing data and alignment information to enhance reproducibility and facilitate new discoveries through data analysis.

- eFP browser: <http://bar.utoronto.ca/efp2/>



Select a plant



Arabidopsis



Tomato



Potato



Poplar

# Time for questions!



# Questions

1. What is a transcriptome?
2. Which elements form a eukaryotic gene?
3. Which are the three most abundant RNA types?
4. Which methods can be used to measure gene expression?
5. How does RT-qPCR work?
6. How does ddPCR work?