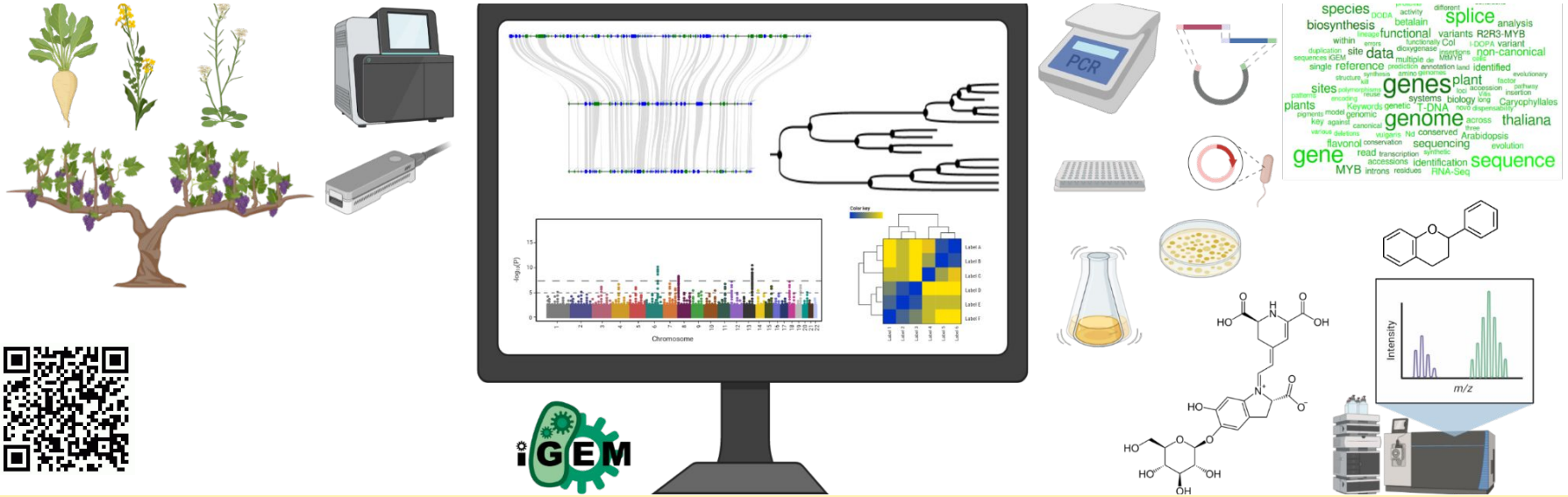
Technische Universität Braunschweig



Latest technological developments

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

Availability of slides

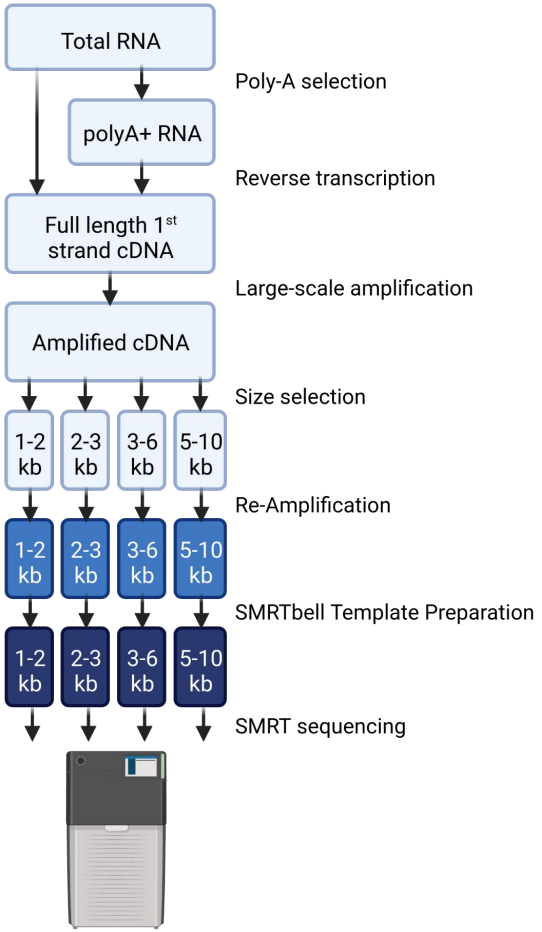
- All materials are freely available (CC BY) - after the lectures:
  - StudIP: **Applied Plant Transcriptomics**
  - GitHub: https://github.com/bpucker/teaching

- Questions: Feel free to ask at any time

- Feedback, comments, or questions: b.pucker[a]tu-bs.de

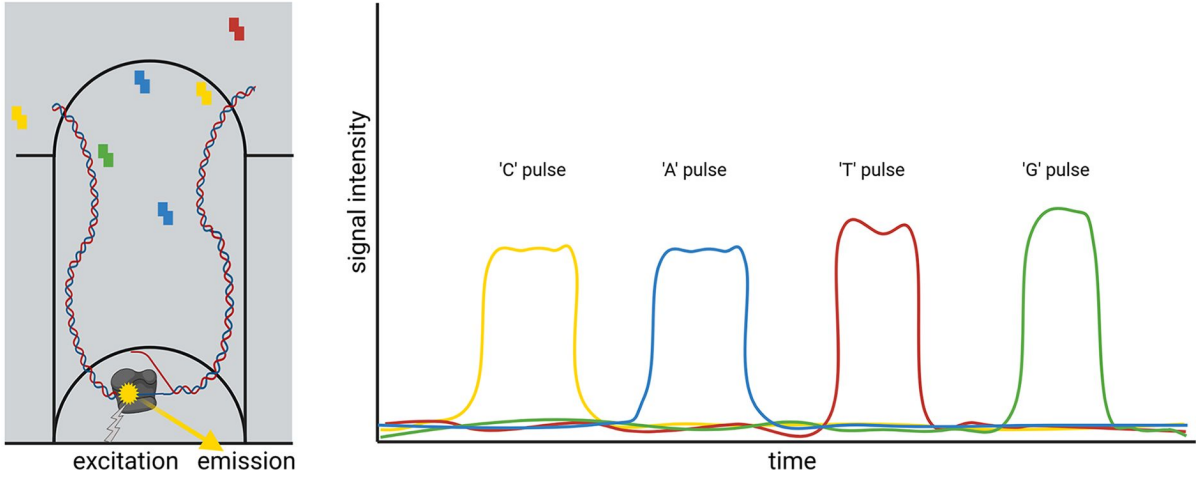
PacBio Iso-Seq (cDNA sequencing)

- SMRT = Single Molecule Real Time sequencing
- Full length cDNA sequencing is beneficial for gene prediction
- Iso-Seq generates several kb long reads and not only 2x300bp



PacBio (SMRT) sequencing

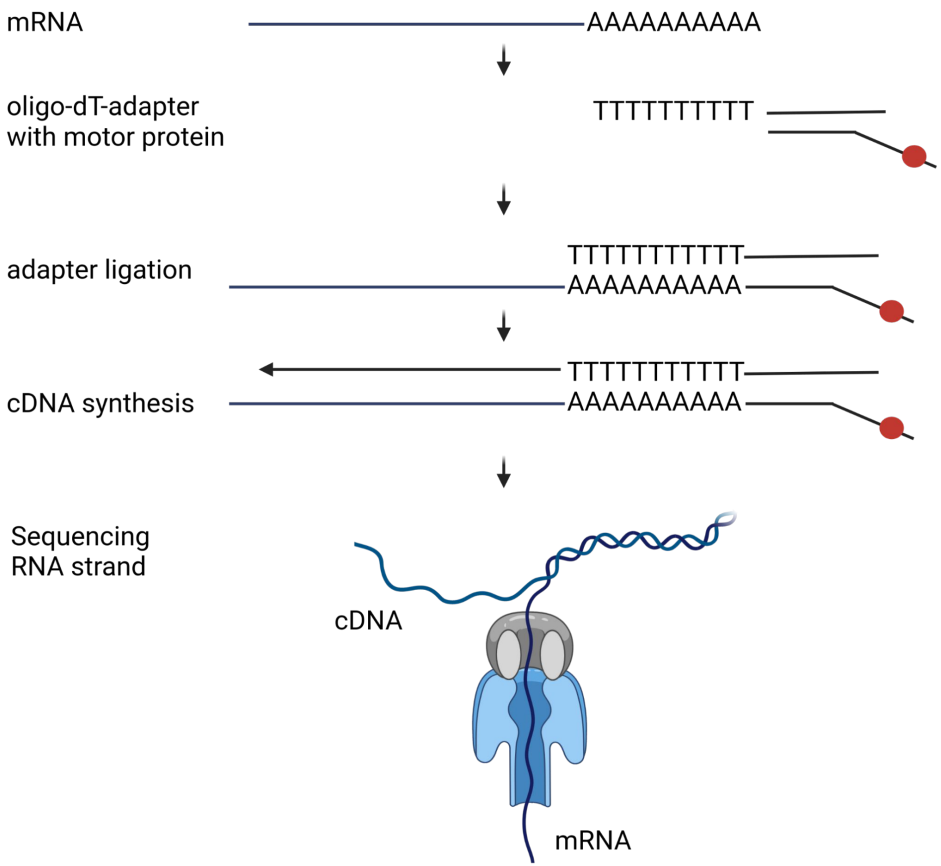
- Single DNA molecule is processed
- DNA polymerase synthesizes new strand
- Nucleotides are labeled with fluorescence
- Multiple rounds of sequencing increases circular consensus sequence (CCS) accuracy



https://doi.org/10.1017/qpb.2021.18

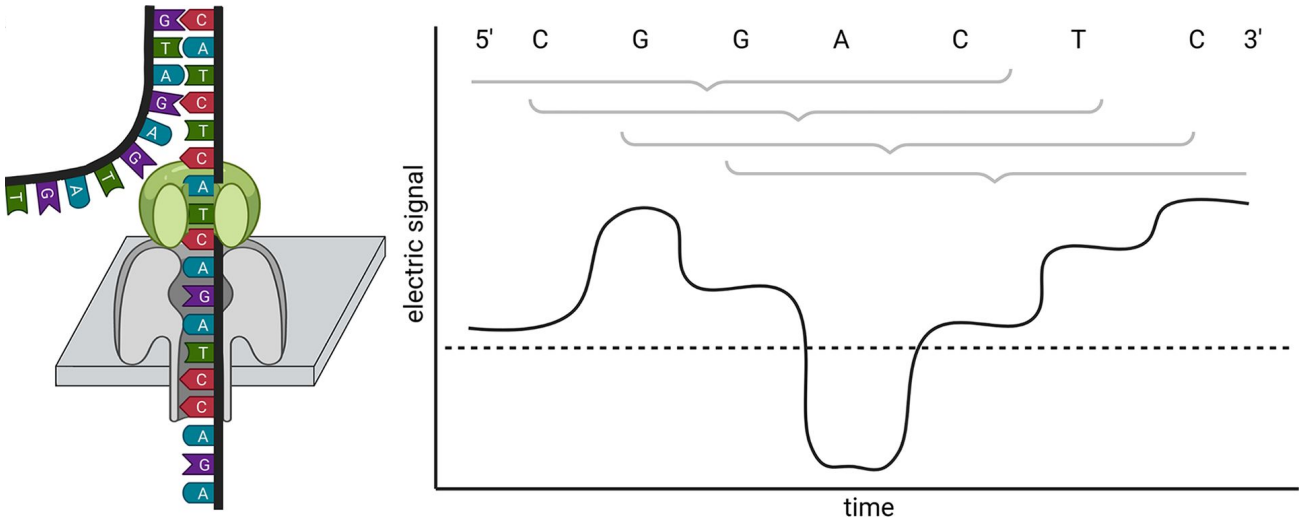
Direct RNA sequencing

- Only sequencing technology to analyze RNA directly at high throughput
- RNA sequencing requires adjusted data processing
- Full length sequences of RNAs are generated



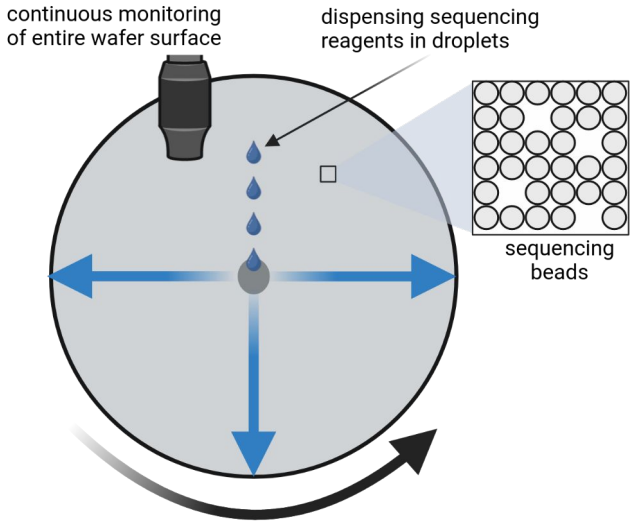
Nanopore sequencing

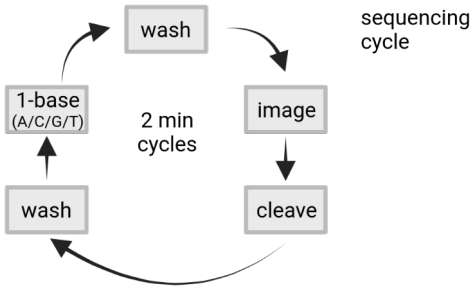
- Single DNA/RNA strand passes through a nanopore in a synthetic membrane
- Blockage of the pore is measured (electric signal)
- Sequence is inferred from changes in electric signal over time



New seq technology

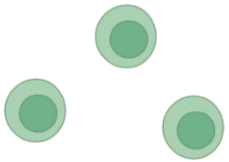
- About 300 nt read length possible
- Base quality: Q15-Q40
- Homopolymers pose an issue (up to 4 possible)
- More natural than labeled nucleotides supplied per sequencing cycle



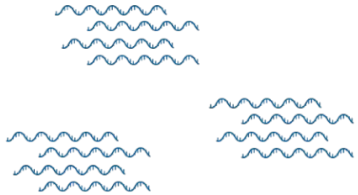


https://doi.org/10.1101/2022.05.29.493900

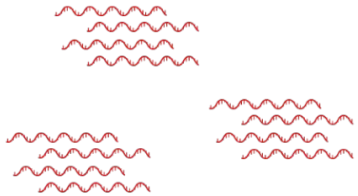
Single cell transcriptomics



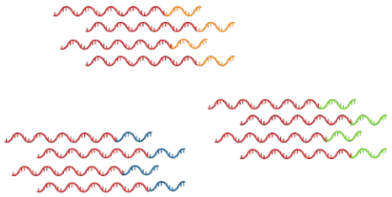
separating cells



RNA extraction

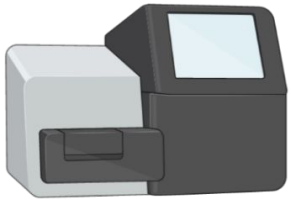


cDNA conversion

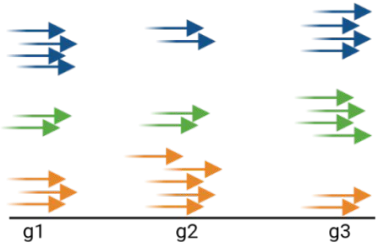


barcoding (UMI)

UMI = Unique Molecular Identifiers



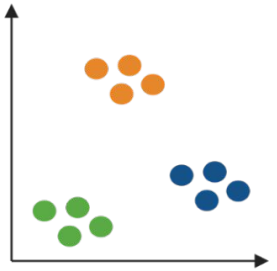
sequencing



read mapping

	s1	s2	s3
g1	0.6	0.3	0.6
g2	0.8	0.8	1.6
g3	0.8	1.2	0.6

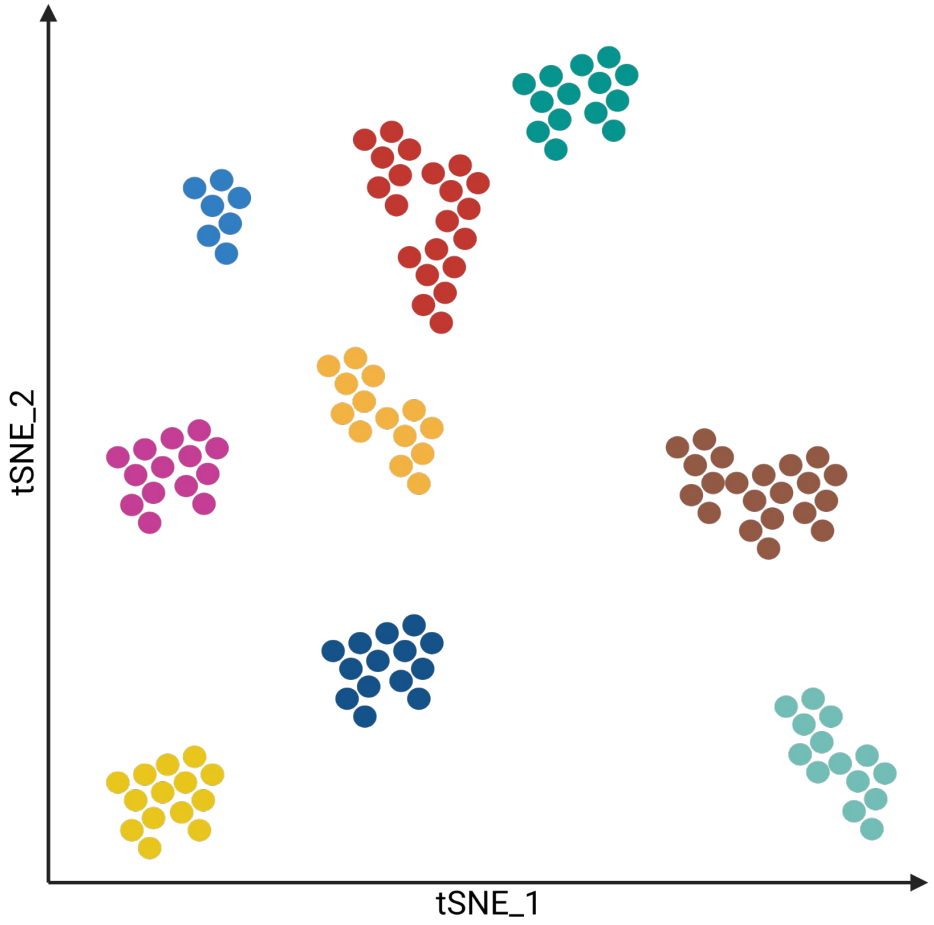
normalization



clustering

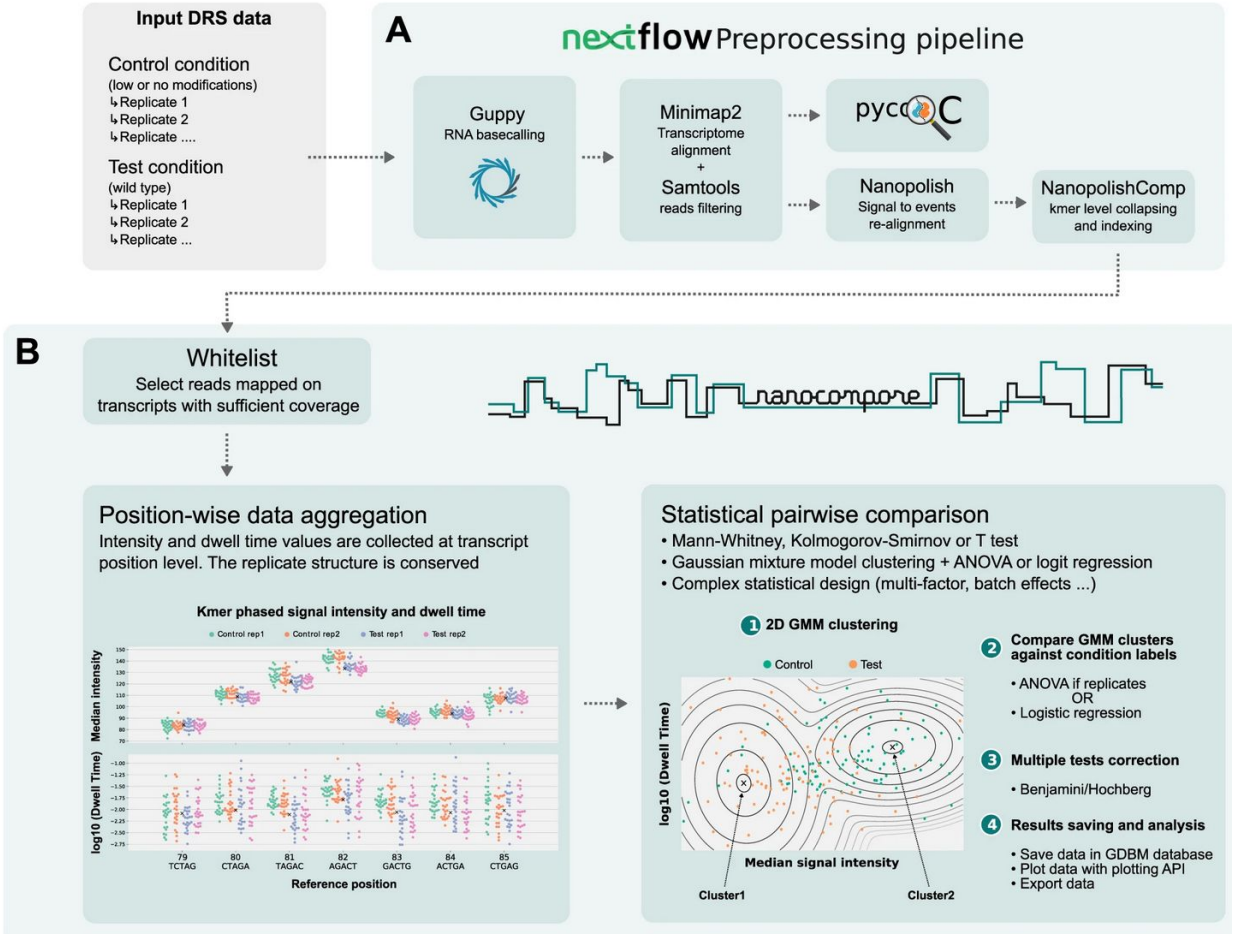
Molecular relationships among individual plant cells

t-distributed stochastic neighbor embedding (tSNE)



Inspired by Ryu et al., 2019: 10.1104/pp.18.01482

RNA modification analysis



https://doi.org/10.1038/s41467-021-27393-3

Time for questions!

Questions

1. How does Iso-Seq work?
2. What is direct RNA sequencing?
3. What are the important steps of single cell transcriptomics?
4. How to study RNA modifications?