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What is HTS?

General presentation on the technology

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Terms related to HTS

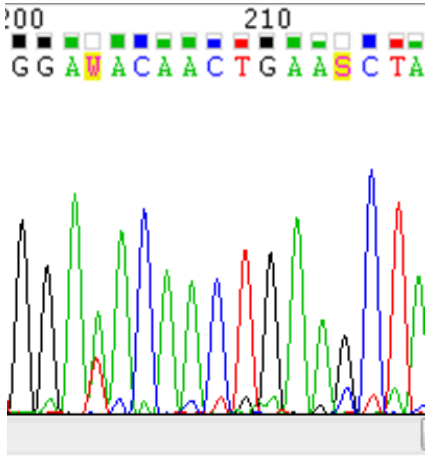
- High throughput sequencing (HTS) = Next generation gequencing (NGS) = deep sequencing
- Different platforms: Illumina dye sequencing, PacBio's SMRT, Ion Torrent, Oxford Nanopore, SOLiD…
- Different tecniques: amplicon sequencing (eg. metabarcoding), shotgun sequencing (eg. whole genome sequencing, siRNA sequencing)

Sanger sequencing vs HTS

- Sanger sequencing:
- Targeted
- Nucleic acids are prepared for sequencing by PCR-reaction that produces different lenght products ending with labeled nucleotides
- Sequencer separates the products by lenght and detects the labeled nucleotides
- One consensus sequence is produced

Extracted nucleic acids

Preaparation for sequencing

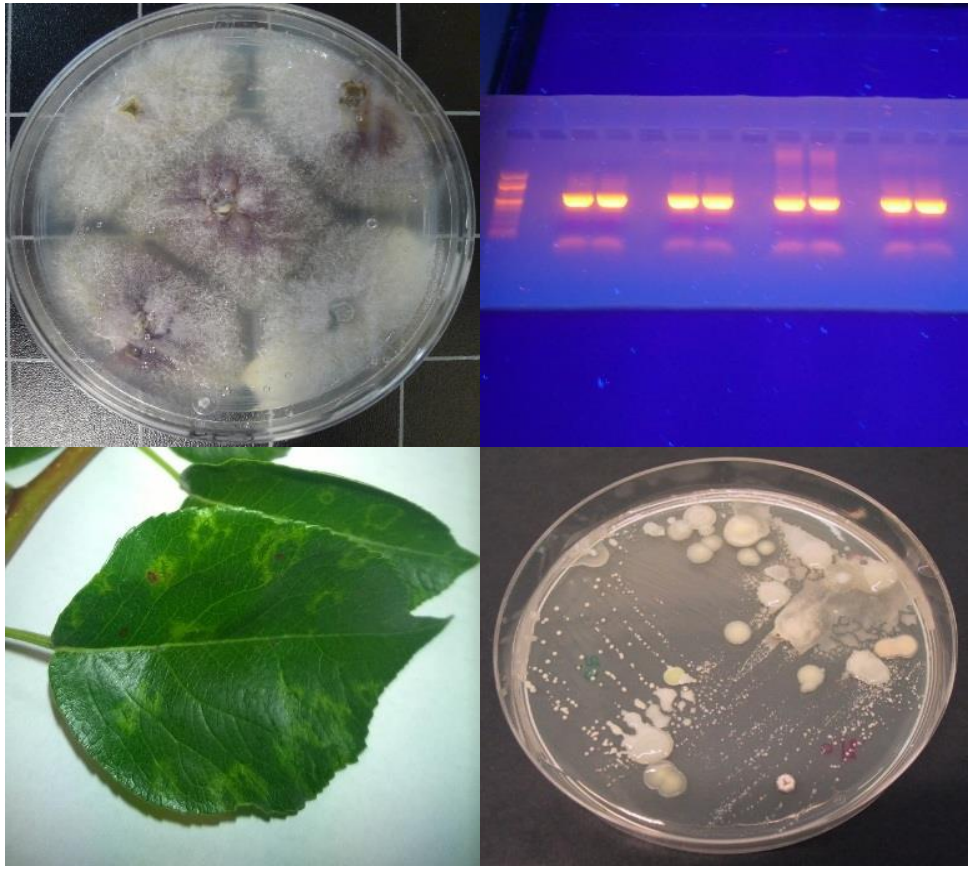


Output GGAAACAACTGAAGCTA
GGAAACAACTGAACCTA
GGATACAACTGAAGCTA

- HTS
- Targeted or non-targeted
- Nucleic acids are prepared for sequencing by library preparation to obtain right amount of right size nucleic acids with flanking adapters (depending on the platform)
- Sequencers detect the nucleic acid incorporation event during elongation or read the strand directly (depending on the platform)
- Individual sequences for each nucleic acid in the sample is produced

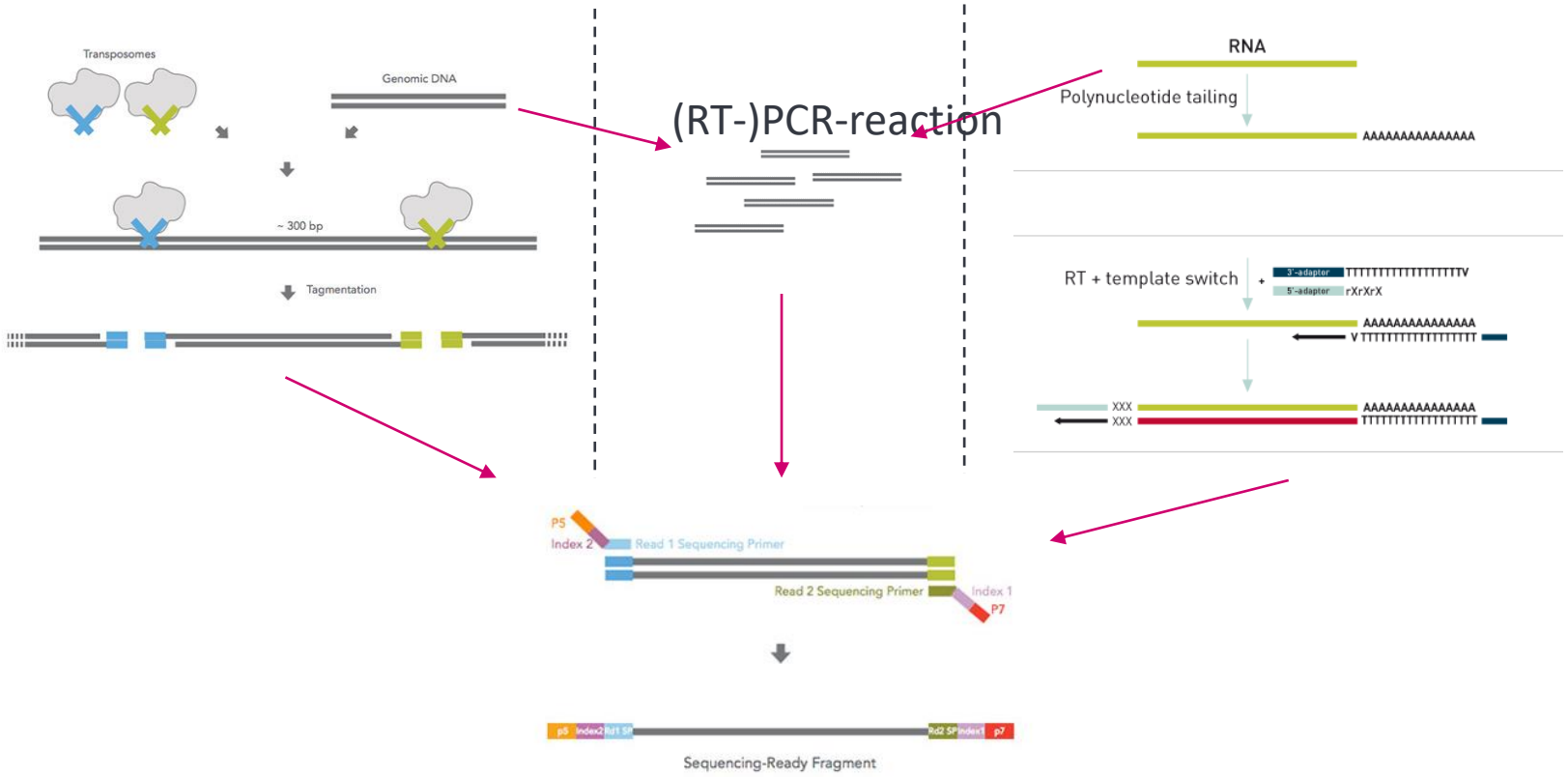
HTS procedure starts with (sampling and) nucleic acid extraction

- matrix with multiple organisms, e.g.
  - plant tissue containing microorganisms
  - environmental samples
  - spore traps
- isolated organisms e.g. microbial colonies on artificial media
- The nucleic acids can be
  - genomic DNA or RNA
  - total DNA or RNA
  - small RNAs
  - double-stranded RNAs



Library preparation

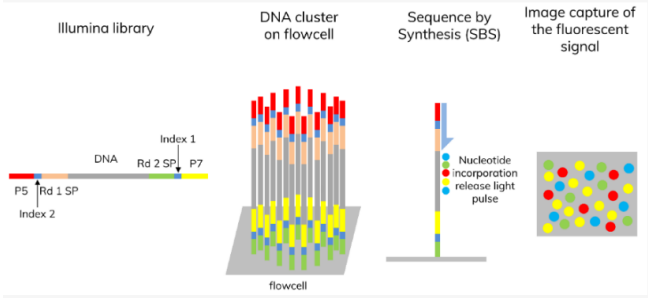
- The library preparation produces a sufficient amount of nucleic acids of appropriate size that are flanked with adapters (oligonucleotide sequences) required for sequencing
- Note, direct RNA sequencing also possible



Sequencing

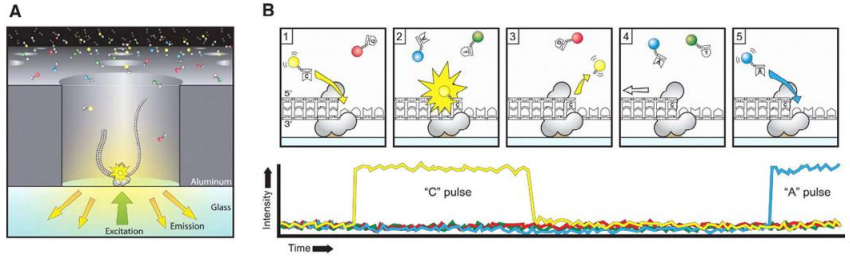
- This step produces the sequences and corresponding quality scores usually in FASTQ format

Illumina dye sequencing capturing fluorescent signals caused by incorporation of labeled nucleotides



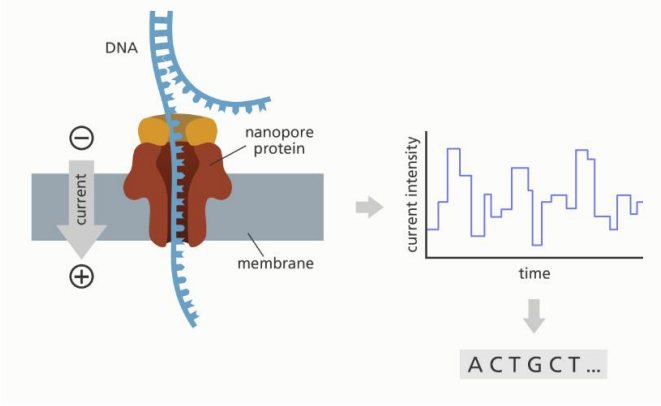
https://www.mdpi.com/2075-4418/13/3/373

PacBio Single molecule real-time sequencing measuring kinetic changes caused by nucleotide incorporation



https://www.cd-genomics.com/pacbio-smrt-system-single-molecule-real-time-sequencing.html

Nanopore sequencing monitoring changes to an electrical current as nucleic acids pass through a protein nanopore



https://www.yourgenome.org/facts/what-is-oxford-nanopore-technology-ont-sequencing/

Analysis of raw reads (including quality control)

- Bioinformatic analysis that may include:
  - Elimination of low quality reads
  - Demultiplexing for pooled samples
  - Primer/adapter/index removal
  - Dublicated or background reads removal
  - Pairing and merging reads
  - Artefacts removal

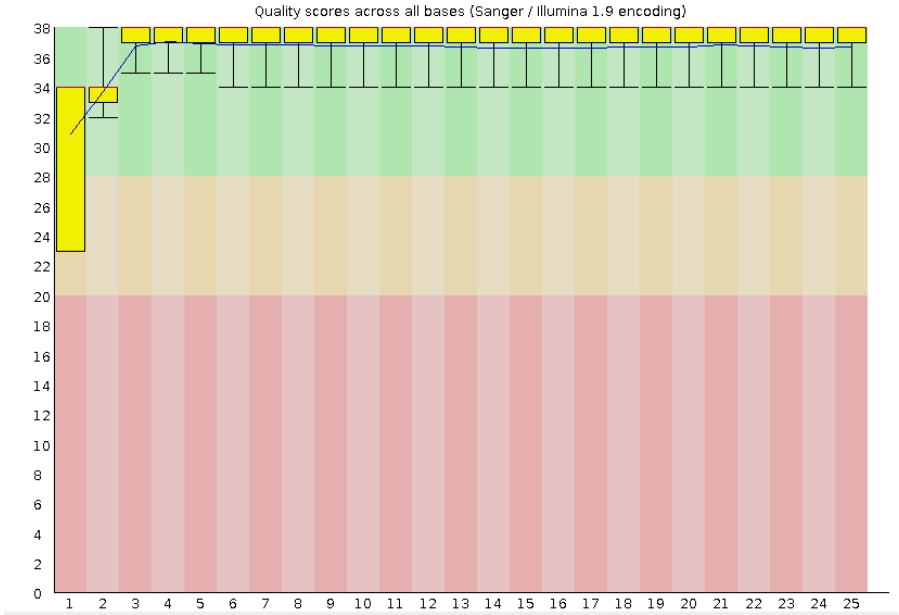
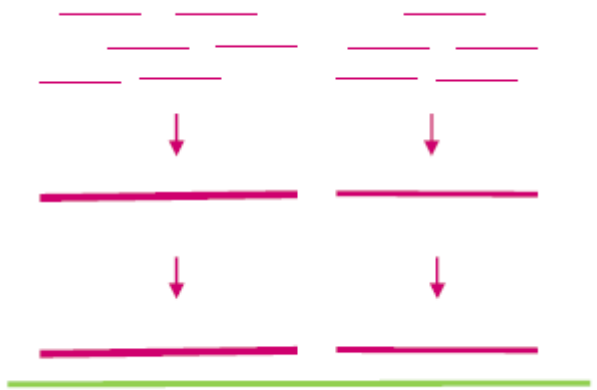


Table 1: Quality Scores and Base Calling Accuracy

Phred Quality Score	Probability of Incorrect Base Call	Base Call Accuracy
10	1 in 10	90%
20	1 in 100	99%
30	1 in 1,000	99.9%
40	1 in 10,000	99.99%
50	1 in 100,000	99.999%

Identification of targets

- Bioinformatic step including optional steps
  - Direct annotation of individual reads
  - De novo assembly of (short) reads to create longer contigs
  - Clustering of amplicon sequences based on similarity
  - Mapping of reads on reference sequence
- Leading to annotation of the sequences, e.g
  - Taxonomic classification
  - Functional annotation
  - Variant calling



Analysis of controls

- Bioinformatic step in which the controls used in the experiment are checked against predefined quality metrics and thresholds to
  - Evaluate contamination
  - Evaluate whether targets are found as expected
  - Find possible sequencing errors
  - Evaluate whether the results need to be confirmed

Applications in plant health

- Identification of pests causing novel diseases
- Development of specific tests (generating sequence data)
- Surveillance programmes
- Detection of pests in propagation material and nuclear stocks
- Post-entry quarantine testing
- Other rutine testing

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Thank you

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