AZ Sint-Jan Brugge

MB&C2026

Workshop NGS

6-2-2026, UCLL Leuven

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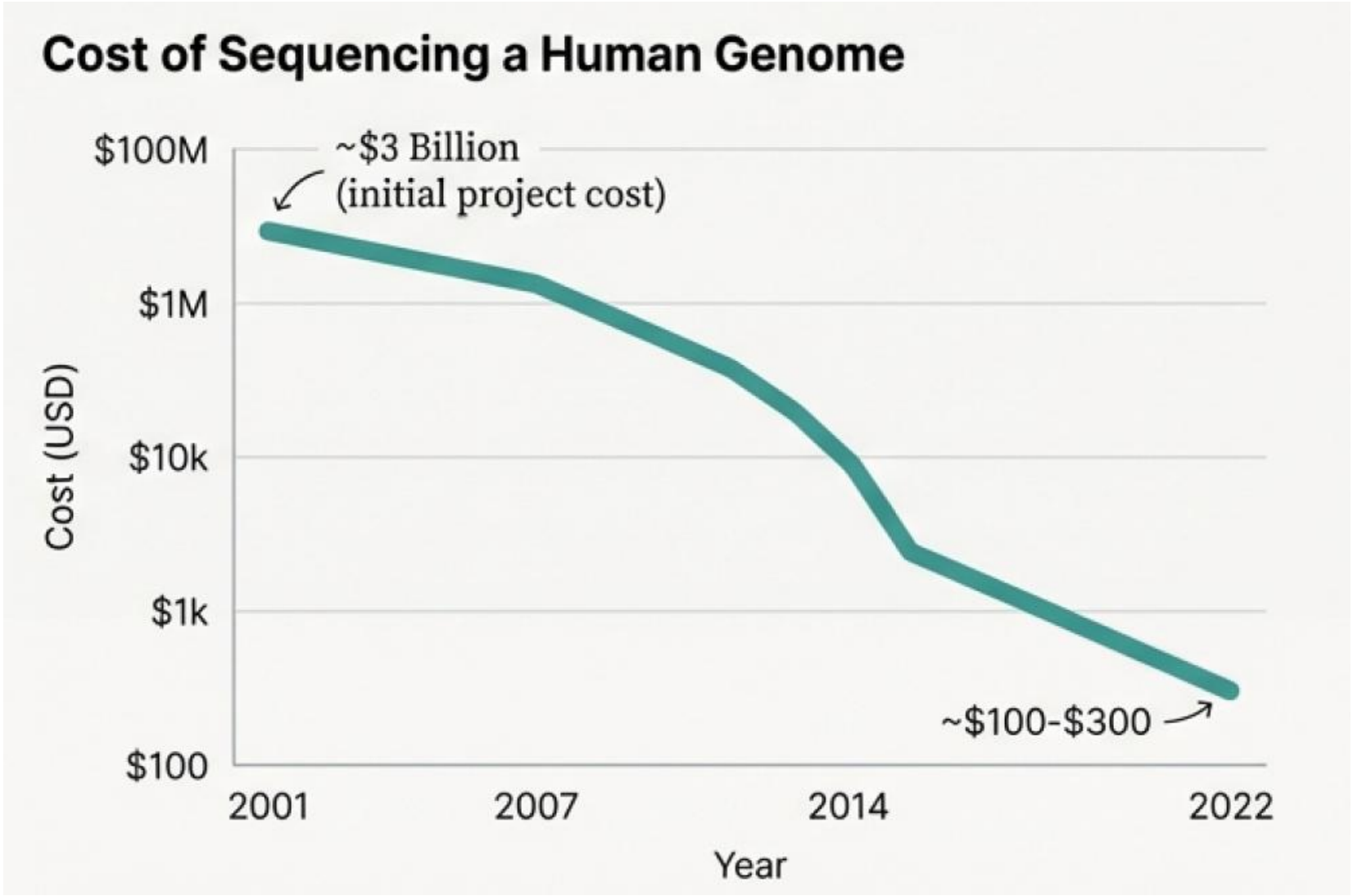
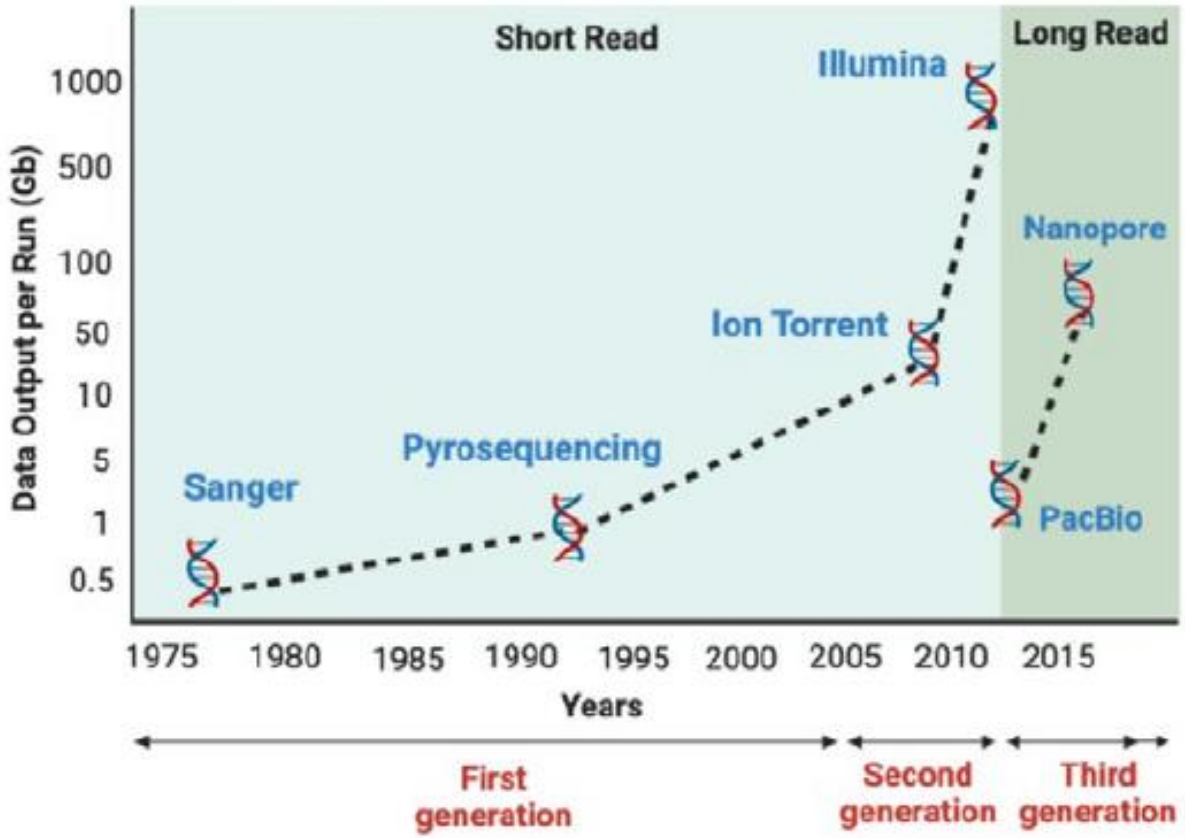
Laboratory Medicine
AZ Sint-Jan Brugge-Oostende AV



Evolution of sequencing technologies

FIGURE 3.1. EVOLUTION OF SEQUENCING TECHNOLOGIES.

The amount of data output possible by each major type of sequencing, split by generation of sequencing chemistry. Source: Satam, et al. 2023[1].



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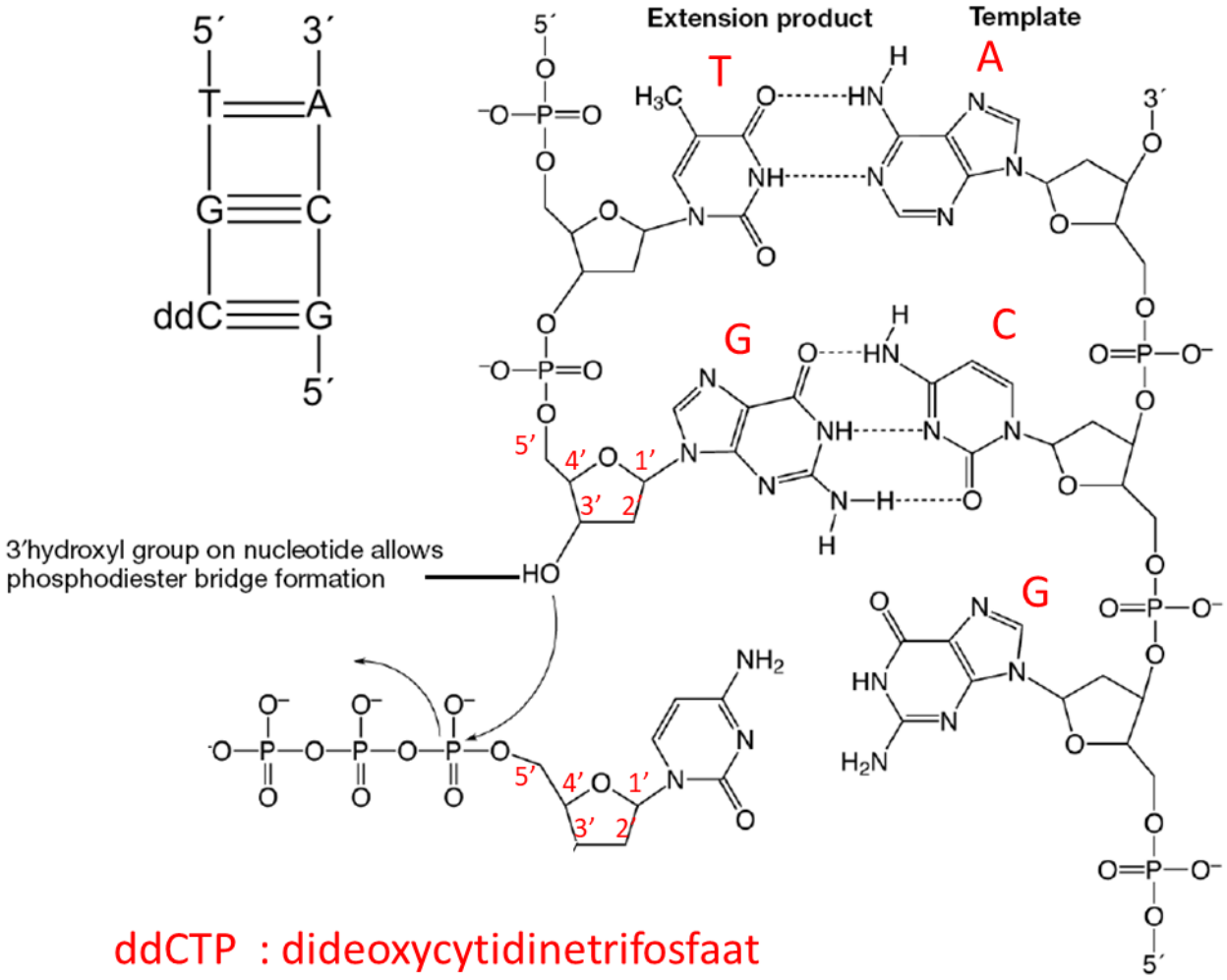
Sanger sequencing - Dye Terminator Sequencing



ddCTP : dideoxycytidinetrifosfaat

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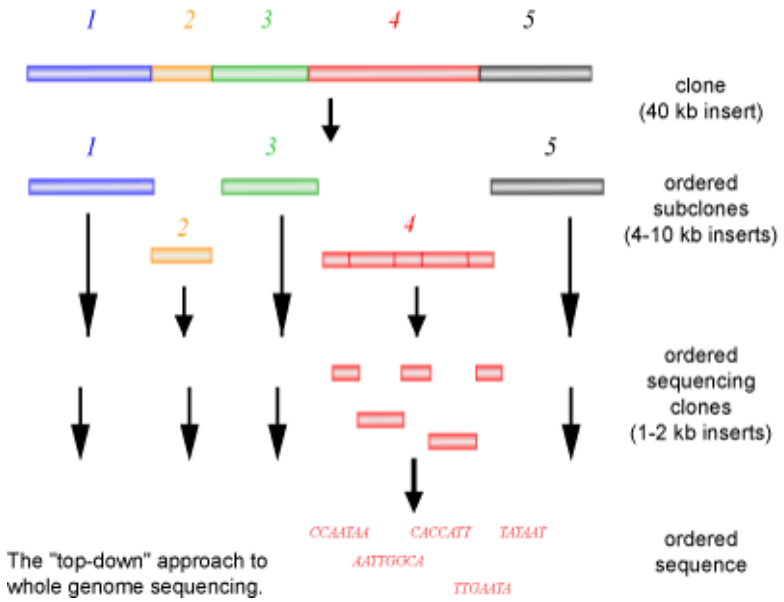
Human genome project (2001 (90%) / 2003 (99%))

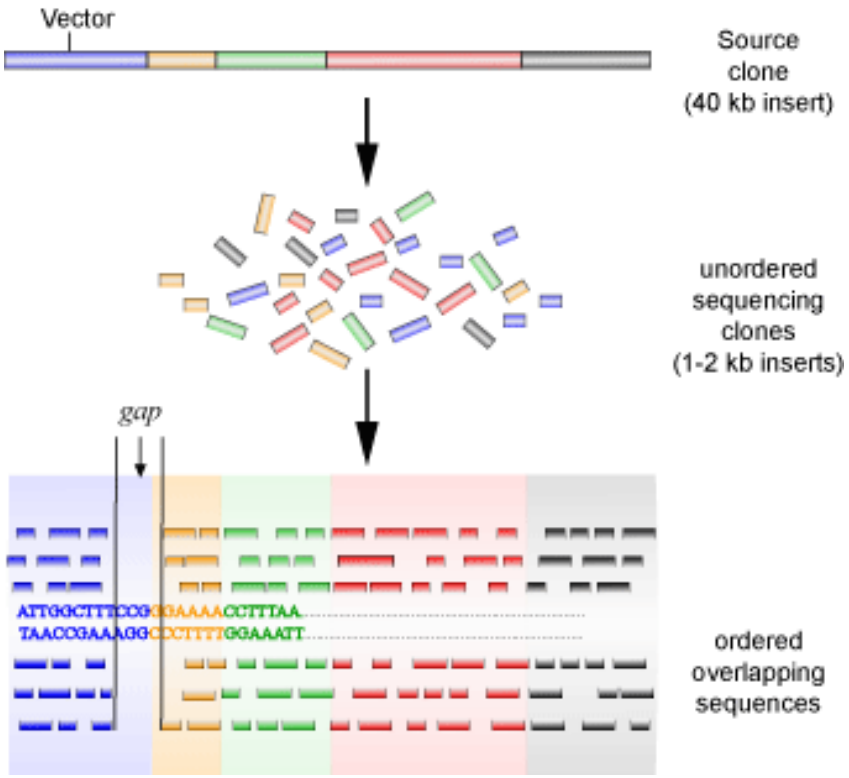
BAC-to-BAC sequencing



The "top-down" approach to whole genome sequencing.

strategie publiek consortium

Shotgun sequencing



strategie Celera Genomics

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The Human Genome

1 genome = 3.3 Gigabase (Gb)

127 volumes
1000 pages/volume
26.000 bases per page

Computer data:
3.3 billion basen
2 bits per base = 786 Mb

Wellcome Collection, London

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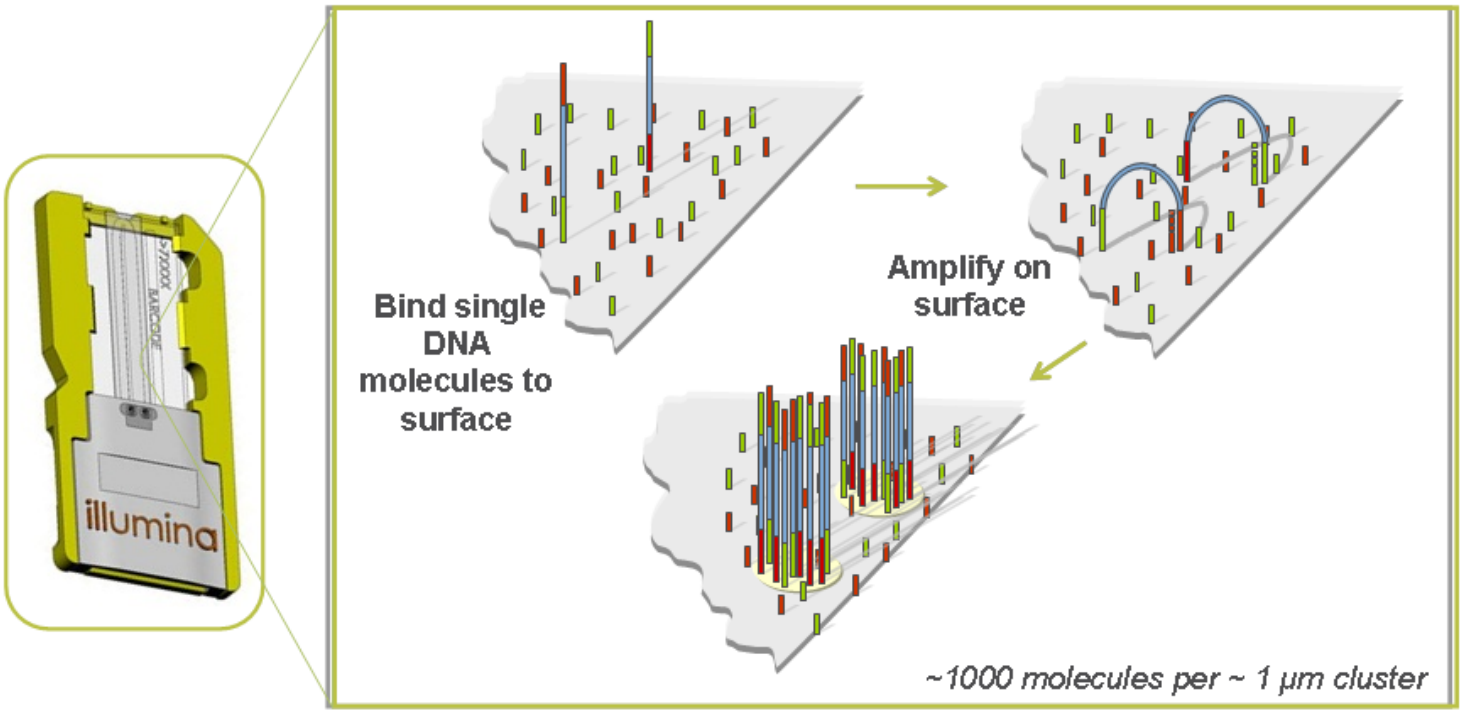
Illumina ~90% market share- Bridge amplification



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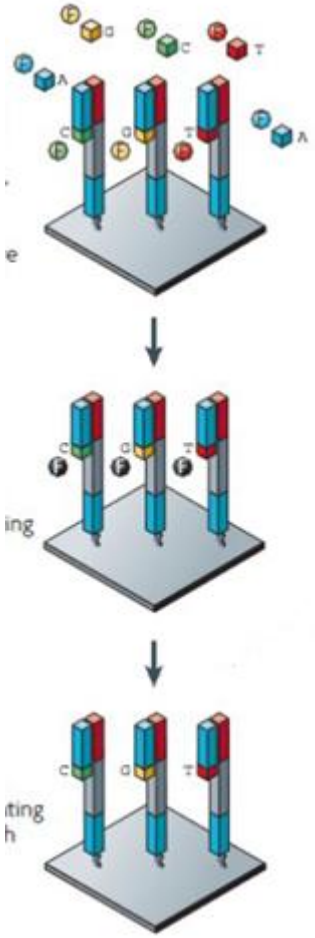
Illumina reversible dye terminator sequencing

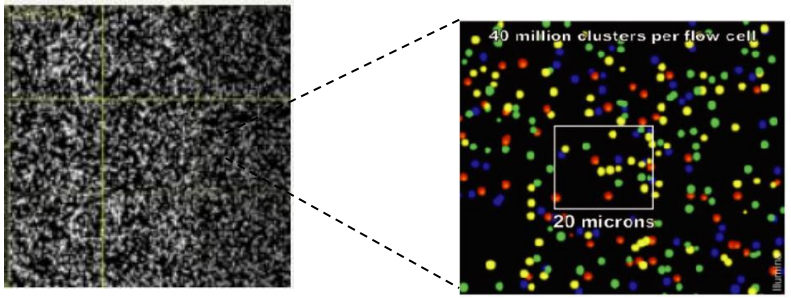
Sequencing cycle



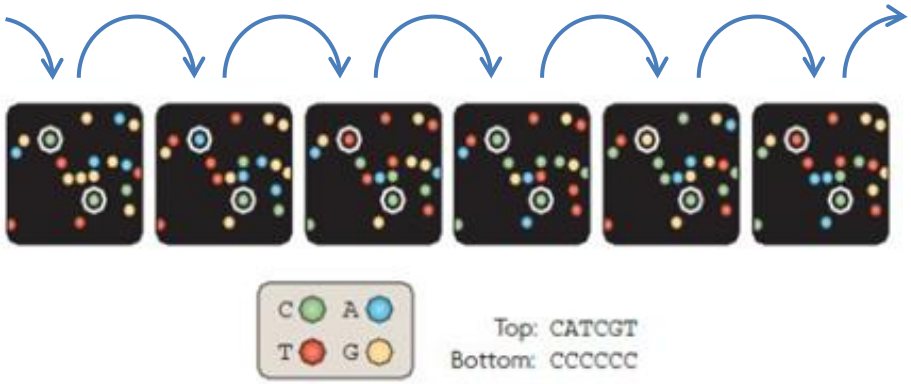
Incorporate all four nucleotides, each labeled with a different dye

Wash and 4-color image

Cleave dye and terminating groups, wash



10^6 clusters/mm2



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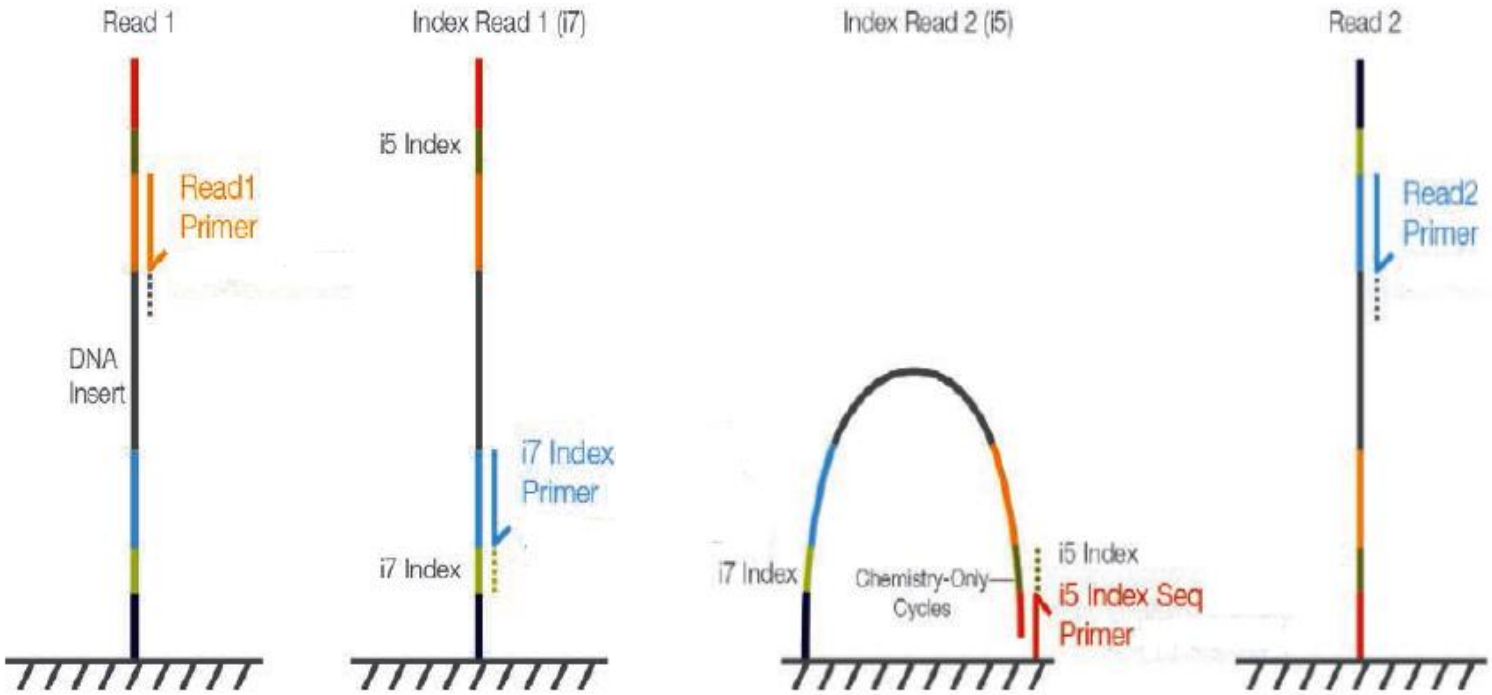
Paired-end sequencing



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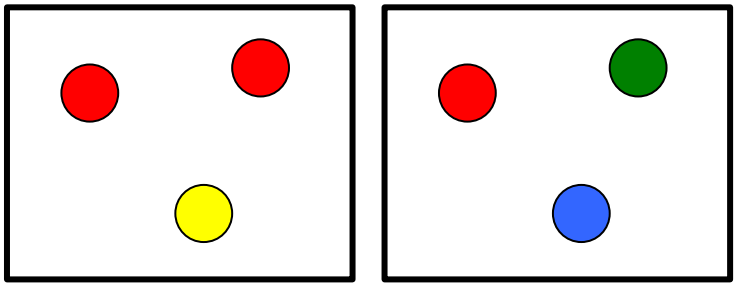
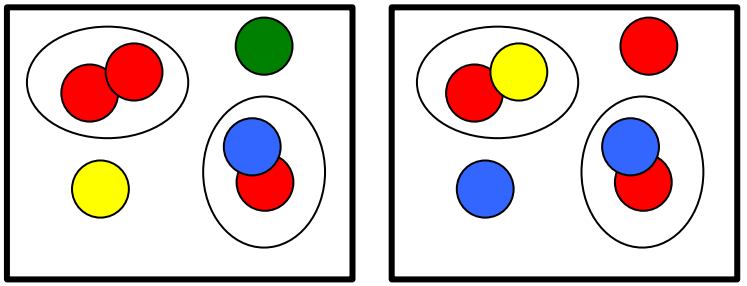
Cluster densiteit

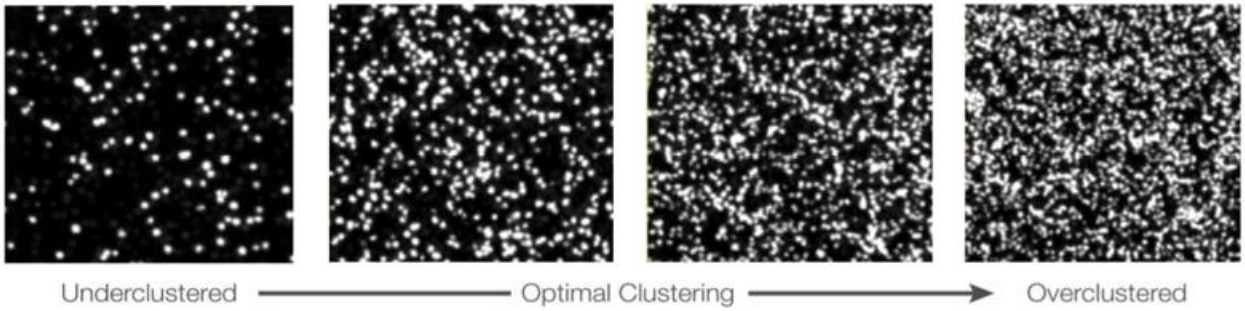


Well-spaced clusters easier to call



Densely-packed clusters difficult to call

- Optimale cluster densiteit
  - Miseq v2 1000-1200 K/mm2; v3 1200-1400 K/mm2
  - NextSeq550 : 170-220 K/mm2
- Accurate kwaliteitscontrole en kwantificatie (pM) van de library is essentieel!



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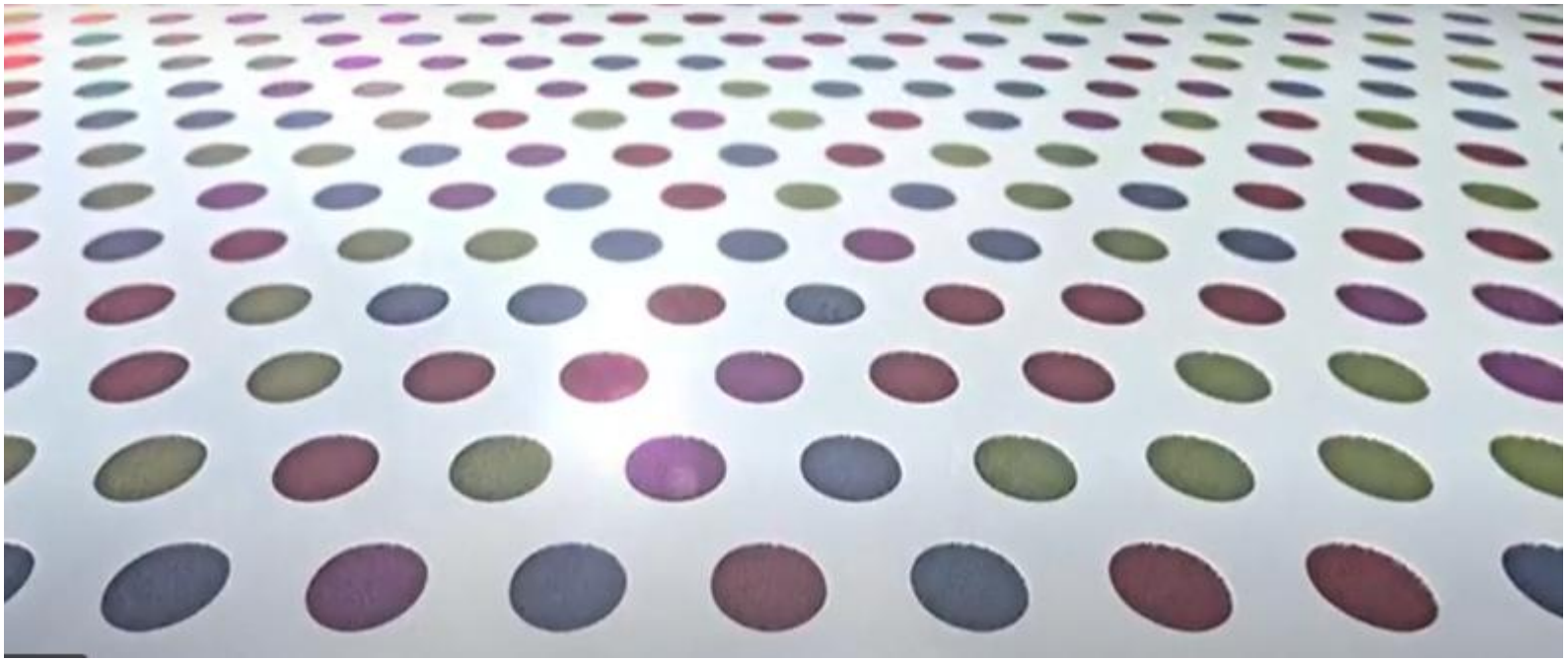
Patterned flow cell technology



- less susceptible to overloading, more tolerant to a broader range of library densities.
- no need to map cluster sites (saves time).

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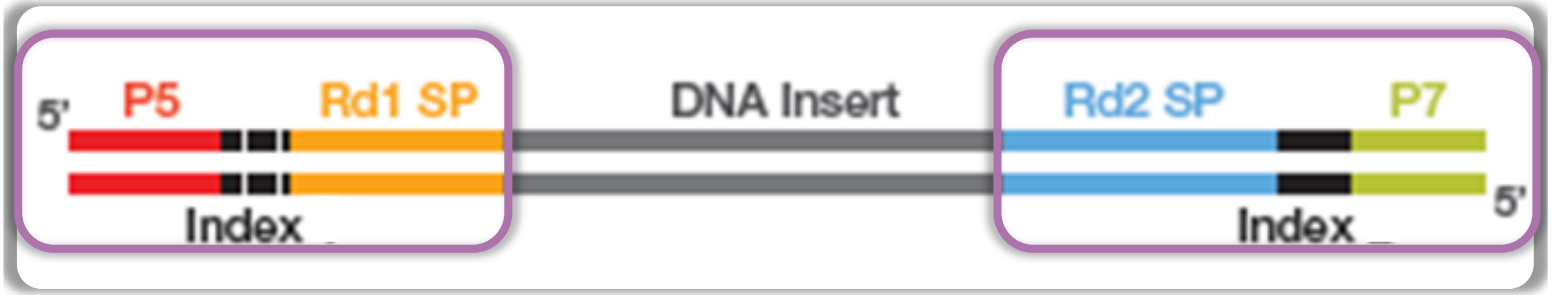
Library prep

Library molecules voor illumina



- **P5 and P7** sequenties aan uiteinden van de adapters binden aan de flowcell
- **Index** sequentie : identificeren van stalen
- **Rd1 SP en Rd2 SP** : Read-1 en -2 sequencing primer sequentie
- **DNA insert** meestal ~200bp tot 1kb

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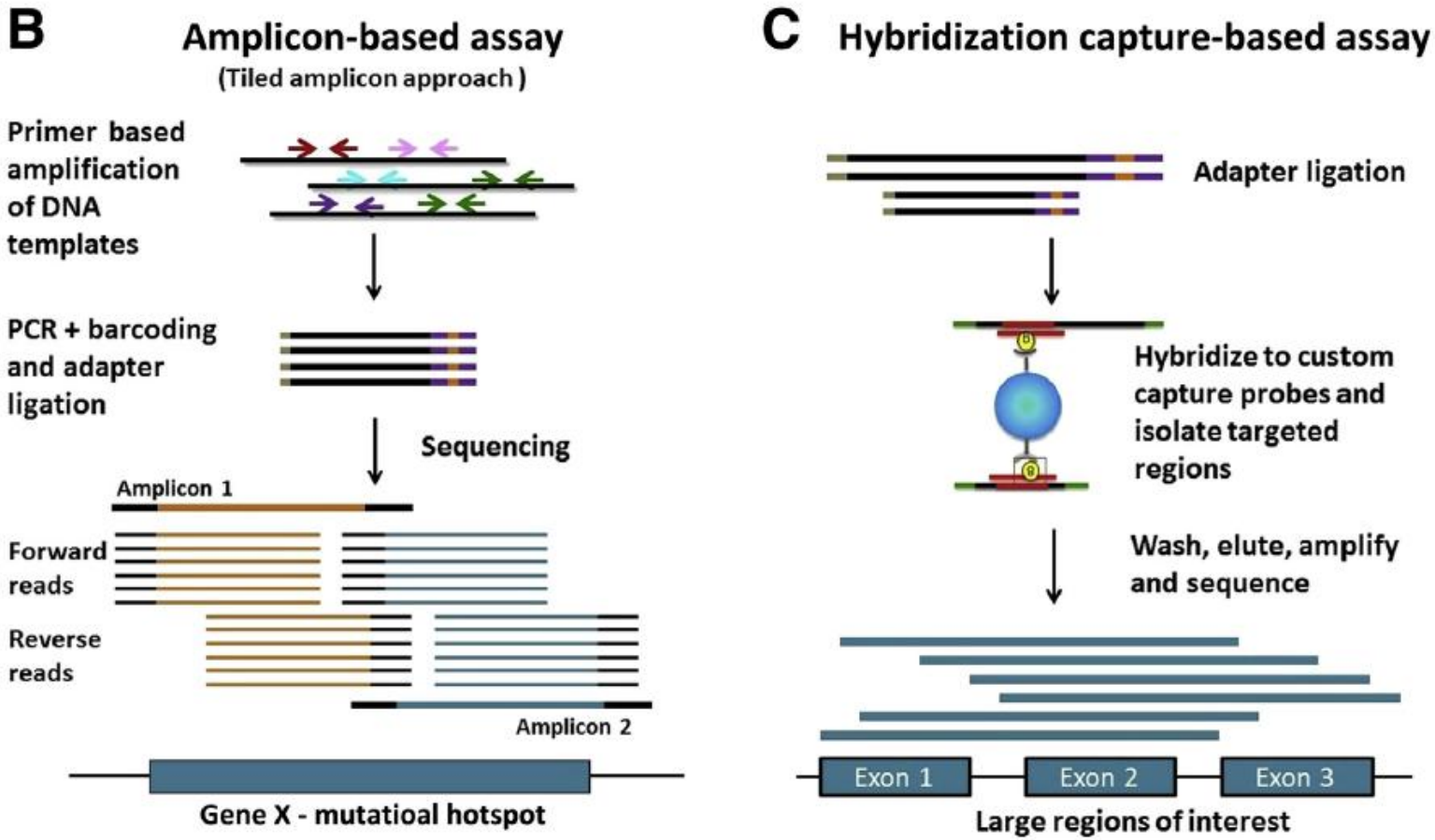
Library prep approaches



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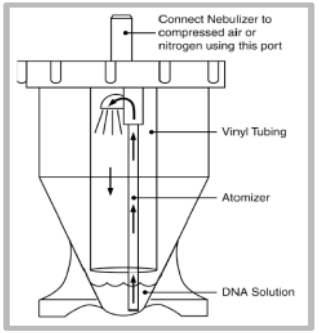
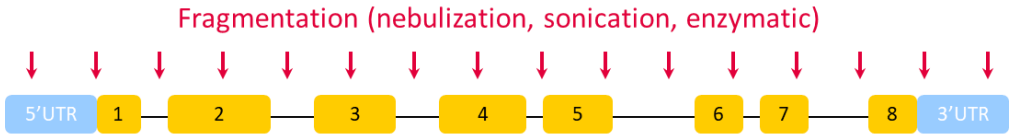
Library prep - VOORBEELD

- Stap-1 : Fragmentatie
  - Nebulizer, goedkoop, OK voor 1-5 µg starting input
  - Andere methodes : Covaris™, Sonication, HydroShear®, Enzymatic!,…





- Stap-2 : End-repair, 5' fosforylatie en dA-tailing
  - Creeërt blunt ends : dNTP, T4 DNA polymerase, Klenow polymerase
  - 5' end fosforylatie : kinase, ATP
  - Converts 3' A-overhangs: dNTP, T4 DNA polymerase, Klenow polymerase

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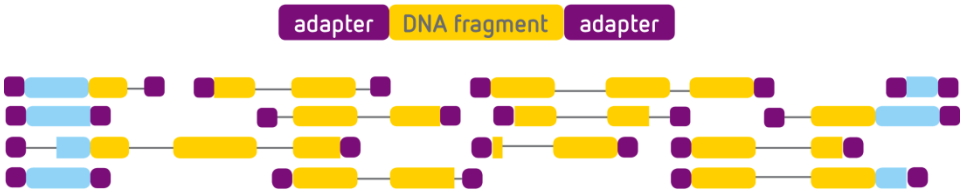
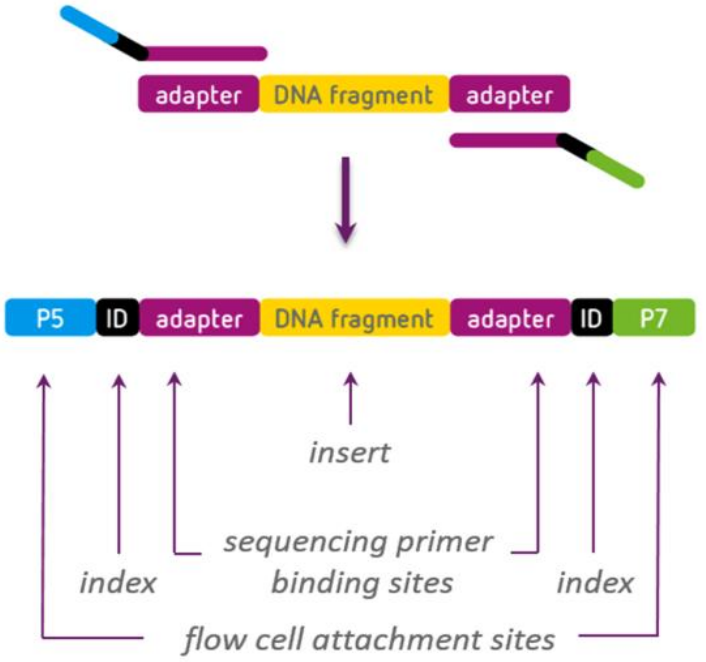
Library prep - VOORBEELD

- Stap-3 : Adapter ligatie
  - DNA ligase
  - (10:1 molar ration of adaptor to insert DNA)



- Stap-4 : Index PCR
  - Single of dual barcoding



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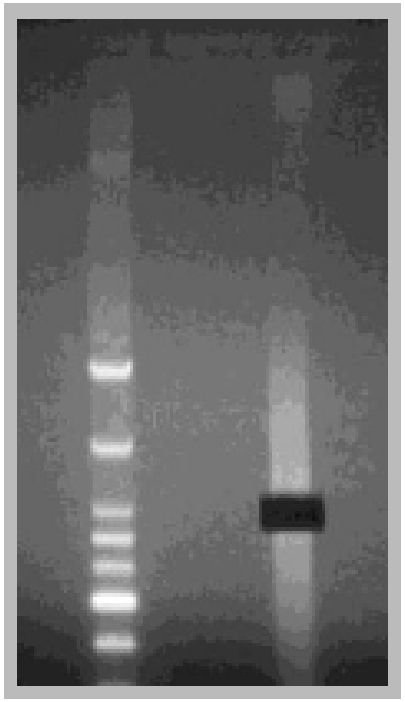
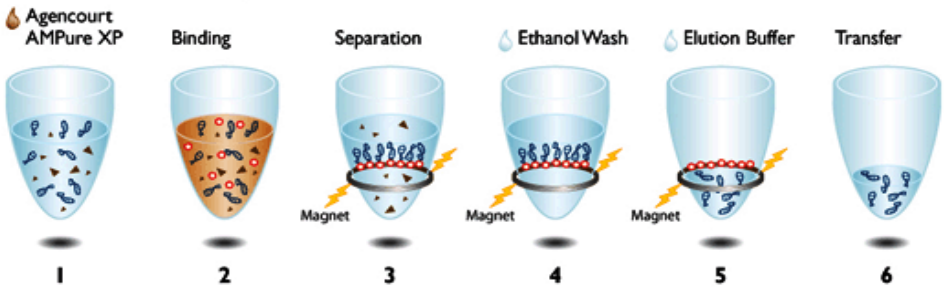
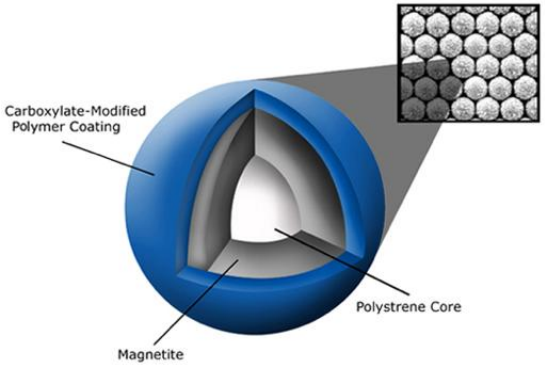
Size selection

- On gel :
- <u>SPRI beads (AMPure beads)</u>
  Solid Phase Reversible Immobilization
  (crowding agent Polyethylene glycol, PEG)
  1. PCR reactie
  2. Binding van de PCR amplicons aan de magnetische beads
  3. PCR amplicons gebonden aan de magnetische beads wordt gescheiden van de contaminanten
  4. Wassen van de PCR amplicons met ethanol
  5. Elutie van PCR amplicons van de magnetische beads
  6. Transfer van de PCR amplicons in een nieuwe reactietube.



Reference : Hawkins TL et al., NAR 1994 22:4543-4





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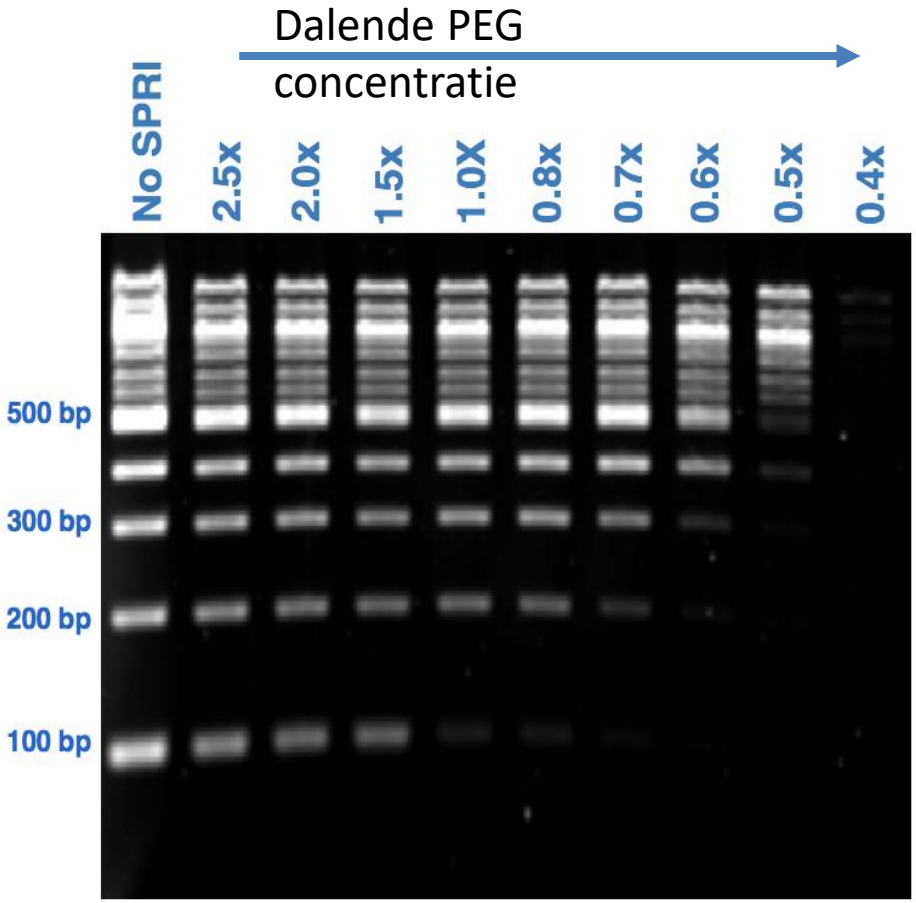
SPRI bead:DNA ratio



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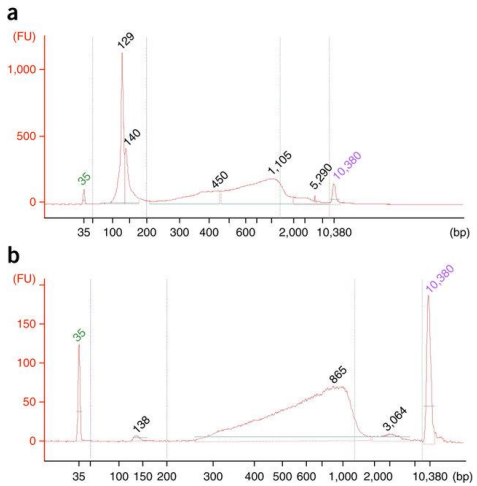
Library zuiveren, poolen en sequencen

- Library clean-up en size selectie met 0.6x SPRI beads (bead:DNA ratio)
- kwantificatie van de stalen met Qubit fluorometer
- Pooling
- Library kwantificatie met Qubit fluoromet
- Eventueel lengte bepaling met Bio-Analyzer of TapeStation (electroforese)
- Verdunnen naar een geoptimaliseerde concentratie (bvb. 10 pM)
- Library denaturatie (met NaOH)
- Laden op de MiSeq



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Illumina: MiSeq i100 Series

Size	Benchtop (402 mm x 256 mm x 448 mm, W = 36.0 kg)
Sequencing chemistry	Sequencing by synthesis
Output per run	1.5 Gb – 30 Gb
Number of paired-end reads per run	Up to 200 million
Max read length	2 x 300 bp
Max run duration	~15.5 hours
Quality score	≥ 90% bases higher than Q30 (PE150), ≥ 85% bases higher than Q30 (PE300)
Typical applications	Transcriptomics, microbial genomics, targeted gene sequencing

Illumina: iSeq100™

Illumina have announced that the iSeq 100 Sequencing System will become obsolete. It will be available for order until 30th September 2025 with full system support until 31st December 2029.

Size	Benchtop (425 mm x 305 mm x 330 mm, W = 15.9 kg)
Sequencing chemistry	Sequencing by synthesis
Output per run	144 Mb – 1.2 Gb
Number of paired-end reads per run	Up to 8 million
Max read length	2 x 150 bp
Max run duration	19 hours
Quality score	80% bases higher than Q30 (PE150)
Typical applications	Small whole-genome sequencing, targeted gene sequencing, gene expression profiling

Illumina: MiSeqDx™

Size	Benchtop (686 mm x 565 mm x 523 mm, W = 54.5 kg)
Sequencing chemistry	Sequencing by synthesis
Output per run	≥ 5 Gb
Number of paired-end reads per run	≥ 15 million
Max read length	2 x 150 bp
Max run duration	24 hours
Quality score	≥ 80% bases > Q30 (PE150)
Typical applications	In vitro diagnostic use. cystic fibrosis testing, IVD assay development, targeted enrichment and cancer companion diagnostics

***Note:** Values above are for Diagnostic mode. In Research (RUO) mode, the Dx has the same performance specifications as the MiSeqDx™.*

Illumina: NextSeq™ 550*

Size	Benchtop (533 mm x 635 mm x 584 mm, W = 83 kg)
Sequencing chemistry	Sequencing by synthesis
Output per run	16 Gb – 120 Gb
Number of paired-end reads per run	130 million – 800 million
Max read length	2 x 150 bp
Max run duration	26 hours
Quality score	> 80% bases > Q30 (PE150)
Typical applications	Exome sequencing, mRNA sequencing, small whole-genome sequencing, transcriptomes sequencing, targeted gene sequencing, microarray sequencing

**Install specifications are based on Illumina PhiX control library at supported cluster densities (between 129 and 165 k/mm2 clusters passing filter). Actual performance parameters may vary based on sample type, sample quality, and clusters passing filter. The percentage of bases > Q30 is averaged over the entire run.*

Illumina: MiniSeq™

Illumina have announced that the MiniSeq Sequencing System will become obsolete. It will be available for order until 30th September 2025 with full system support until 31st December 2029.

Size	Benchtop (456 mm x 480 mm x 518 mm, W = 45 kg)
Sequencing chemistry	Sequencing by synthesis
Output per run	1.65 Gb - 7.5 Gb
Number of paired-end reads per run	14 million – 50 million
Max read length	2 x 150 bp
Max run duration	~ 24 hours
Quality score	> 80% bases higher than Q30 (PE150)
Typical applications	Small whole-genome sequencing, targeted gene sequencing, targeted gene expression profiling, 16S metagenomic sequencing

Illumina: MiSeq™

Illumina have announced that the MiSeq Sequencing System will become obsolete. It will be available for order until 30th September 2025 with full system support until 31st December 2029.

Size	Benchtop (686 mm x 565 mm x 523 mm, W = 57.2 kg)
Sequencing chemistry	Sequencing by synthesis
Output per run	540 Mb – 15 Gb
Number of paired-end reads per run	24 million – 50 million
Max read length	2 x 300 bp
Max run duration	24 hours (PE150), 56 hours (PE300)
Quality score	80% bases > Q30 (PE150), 70% bases > Q30 (PE300)
Typical applications	Small whole-genome sequencing, targeted gene sequencing, targeted gene expression profiling, 16S metagenomic sequencing

Illumina: NextSeq™ 550Dx*

Size	Benchtop (540mm x 690 mm x 580 mm, W = 84.4 kg)
Sequencing chemistry	Sequencing by synthesis
Output per run	16.25 Gb - 120 Gb
Number of paired-end reads per run	> 300 million
Max read length	2 x 150 bp
Max run duration	≤ 35 hours
Quality score	> 75% bases > Q30 (PE150)
Typical applications	In vitro diagnostic use. IVD assay development, targeted enrichment, non-invasive prenatal testing, comprehensive genomic profiling

***Note:** Values above are for Diagnostic mode. In Research (RUO) mode, the Dx has the same performance specifications as the NextSeq™ 550. **European Union Intended Use Statement:** The NextSeq 550Dx instrument is intended for*

Illumina: NextSeq™ 1000 & 2000

Size	Benchtop (550 mm x 650 mm x 600 mm, W = 141 kg)
Sequencing chemistry	Sequencing by synthesis
Output per run	10 Gb - 540 Gb
Number of paired-end reads per run	200 million – 3.6 billion
Max read length	2 x 300 bp
Max run duration	44 hours
Quality score	1 × 50 bp, 2 × 50 bp, 2 × 100 bp, 2 × 150 bp ≥ 90% of bases higher than Q30 2 × 300 bp ≥ 85% of bases higher than Q30
Typical applications	Small whole-genome sequencing, exome & large panel sequencing, targeted gene sequencing, targeted gene expression, chromatin analysis, methylation sequencing, single-cell profiling, transcriptome sequencing, metagenomic profiling, proteogenomics, 16S metagenomic sequencing

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Illumina: NovaSeq™ 6000

Size	Production-Scale (800mm x 945 mm x 1656 mm, W = 481 kg)
Sequencing chemistry	Sequencing by synthesis
Output per run	65 Gb – 3 Tb
Number of paired-end reads per run	1.3 billion – 20 billion
Max read length	2 x 250 bp
Max run duration	~ 44 hours
Quality score	≥ 85% bases > Q30 (PE150)
Typical applications	Large whole-genome sequencing, exome & large panel sequencing, single-cell profiling, transcriptome sequencing, chromatin analysis, methylation sequencing, metagenomic profiling, proteogenomics, cell-free sequencing & liquid biopsy analysis

Illumina: NovaSeq™ 6000Dx

Size	Production-Scale (800mm x 945 mm x 1656 mm, W = 481 kg)
Sequencing chemistry	Sequencing by synthesis
Output per run	1 Tb – 6 Tb
Number of paired-end reads per run	6.67 billion – 40 billion
Max read length	2 x 150 bp
Max run duration	≤ 45 hours
Quality score	≥ 85% bases > Q30 (PE150)
Typical applications	In vitro diagnostic use, targeted enrichment, IVD assay development.

***Note:** Values above are for Diagnostic mode. In Research (RUO) mode, the Dx has the same performance specifications as the NovaSeq™ 6000.*

Thermo Fisher Scientific: Ion GeneStudio™ S5

Size	Benchtop (542 mm x 806 mm x 509 mm, W = 63.5 kg)
Sequencing chemistry	Ion semiconductor sequencing
Output per run	10 Gb – 15 Gb
Number of reads per run	60 million – 80 million
Max read length	600 bp
Max run duration	19 hours
Quality score	> 99% bases > Q30
Typical applications	Targeted DNA sequencing, exome sequencing, targeted RNA sequencing, targeted transcriptome sequencing, whole transcriptome sequencing

Thermo Fisher Scientific: Ion GeneStudio™ S5 Prime

Size	Benchtop (542 mm x 806 mm x 509 mm, W = 63.5 kg)
Sequencing chemistry	Ion semiconductor sequencing
Output per run	20 Gb – 25 Gb
Number of reads per run	100 million – 130 million
Max read length	600 bp
Max run duration	8.5 hours
Quality score	> 99% bases > Q30
Typical applications	Exome sequencing, targeted transcriptome sequencing, whole transcriptome sequencing

Illumina: NovaSeq™ X and X Plus

Size	Production-Scale (864 mm x 933 mm x 1588 mm, W = 532 kg)
Sequencing chemistry	Sequencing by synthesis
Output per run	165 Gb – 16 Tb
Number of paired-end reads per run	3.2 billion – 52 billion
Max read length	2 x 150 bp
Max run duration	~ 48 hours
Quality score	≥ 85% bases > Q30 (PE150)
Typical applications	Large whole-genome sequencing, whole exome & large panel sequencing, single-cell profiling, whole transcriptome sequencing, chromatin analysis, methylation sequencing, metagenomic profiling, proteogenomics, cell-free sequencing & liquid biopsy analysis

Thermo Fisher Scientific: Ion Torrent GeneXus™

Size	Benchtop (815 mm x 1065 mm x 1678 mm, W = 68 kg)
Sequencing chemistry	Ion semiconductor sequencing
Output per run	15 Gb – 60 Gb
Number of reads per run	15 million – 60 million
Max read length	400 bp
Max run duration	24 hours
Quality score	> 99% bases > Q30
Typical applications	Whole genome sequencing, whole exome sequencing, targeted genome sequencing

MGI: DNBSEQ-E25

Size	Benchtop (348 mm x 312 mm x 257 mm)
Sequencing chemistry	DNA nanoball
Output per run	7.5 Gb
Number of reads per run	25 million
Max read length	2 x 150 bp
Max run duration	20 hours
Quality score	> 80% bases > Q30 (PE150)
Typical applications	Remote site sequencing, pathogen and microbiome study, small whole genome sequencing, targeted sequencing, forensics and education

MGI: DNBSEQ-G50

Size	Benchtop (654 mm x 489 mm x 545 mm, W = 85 kg)
Sequencing chemistry	DNA nanoball
Output per run	150 Gb (PE150-FCL)
Number of reads per run	100 million (FCS) - 500 million (FCL)
Maximum paired-ends read length	2 x 150 bp
Max run duration	28 hours (PE150-FCS) - 40 hours (PE150-FCL)
Quality score	> 80% bases > Q30 (PE150-FCL)
Typical applications	Small Genome sequencing, Low-pass whole genome sequencing, target sequencing, RNA-Seq

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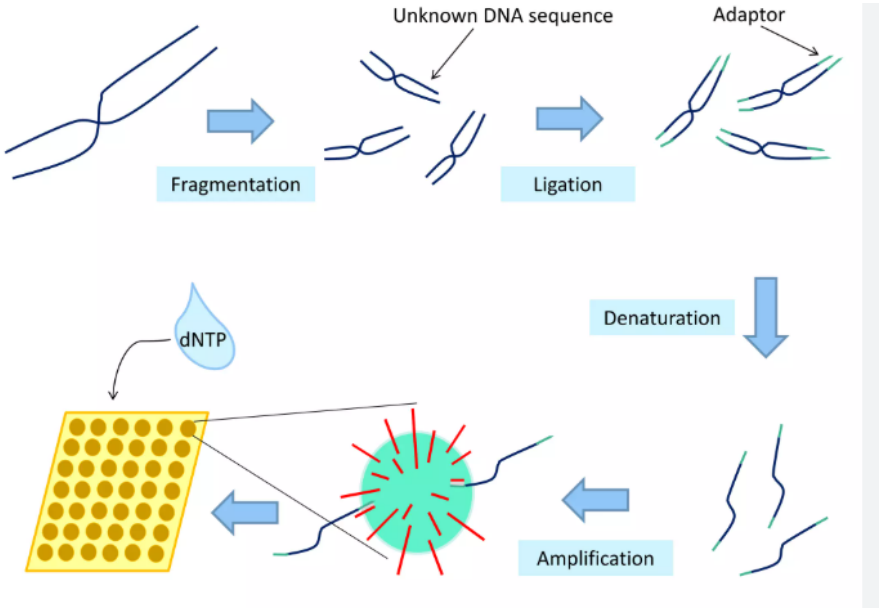
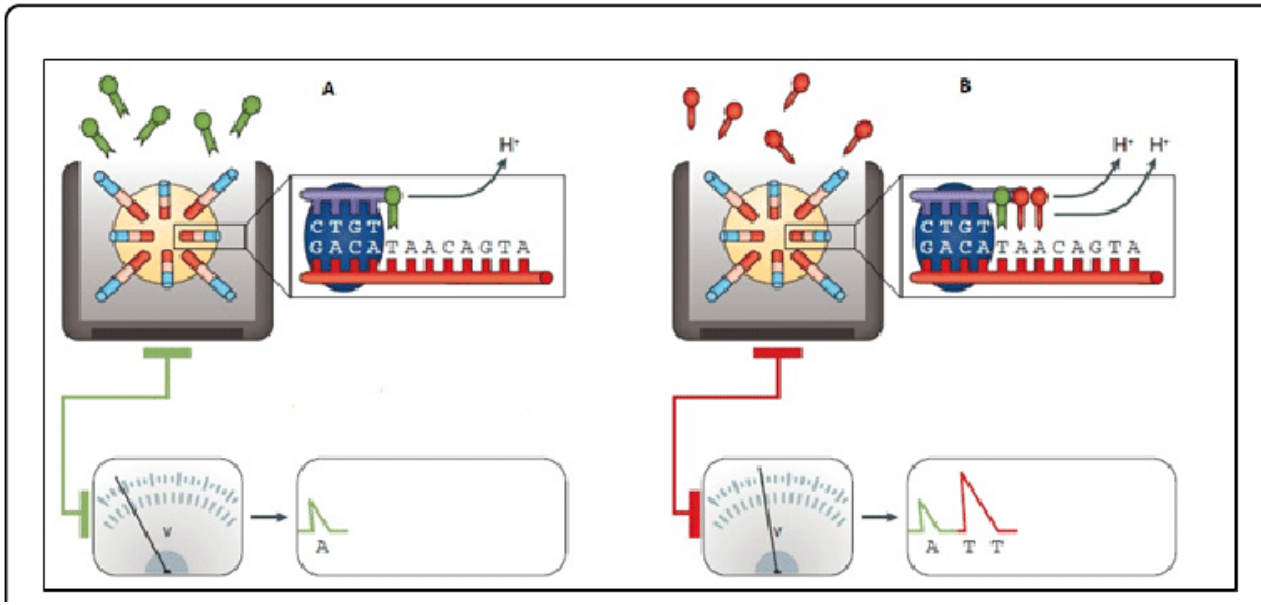
ThermoFisher Ion semi conductor sequencing





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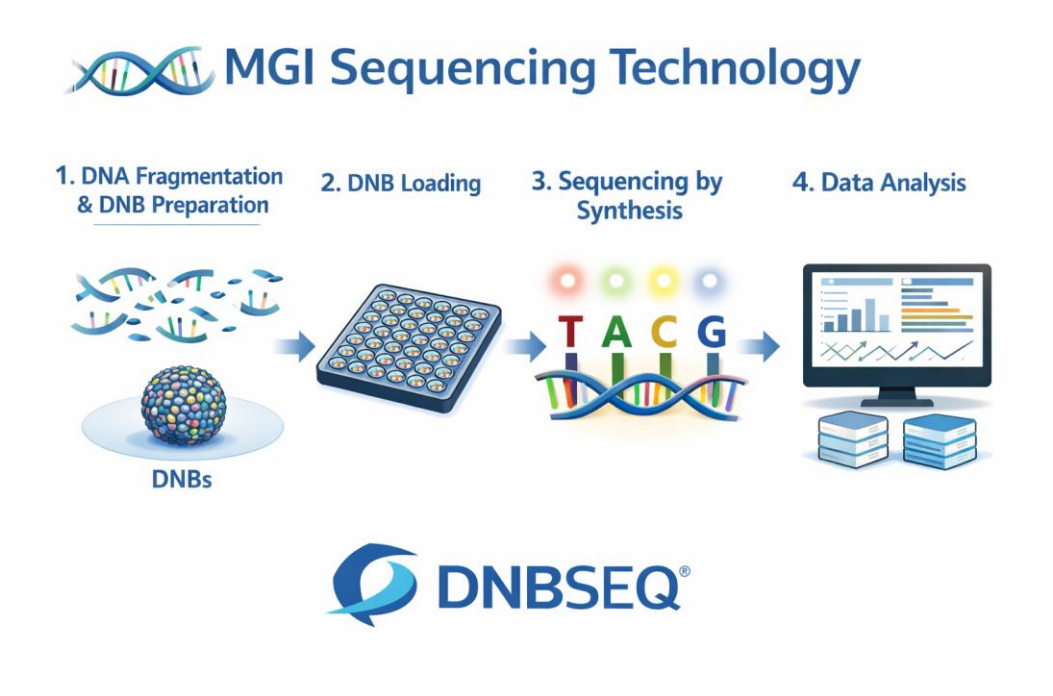
MGI DNA nanoball sequencing (DNBSEQTM)

1. MGI **Rolling Circle Replication (RCR)**.
2. Patterned Array Flow Cells
3. cPAS Chemistry (Combinatorial Probe-Anchor Synthesis)



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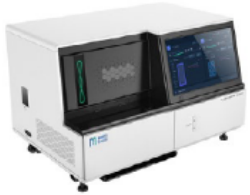
MGI: DNBSEQ-G99

Size	Benchtop (607 mm x 680 mm x 640 mm, W = 140 kg)
Sequencing chemistry	DNA nanoball
Output per run	96 Gb (PE300)
Number of reads per run	80 million x 2
Maximum paired-ends read length	2 x 300 bp
Max run duration	12 hours (PE150), 30 hours (PE300)
Quality score	> 90% bases > Q30 (PE150)
Typical applications	Target sequencing, small genome sequencing, forensic, 16s sequencing

MGI: DNBSEQ-G400



Size	Benchtop (1086 mm x 756 mm x 710 mm, W = 200 kg)
Sequencing chemistry	DNA nanoball
Output per run	1,440 Gb (PE200-FCL; SE400-FCL)
Number of reads per run	1.8 billion x 2
Maximum paired-ends read length	2 x 300 bp (FCS)
Max run duration	31 hours (PE150-FCS), 98 hours (PE300-FCS), 50 hours (PE150-FCL) , 109 hours (SE400-FCL)
Quality score	> 85% bases > Q30 (PE150-FCS; PE150-FCL)
Typical applications	Whole-genome sequencing, whole exome sequencing, transcriptome sequencing, single-cell sequencing, methylation sequencing, 16s sequencing, STOmics sequencing, small RNA sequencing, metagenomics, large/small panel sequencing.

MGI: DNBSEQ-G400RS FluoXpert

Size	Benchtop (1086 mm x 756 mm x 710 mm, W = 200 kg)
Modes	Sequencing and Multiplex Immunofluorescence Staining

Sequencing Mode:	
Sequencing chemistry	DNA nanoball
Output per run	1,440 Gb (PE200-FCL; SE400-FCL)
Number of reads per run	1.8 billion x 2
Maximum paired-ends read length	2 x 300 bp (FCS)
Max run duration	31 hours (PE150-FCS), 98 hours (PE300-FCS), 50 hours (PE150-FCL) , 109 hours (SE400-FCL)
Quality score	> 85% bases > Q30 (PE150-FCS; PE150-FCL)
Typical applications	Whole-genome sequencing, whole exome sequencing, transcriptome sequencing, single-cell sequencing, methylation sequencing, 16s sequencing, STOmics sequencing, small RNA sequencing, metagenomics, large/small panel sequencing.

Multiplex Immunofluorescence Staining Mode:	
Staining technology	FluoXpert™ multiplex immunofluorescence staining
Tissue format	FFPE and fresh frozen tissue sections
Flowcell types	SS, SL, DL
Throughput per week (calculated under 7*24 h/week)	6-plex: 84 sections (SS/SL), 140 sections (DL)*; 24-plex: 32 sections (SS/SL), 53 sections (DL)*
Maximum plex per run	24 plex
Resolution	20X objective lens, 0.36 μm/pixel
File formats	TIFF, OME-TIFF, JPEG, PNG, BMP
Image analysis platform	FluoXpert Vision
Typical applications	multiplex immunofluorescence staining, tumor microenvironment, translational research, immune checkpoint blockade, drug screening.

MGI: DNBSEQ-T7

Size	Production-Scale (1656 mm x 903 mm x 1815 mm, W = 765 kg)
Sequencing chemistry	DNA nanoball
Output per run	7 Tb
Number of reads per run	5.8 billion x 4
Maximum paired-ends read length	2 x 150 bp
Max run duration	24 hours
Quality score	> 85% bases > Q30 (PE150)
Typical applications	Deep whole-genome sequencing, deep exome sequencing, transcriptome sequencing, targeted panel projects, single-cell sequencing, stereo-seq

MGI: DNBSEQ-T20x2

Size	Production-Scale (4200 mm x 4800 mm x 2000 mm, W ~ 3700 kg)
Sequencing chemistry	DNA nanoball
Output per run	72 Tb (PE150)
Number of reads per run	40 billion x 6
Maximum paired-ends read length	2 x 150 bp
Max run duration	80 hours (PE150)
Quality score	≥ 85% bases > Q30 (PE150)
Typical applications	Large-scale population studies, ultra-high-depth whole genome sequencing, metagenomics

MGI: DNBSEQ-T10x4RS (customised product)

Size	Production-Scale (7200 mm x 5000 mm x 1950 mm, W = 10,000 kg)
Sequencing chemistry	DNA nanoball
Output per run	76.8 Tb (PE150)
Number of reads per run	32 billion – 45 billion x 8
Maximum paired-ends read length	2 x 150 bp
Max run duration	106 hours (PE150)
Quality score	> 85% bases > Q30 (PE150)
Typical applications	Large-scale population studies, ultra-high-depth whole genome sequencing

Singular Genomics: G4

Size	Benchtop (982 mm x 583 mm x 813 mm, W = 135 kg)
Sequencing chemistry	Rapid sequencing by synthesis
Output per run	Up to 480 Gb
Number of reads per run	Flexible; 1 billion – 3.2 billion
Maximum paired-ends read length	2 x 150 bp
Max run duration	24 hours
Quality score	80% - 90% bases > Q30
Typical applications	RNA gene expression; single cell RNAseq; total RNA-seq; exome sequencing; target enrichment, whole genome sequencing

Note: G4X combines the power of high-throughput in situ multi-omics with fast, flexible next generation sequencing (NGS).

PacBio: Onso

Size	Benchtop (940 mm x 686 mm x 762 mm, W = 123.3 kg)
Sequencing chemistry	Sequencing by binding
Output per run	120 – 150 Gb (PE150)
Number of reads per run	800 million – 1 billion (PE150)
Maximum paired-ends read length	2 x 150 bp
Max run duration	48 hours (PE150)
Quality score	> 90% bases Q40
Typical applications	Cancer research, gene editing, whole exome sequencing, single-cell analysis

Element Biosciences: AVITI System

Size	Benchtop (295 mm x 376 mm x 285 mm, W = 155.1 kg)
Sequencing chemistry	Avidity Based Chemistry (ABC)
Output per run	300 Gb (600 Gb – dual flow cell run) (PE150)
Number of reads per run	1 billion
Maximum read length	2 x 150 bp or 2 x 300 bp
Max run duration	38 hours (PE150 at 1 billion rads)
Quality score	> 90% bases > Q30 (PE150)
Typical applications	Single-cell RNA sequencing, whole genome sequencing, whole exome sequencing, targeted sequencing, SARS-CoV-2 sequencing

Note: AVITI is an alternative version of the system that runs low and medium throughput kits and Elembio Cloud is an online platform to monitor sequencing runs with real-time updates.

Ultima Genomics: UG100™

Size	Production-Scale
Sequencing chemistry	Mostly natural sequencing-by-synthesis
Output per run	3 Tb
Number of reads per run	~ 10 billion
Maximum read length	2 x 300 bp
Max run duration	< 20 hours
Quality score	> 85% bases > Q30
Typical applications	Single-cell RNA sequencing, whole genome sequencing, whole exome sequencing, multi-omics, clinical

Note: Details acquired from non-peer reviewed publication from Ultima Genomics team[1].

So, how do you decide whether to use a newer short-read NGS technology over those that are more widely used? We heard from **Michael Peterson**, Scientist I at the **Stowers Institute for Medical Research**, at our recent Sequencing: A State of Play webinar on the lessons he has learned through the early adoption of new short-read NGS technologies introduced over the last couple of years. He talks about some examples specific to his experiences acquiring NGS sequencers that were new to the market at the time, but highlights that there are general themes present that are good to keep in mind when approaching any new technology.

GeneMind NGS Sequencing Platforms op basis van Doorvoer

GeneMind biedt een compleet assortiment NGS-sequencers met schaalbare doorvoer, die data-output van 24 Gb tot 14 Tb per run leveren. Deze platforms zijn ontworpen om laboratoria van alle groottes te ondersteunen, van kleine academische onderzoeksgroepen tot ultra-high-throughput sequencingfaciliteiten.

FASTASeq S

Snelle NGS-sequencer voor tijdkritische toepassingen

LAAG Doorvoer

2 Gb tot 24 Gb

mNGS / tNGS / NIPT / PGT-A / snelle pathogentest

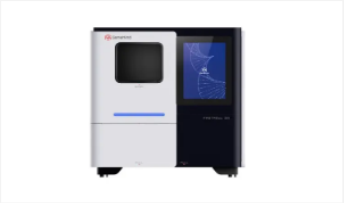
Learn More about FASTASeq S

FASTASeq 300

Desktop Hoge-Throughput NGS Sequencer

GEMIDDELD Doorvoer

5 Gb tot 150 Gb



mNGS / tNGS / Paneel / NIPT / PGT-A / 16s / forensisch / eDNA

Leer meer over FASTASeq 300

SURFSeq 5000

Flexibele Benchtop High-Throughput NGS Platform

HOGE Doorvoer

50 Gb naar 2,2 Tb

WES / WGS / kankerpaneel / Single cell sequencing / Ruimtelijke transcriptomics

Leer meer over SURFSeq 5000

SURFSeq Q

Krachtig bench-top high-throughput sequencing platform

ULTRA-HOOG Doorvoer

0,6 Tb tot 14 Tb

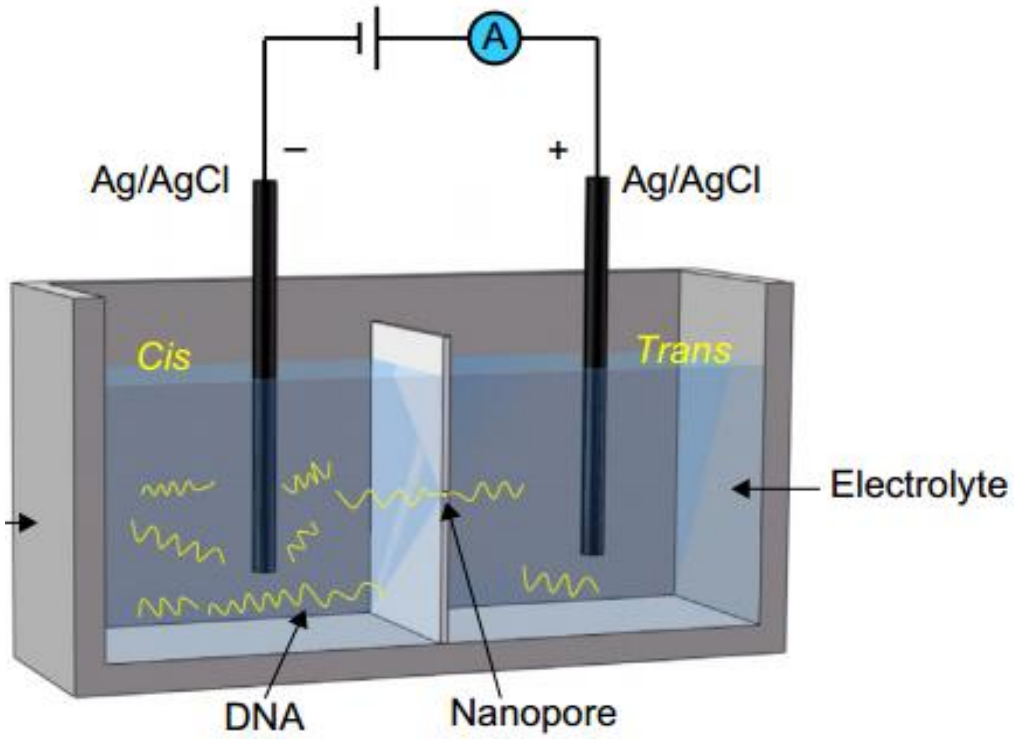
WES / WGS / kankerpaneel / enkelvoudige celsequencing / ruimtelijke transcriptomics

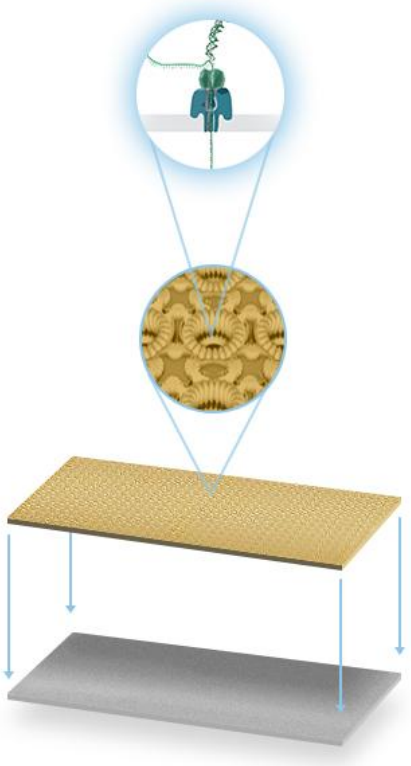
Leer meer over SURFSeq Q

AZ Sint-Jan Brugge

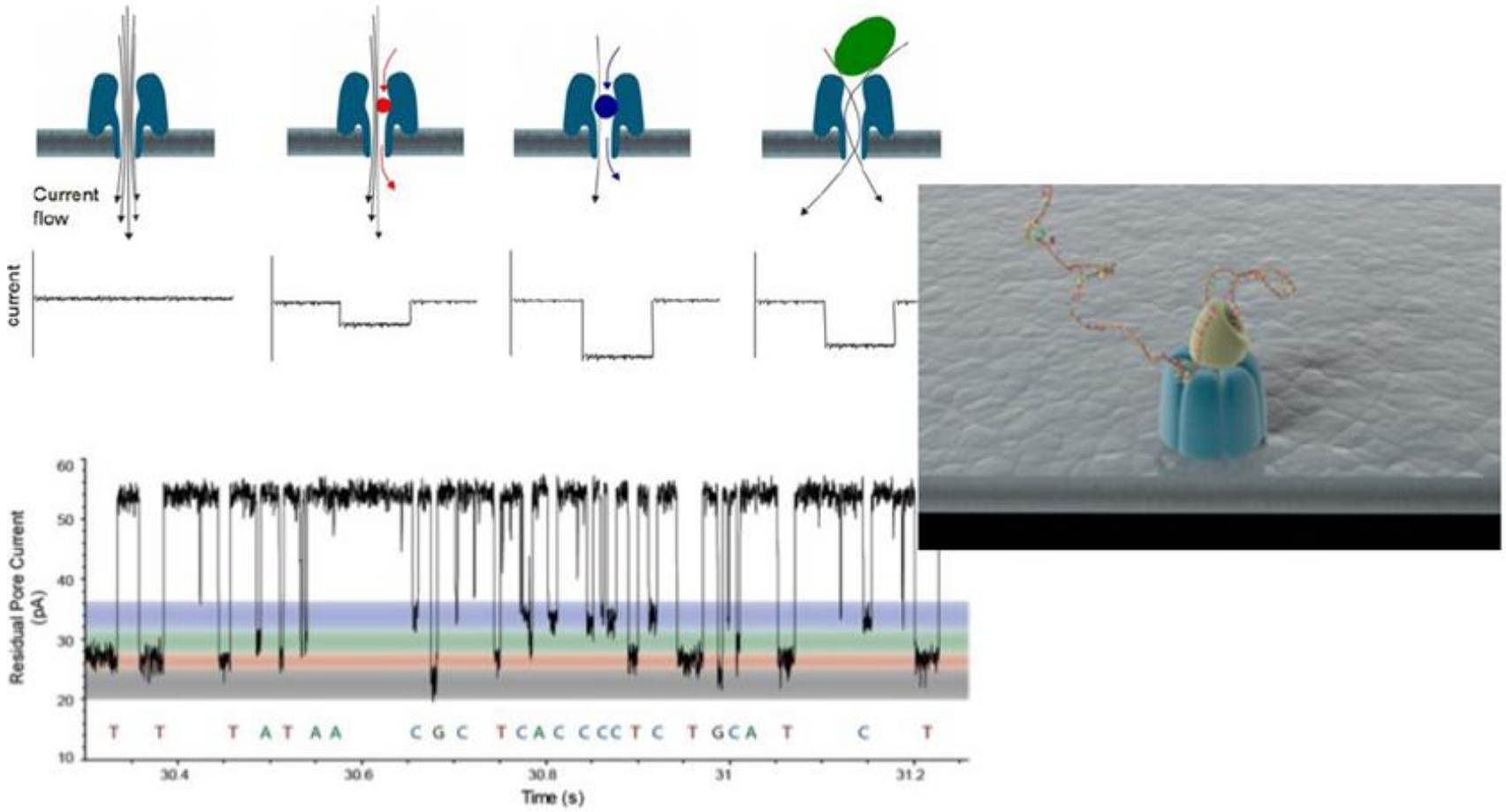
3th Generation Sequencing

Nanopore sequencing





Nanopore sequencing



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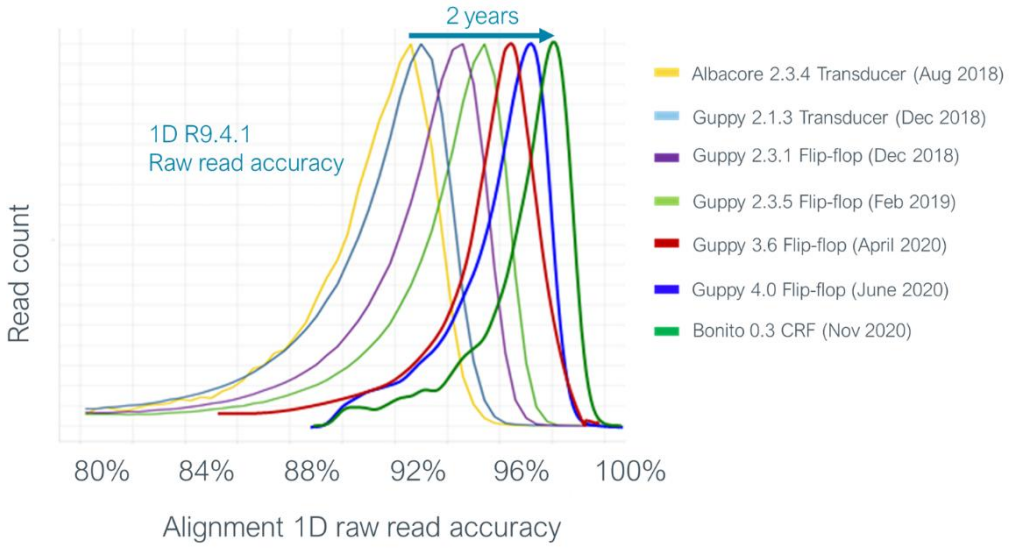
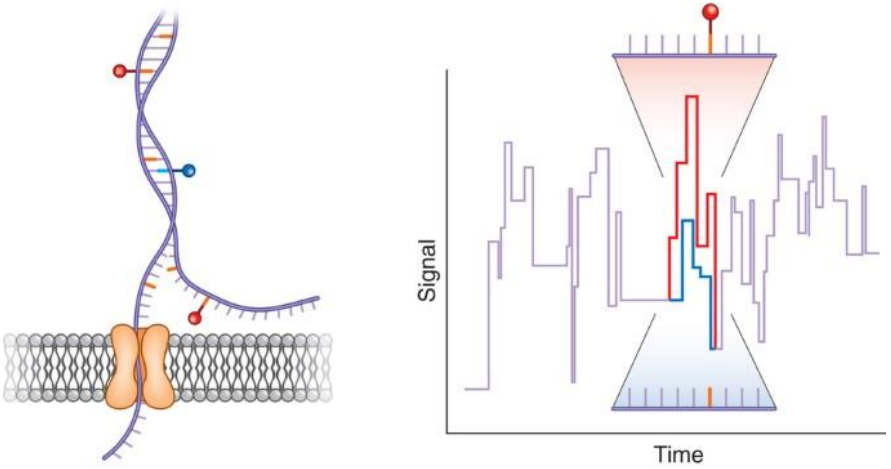
Nanopore sequencing

- Accuracy



- Direct RNA sequencing
- Sequencing epigenetic changes (methylation)



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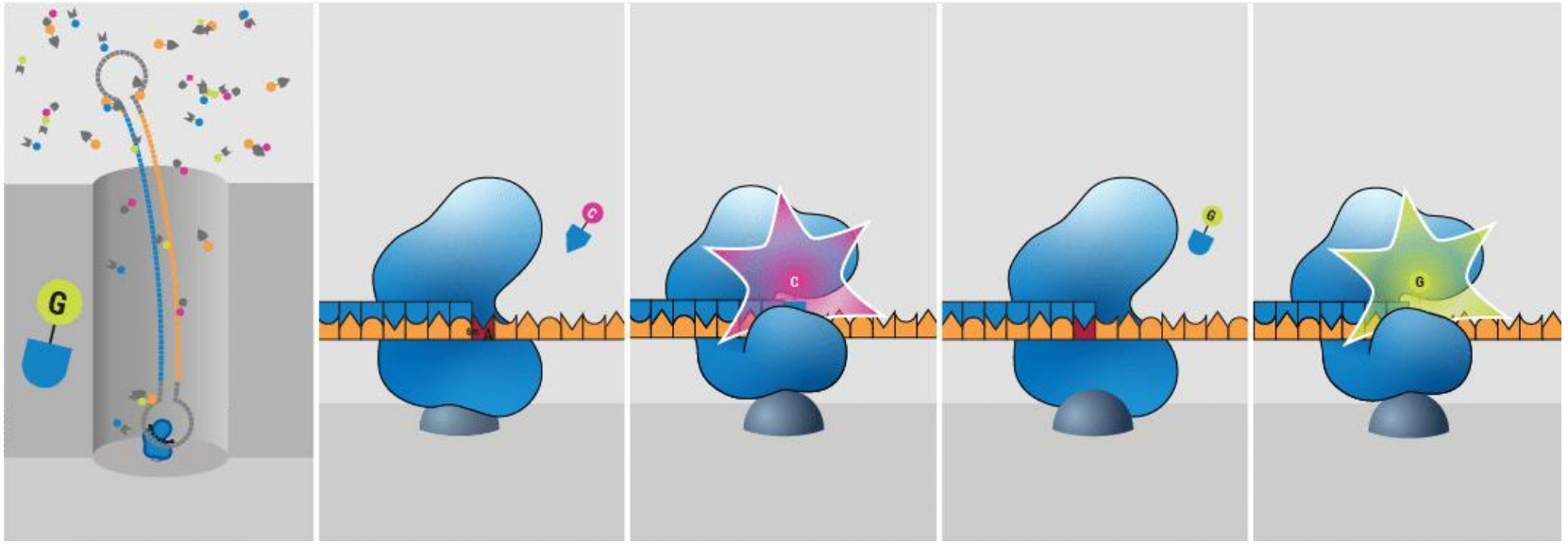
PACBIO sequencing (SMRT bell template



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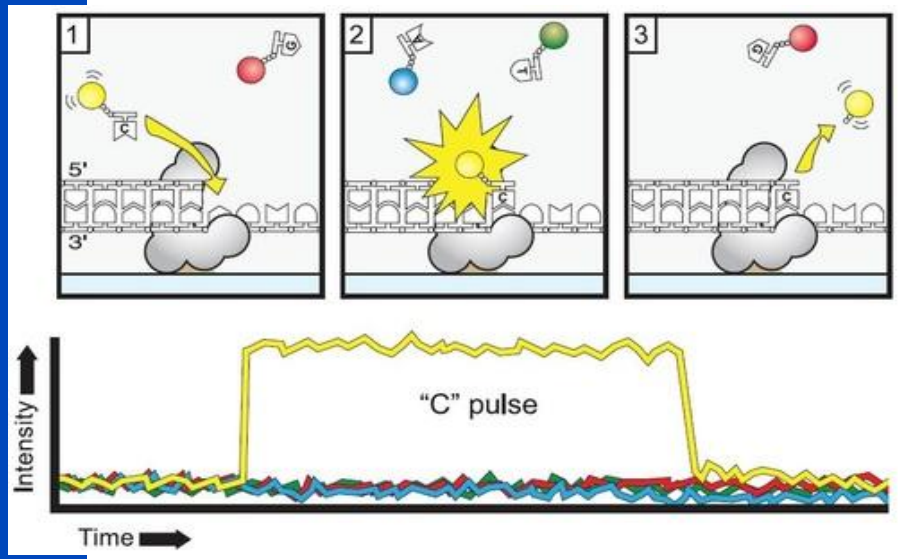
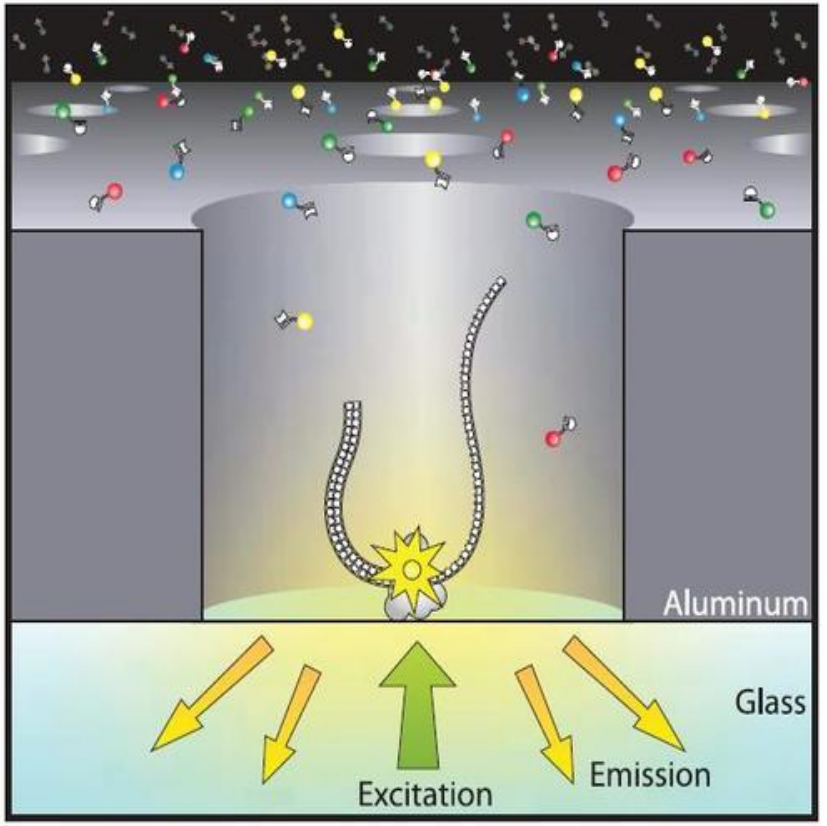
Single Molecule Real Time (SMRT) sequencing (Pacific Biosciences)





zero-mode waveguide

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PacBio: Vega

Size	Benchtop (557 mm x 695 mm x 768 mm, W = 125.2 kg)
Sequencing chemistry	Single molecule real-time (SMRT)
Output per run	~ 60 Gb HiFi
Number of HiFi reads per run	6-8 million reads
Max read length	15 kb – 20 kb
Max run duration	24 hours
Quality score	90% Q30+ bases
Typical applications	Amplicon, microbial genome, whole genome, methylation sequencing, targeted sequencing, RNA sequencing, small genome sequencing

PacBio: Sequel II/IIe

Size	Production-Scale (927 mm x 864 mm x 1676 mm, W = 362 kg)
Sequencing chemistry	Single molecule real-time (SMRT)
Output per run	~ 30 Gb HiFi
Number of HiFi reads per run	Up to 4 million
Max read length	15 kb – 20 kb
Max run duration	Up to 30 hours
Quality score	HiFi reads > 99% accuracy
Typical applications	Whole genome sequencing, RNA sequencing, targeted sequencing, complex populations, epigenetics, metagenome sequencing

PacBio: Revio

Size	Production-Scale (927 mm x 914 mm x 1745 mm, W = 465 kg)
Sequencing chemistry	Single molecule real-time (SMRT)
Output per run	120 Gb x 4
Number of reads per run	6 million - 10 million
Max read length	20 kb – 25 kb
Max run duration	24 hours (15 kb – 20 kb), 30 hours (20 kb – 25 kb)
Quality score	99.95% bases ≥ Q33 (15 kb – 20 kb)
Typical applications	Human genome analysis, single-cell transcriptome analysis, small genome analysis, targeted sequencing, epigenetics, microbial genome analysis.

Note: With the launch of PacBio's new SPRQ chemistry, Revio has a reduced DNA input requirement (a four-fold reduction to 500 ng) and on-instrument epigenetics capabilities (e.g., 5mC calling). The SPRQ chemistry will increase the efficiency of loading on Revio SMRT cells and improve sequencing performance (with a 25% increase in sequencing yield per SMRT cell).

Oxford Nanopore Technologies: MinION MK1B

Size	Portable (105 mm x 23 mm x 33 mm, W = 0.087 kg)
Sequencing chemistry	Nanopore
Output per run	Up to 50 Gb
Number of reads per run	Use case dependent
Max read length	20 bp – 4 Mb
Max run duration	Up to 72 hours
Quality score	Simplex 99% > Q20, duplex 99.9% > Q30
Typical applications	Low pass whole genomes/exomes, metagenomics, targeted sequencing, whole transcriptome, smaller transcriptomes, multiplexing for smaller samples

Oxford Nanopore Technologies: MinION MK1C

Size	Portable (140 mm x 30 mm x 116 mm, W = 0.45 kg)
Sequencing chemistry	Nanopore
Output per run	Up to 50 Gb
Number of reads per run	Use case dependent
Max read length	20 bp – 4 Mb
Max run duration	Up to 72 hours
Quality score	Simplex 99% > Q20, duplex 99.9% > Q30
Typical applications	Low pass whole genomes/exomes, metagenomics, targeted sequencing, whole transcriptome, smaller transcriptomes, multiplexing for smaller samples

Oxford Nanopore Technologies: MinION MK1D

Size	Portable
Sequencing chemistry	Nanopore
Output per run	Up to 50 Gb
Number of reads per run	Use case dependent
Max read length	20 bp – 4 Mb
Max run duration	Up to 72 hours
Quality score	Simplex 99% > Q20, duplex 99.9% > Q30
Typical applications	Low pass whole genomes/exomes, metagenomics, targeted sequencing, whole transcriptome, smaller transcriptomes, multiplexing for smaller samples

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Oxford Nanopore Technologies: GridION MK1

Size	Benchtop (370 mm x 220 mm x 365 mm, W = 11 kg)
Sequencing chemistry	Nanopore
Output per run	Up to 240 Gb
Number of reads per run	Use case dependent
Max read length	20 bp – 4 Mb
Max run duration	Up to 72 hours
Quality score	Simplex 99% > Q20, duplex 99.9% > Q30
Typical applications	Low pass whole genomes/exomes, metagenomics, targeted sequencing, whole transcriptome, smaller transcriptomes, multiplexing for smaller samples

Oxford Nanopore Technologies: PromethION 2/PromethION 2 Solo

Size	P2 Solo = Portable (110 mm x 87 mm x 152 mm, W = 1.5 kg)
Sequencing chemistry	Nanopore
Output per run	Up to 580 Gb
Number of reads per run	Use case dependent
Max read length	20 bp – 4 Mb
Max run duration	Up to 72 hours
Quality score	Simplex 99% > Q20, duplex 99.9% > Q30
Typical applications	Human, plant and animal whole genome sequencing, large genome assembly, targeted sequencing, transcriptomics, single cell, highly multiplexed sequencing, high depth metagenomics, epigenetics

Oxford Nanopore Technologies: PromethION 24

Size	Benchtop (Sequencing module – 590 mm x 190 mm x 430 mm, W = 28 kg, Computing module – 178 mm x 440 mm x 470 mm, W = 25 kg)
Sequencing chemistry	Nanopore
Output per run	Up to 7 Tb
Number of reads per run	Use case dependent
Max read length	20 bp – 4 Mb
Max run duration	Up to 72 hours
Quality score	Simplex 99% > Q20, duplex 99.9% > Q30
Typical applications	Human, plant and animal whole genome sequencing, large genome assembly, targeted sequencing, transcriptomics, single cell, highly multiplexed sequencing, high depth metagenomics, epigenetics

Oxford Nanopore Technologies: PromethION 48

Size	Benchtop (Sequencing module – 590 mm x 190 mm x 430 mm, W = 28 kg, Computing module – 178 mm x 440 mm x 470 mm, W = 25 kg)
Sequencing chemistry	Nanopore
Output per run	Up to 14 Tb
Number of reads per run	Use case dependent – Theoretical max = 261 million
Max read length	20 bp – 4 Mb
Max run duration	Up to 72 hours
Quality score	Simplex 99% > Q20, duplex 99.9% > Q30
Typical applications	Human, plant and animal whole genome sequencing, large genome assembly, targeted sequencing, transcriptomics, single cell, highly multiplexed sequencing, high depth metagenomics, epigenetics

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Toepassingen

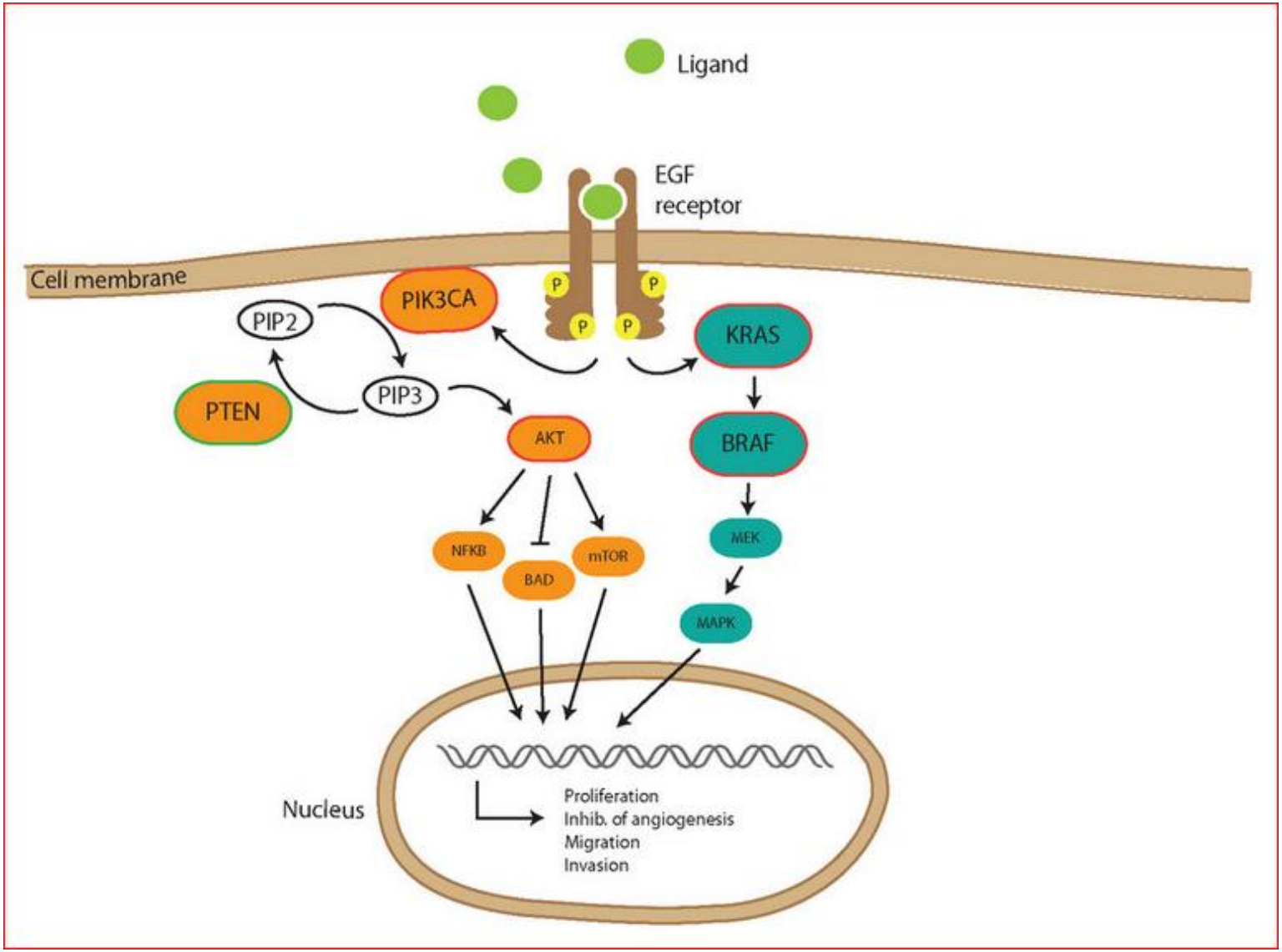
Sequencing in human molecular diagnostics

- Germline mutation detection (hereditary diseases)

- Somatic mutation detection (cancer)
  - mutations in proto-oncogenes:
    - Genes for growth stimulation
    - Mutation of 1 allele is sufficient
    - Point mutations or chromosomal translocations
  - mutations in tumor suppressor genes:
    - Genes which control growth
    - 2 alleles should be inactivated (Knudson two-hit model)

Oncogene signaal pathways

voorbeeld : Epithelial Growth Factor pathway



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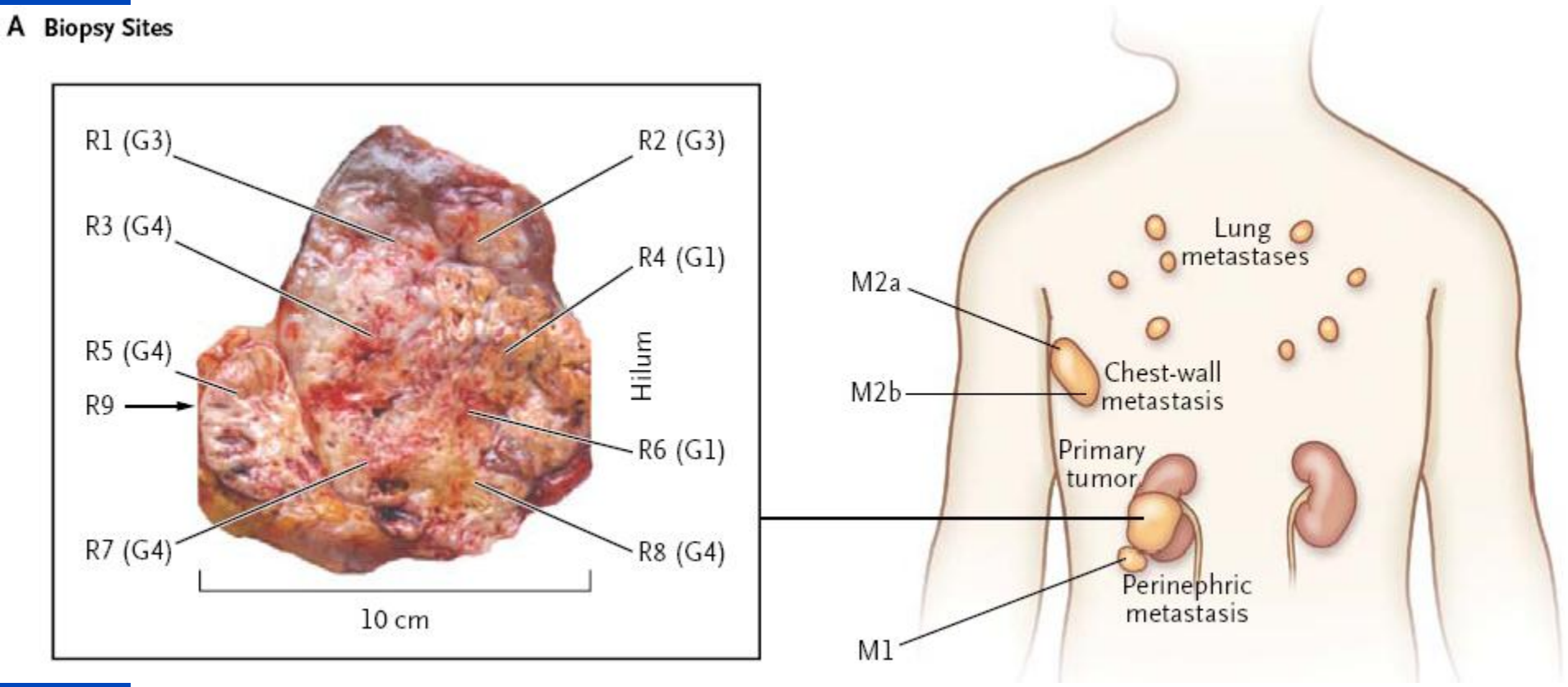
Intratumor Heterogeneity and Branched Evolution Revealed by Multiregion Sequencing

Gerlinger et al., NEJM (2012) 366;10



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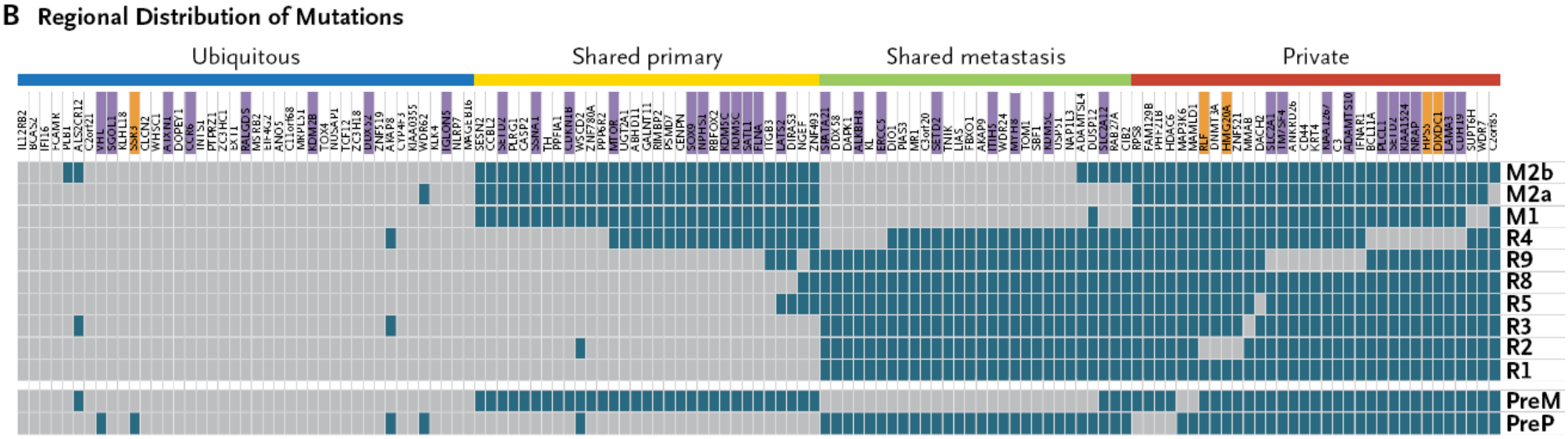

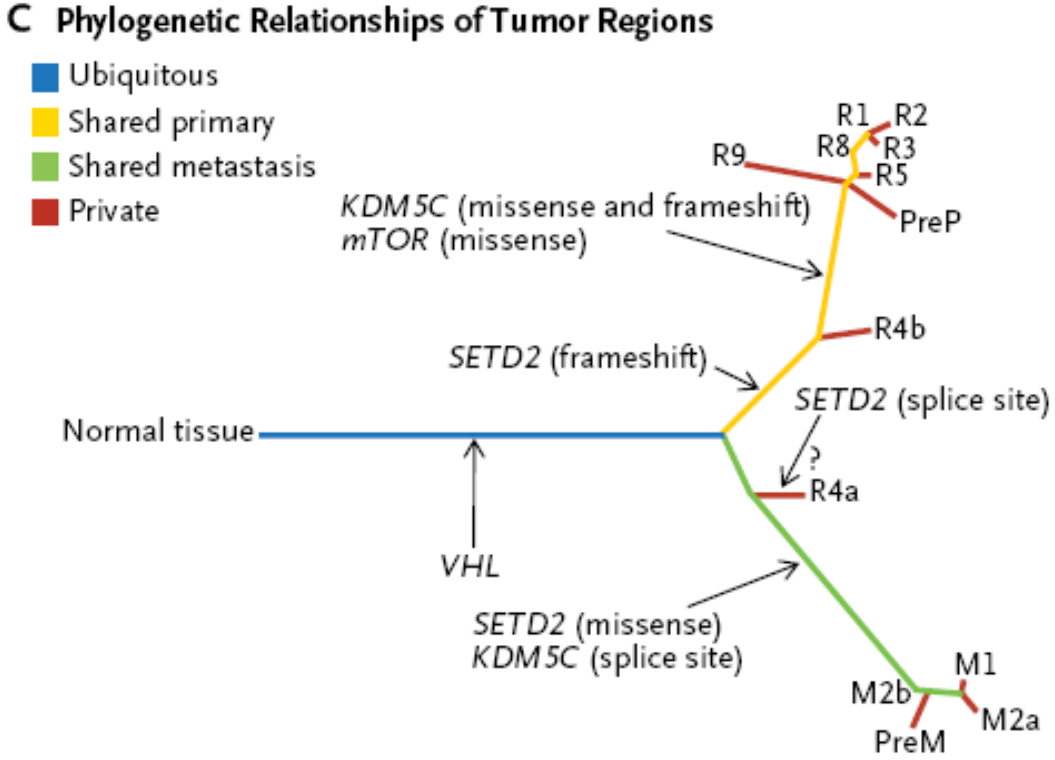

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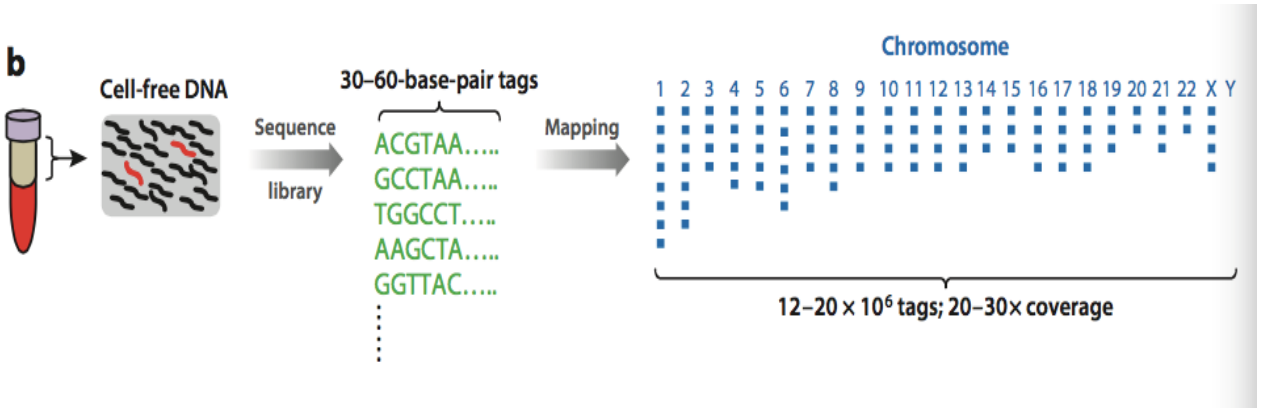
NIPT (non invasive prenatal testing)

- Foetal cfDNA
  - From apoptotic cellen of placenta
  - Short DNA fragments (± 150 bp)
  - Foetal fraction ±10% at 10 w pregnancy

Whole genome NIPT



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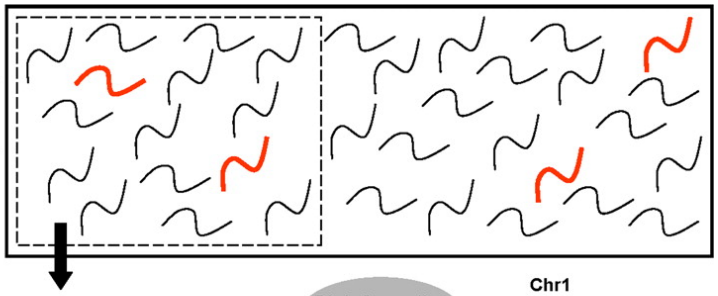
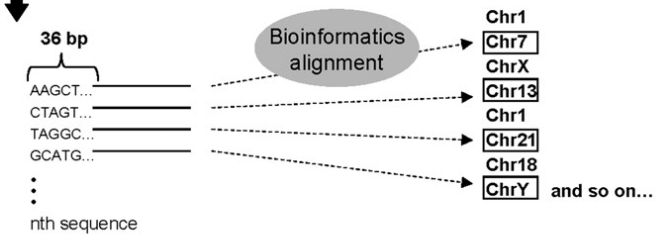
NIPT

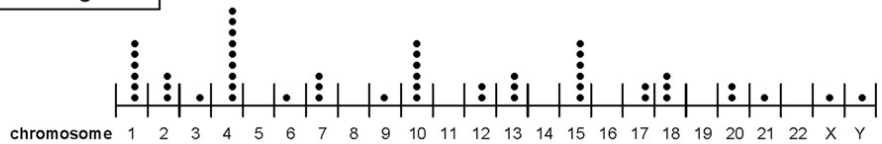
DNA fragments in maternal plasma



Sequence and align



Sequence counting

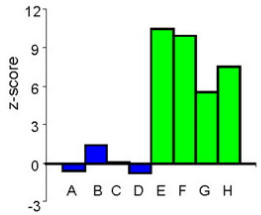


% representation of unique sequences mapped to a chromosome

$$\%\ \text{chrN} = \frac{\text{Unique count for chrN}}{\text{Total unique count}}$$

Disease status determination

$$\text{chrN z-score for test sample} = \frac{\%\ \text{chrN}_{\text{sample}} - \text{mean}\ \%\ \text{chrN}_{\text{reference}}}{\text{S.D.}\ \%\ \text{chrN}_{\text{reference}}}$$



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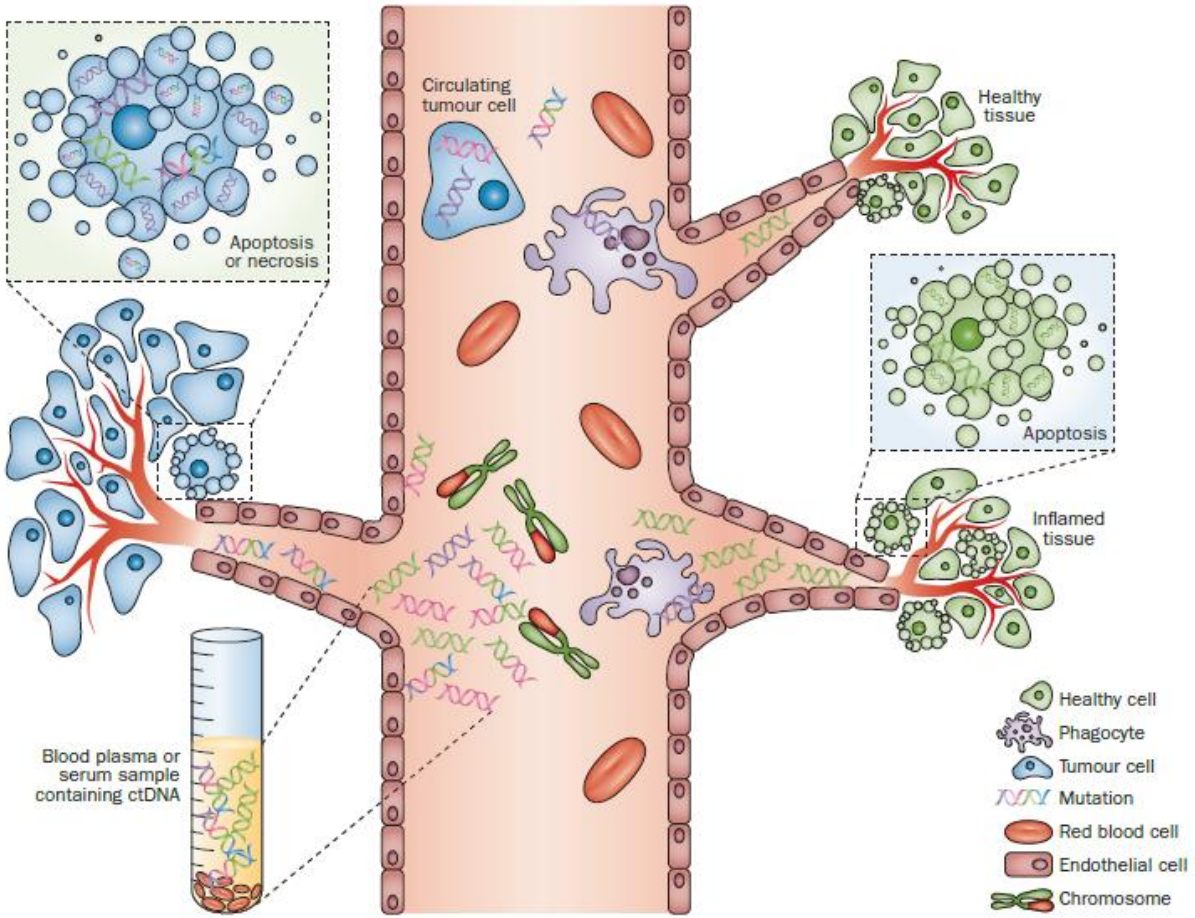
Liquid Biopsy



cfDNA = cfDNA (non-tumoral) + cell free **tumor** DNA

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- Preconception carrier testing (PCT)
- Preimplantation Genetic Testing (PGT)

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Single cell sequencing

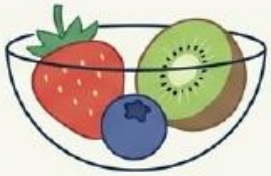
Choosing Your Resolution: A Blended Smoothie or a Bowl of Distinct Fruits?

Bulk Sequencing

Measures the *average* expression or genomic content across thousands or millions of cells. Powerful for understanding the overall state of a tissue.

- Reveals: The dominant signals and pathways.
- Misses: The contributions of rare cell types, subtle shifts in subpopulations, and cellular heterogeneity.

Single-Cell Sequencing



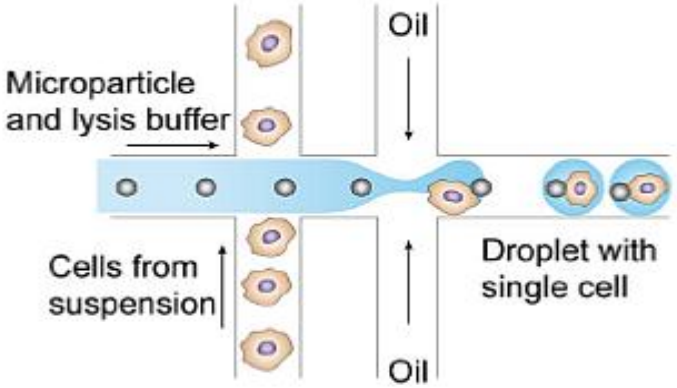
Deconvolutes a tissue into its individual cellular components, providing a high-resolution profile for each cell.

- Reveals: Cellular heterogeneity, rare cell populations, developmental trajectories, and cell-specific responses.
- Enables: Understanding disease at the level at which it acts—the individual cell.

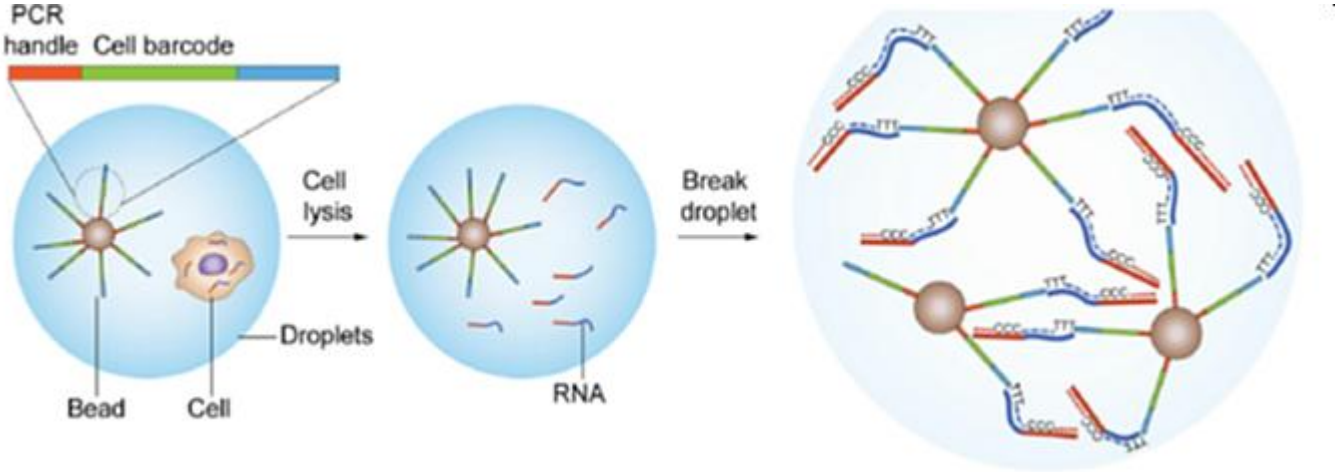
"Single-cell shines when you are working with a heterogeneous mixture, e.g., the tumour microenvironment." — Ojasvi Chaudhary, Senior Scientist, AstraZeneca

Single cell RNA sequencing

Microfluidic technologie voor single cell isolatie



Droplet-gebaseerde library bereiding



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G4X™ Spatial Sequencer: Singular Genomics

The G4X™ Spatial Sequencer is a high-throughput in-situ spatial sequencing platform capable of simultaneous direct RNA sequencing (Direct-Seq™), targeted transcriptomics, proteomics and fluorescent H&E from FFPE tissues. The G4X shares the same platform as the earlier G4® sequencer.

With sub-micron resolution and ultra-high throughput capacity, G4X employs rapid 4-colour sequencing-by-synthesis (SBS) chemistry to profile RNA transcripts and proteins in FFPE tissue. The technique generates fluorescent H&E images, producing multi-modal spatial images of 40 cm2 of tissue across 4 flow cells in less than 24 hours. Transcripts are detected by annealing a padlock probe sequence to a target RNA and additional specificity is conferred by requiring the 3' and 5' end to be adjacent for ligation. Proteins are detected by staining with oligo-conjugated antibodies, which are then targeted with padlock probes that use the ab-oligo as a splint. All ligated padlock probes are then amplified by rolling circle amplification.

AVITI24™: Element Biosciences

The AVITI24™ instrument combines state-of-the-art sequencing alongside cyto-profiling, enabling the simultaneous profiling of DNA, RNA, proteins, phosphoproteins and cell structure within single cells.

Element Biosciences' new Teton™ chemistry means this multi-omics functionality is captured in one read-out in a run that is under 24 hours in length. The 2024 instrument is planned to sequence 350 RNA targets, 50 protein targets and 3 cell morphology markers at subcellular resolution on fixed cell suspensions directly on the flow cell. This will result in spatial multi-omics over a 10 cm2 area with two independent runs on the two flow cells. Plans aim to combine an untargeted transcriptome readout with DNA sequencing, up to 20 morphology markers and custom protein panels as well as additional sample type capacity.

Alongside these, specific single-cell and spatial technologies detailed above also have multi-omic capabilities. For example, the Tapestri platform from Mission Bio can incorporate protein panels to create a multi-omics workflow. Check out the companies' websites (via the relevant hyperlinks on each product above) for more details.

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