



PathoSense



Introduction to NGS

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CEO & CO-FOUNDER**

**WEBINAR FOR THE DEVELOPING COUNTRIES VACCINE
MANUFACTURERS NETWORK 20-07-2022**

Disclaimer

This presentation is intended to give a **global overview of the next generation sequencing landscape**. The speaker made use of publicly available data is not responsible for the accuracy of these data.

The presentation is **not intended to give advice or recommendations** for the use of certain **technologies or brands**.

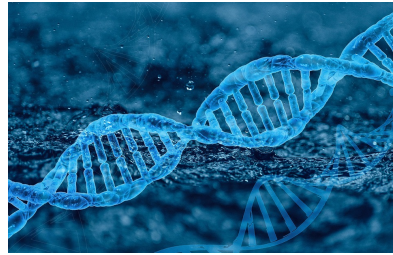
Sebastiaan Theuns is **co-founder and co-owner of PathoSense, a Ghent University spin-off company** that makes commercial use of Oxford Nanopore Technologies for diagnostics of veterinary infectious diseases.

Sequencing: the analysis of the genetic code

**(Micro)
Organisms**



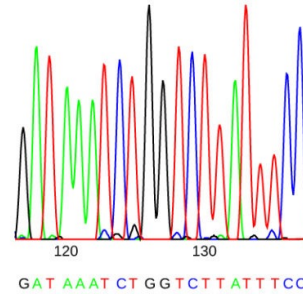
**Genetic code:
DNA/RNA**



**ATCG
AUCG**



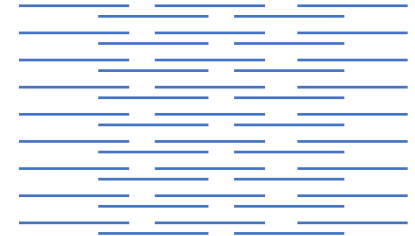
**Sanger
sequencing**



**One sequence
500-1000bp**



**Next-generation
sequencing**



**Multiple sequences
Giga to Tera bases**

Next-generation sequencing technologies

illumina®

iontorrent
by Thermo Fisher Scientific

 Element
Biosciences

 Oxford
NANOPORE
Technologies

 MGI
华大智造

 GENAPSYS™

PacBio●

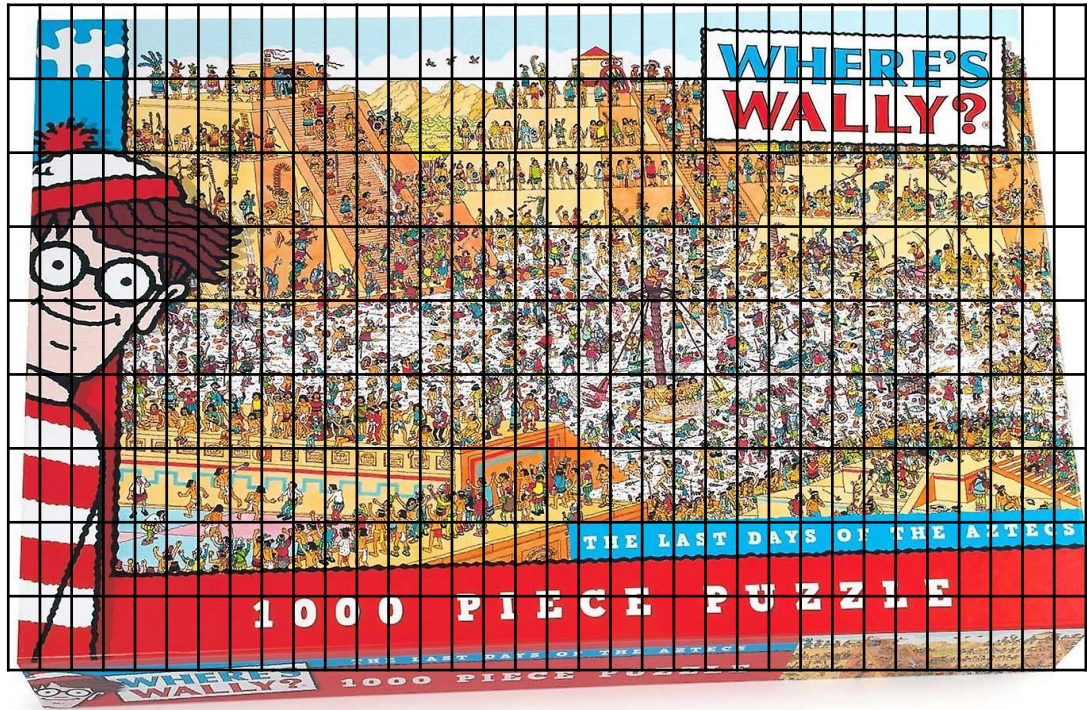
 S I N G U L A R
G E N O M I C S

 ULTIMA
G E N O M I C S

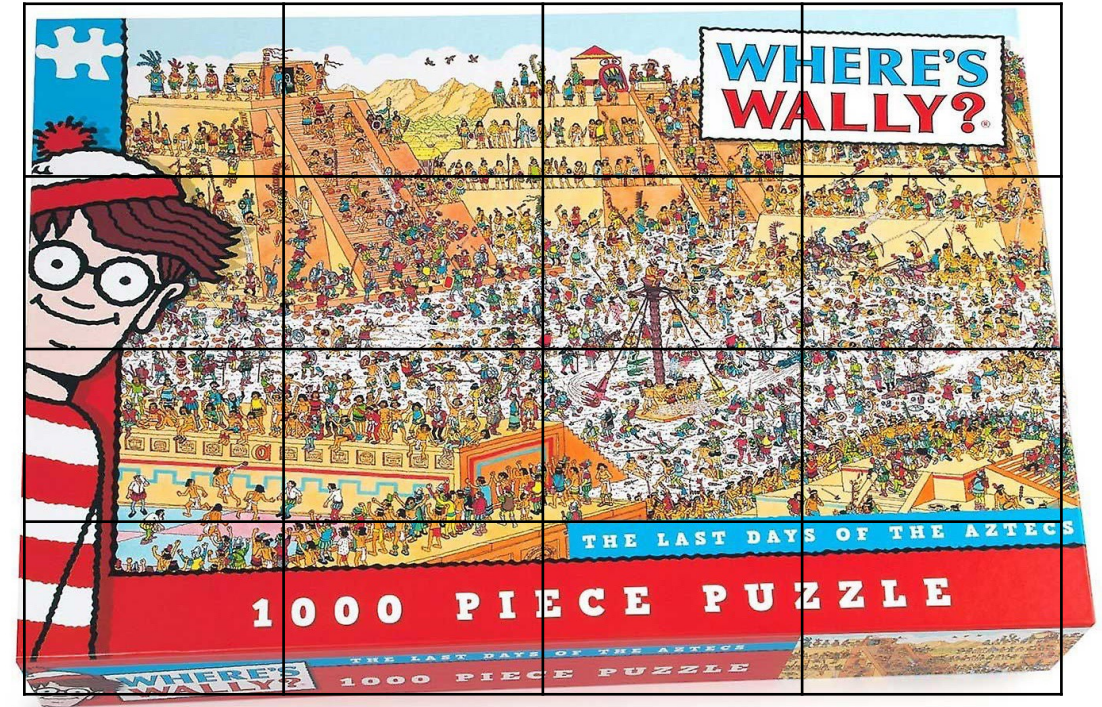
> **Strong competition: market - technology - accuracy - output - read length**

Read-length and genome assembly

Short read



Long read



illumina®

iontorrent
by Thermo Fisher Scientific

MGI
华大智造

Oxford
NANOPORE
Technologies

PacBio

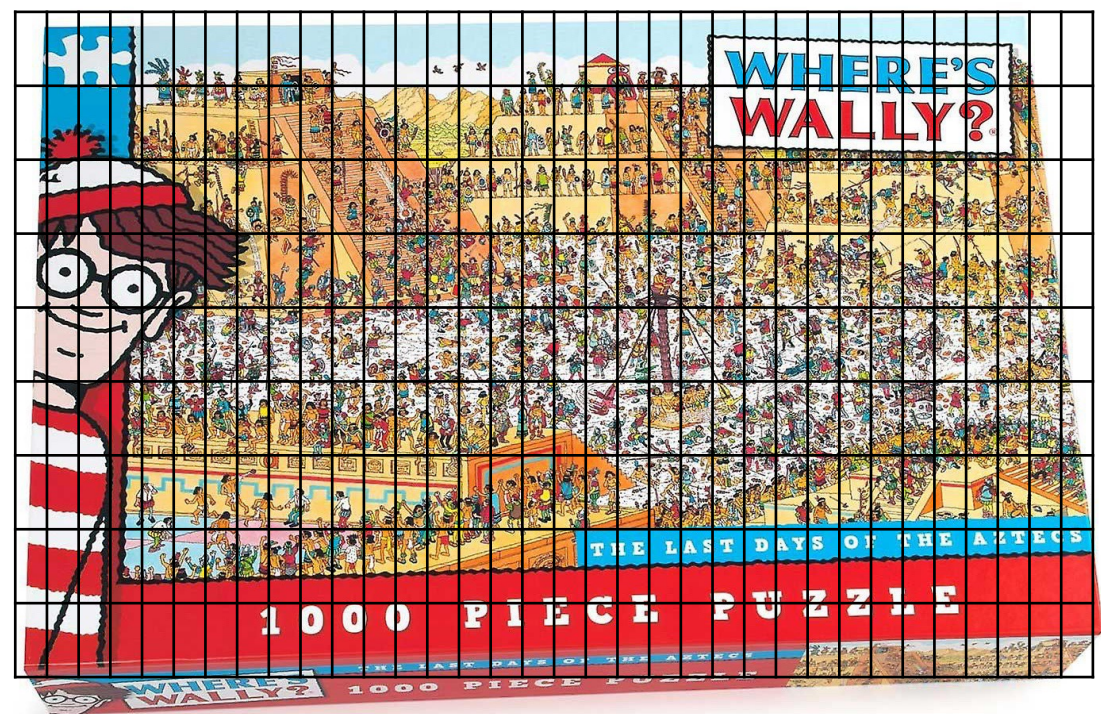
GENAPSYS™

Element
Biosciences

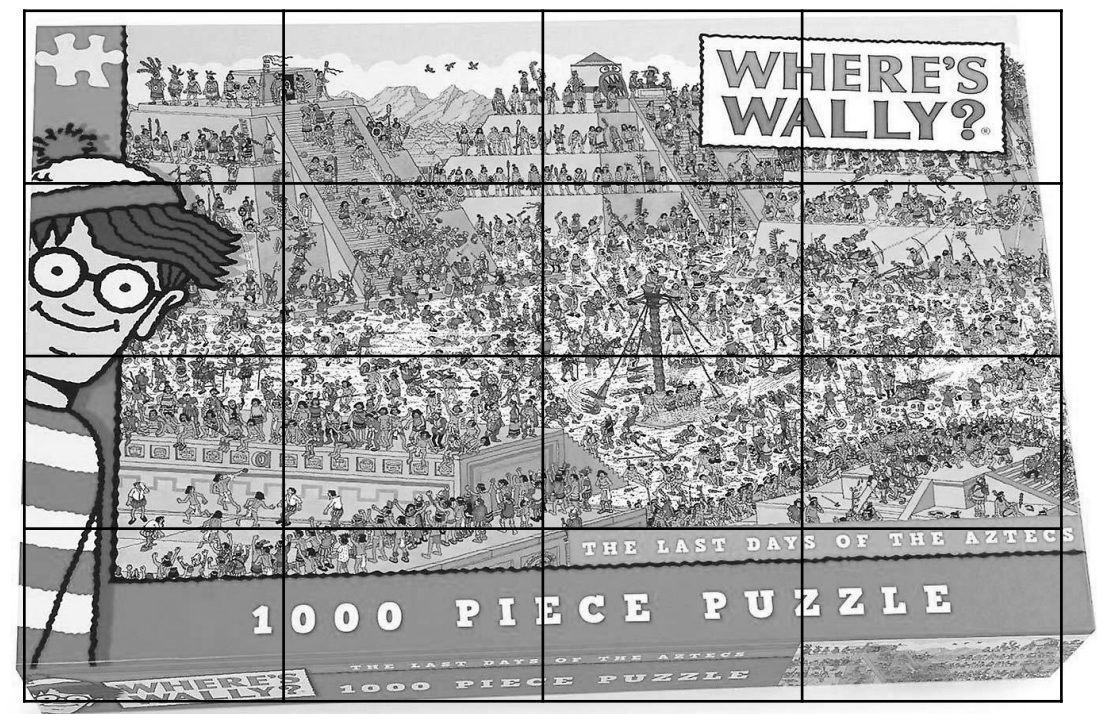
ULTIMA
GENOMICS

Read-length and genome assembly

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Technologies

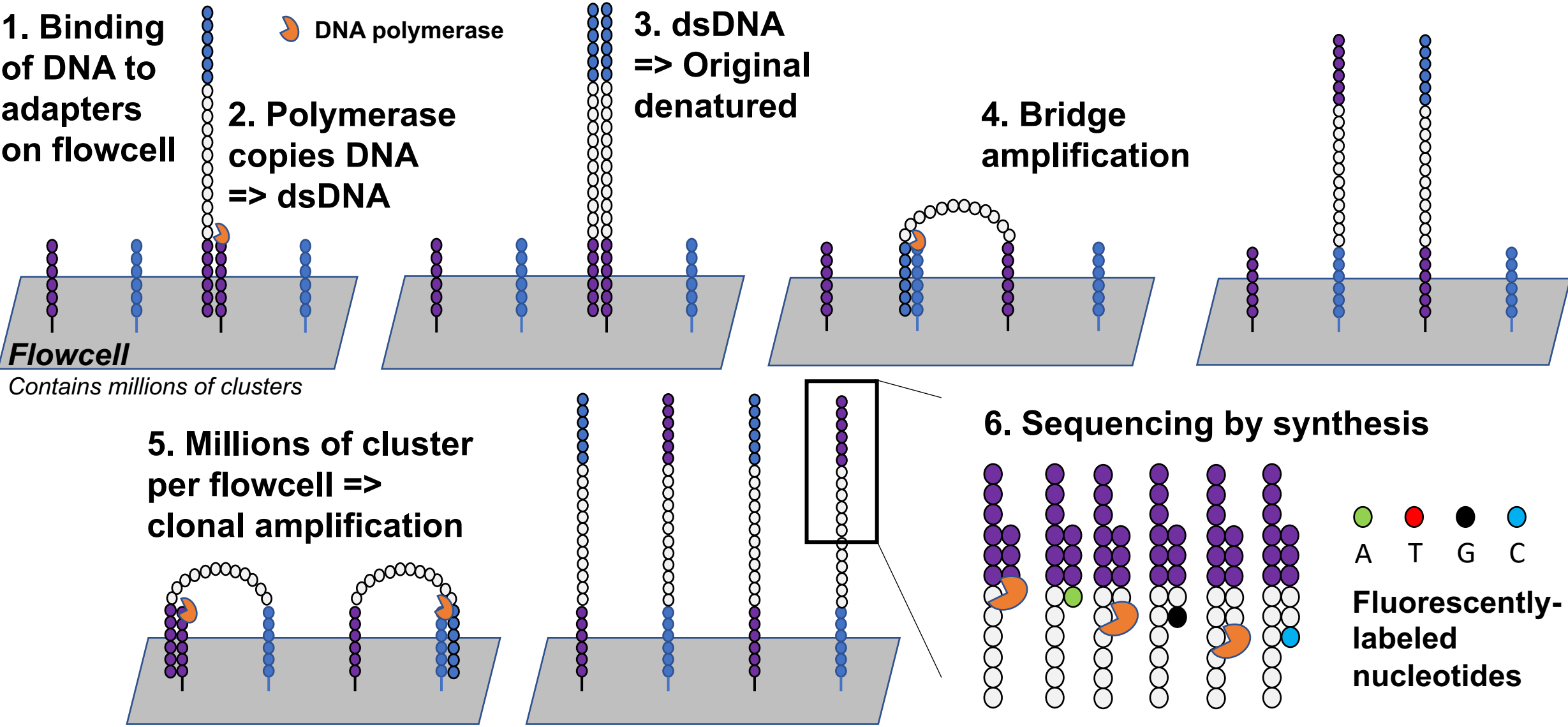
PacBio

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GENOMICS

Illumina - sequencing by synthesis



Illumina sequencing devices



Parameter/device	iSeq 100	MiSeq	NextSeq2000	HiSeq	NovaSeq
Max output	1.2 GB	15GB	360GB	1.5 TB	6 TB
Max # reads	4 million	25 million	2.4 billion	5 billion	20 billion
Max read length	2x150bp	2x300bp	2x150 bp	2x150bp	2x250bp
Accuracy	>80% of bases Q30	>70% of bases Q30	>85% of bases Q30	>75% of bases Q30	>75% of bases Q30
Max seq. time	17.5h	56h	48h	3.5 days	~44h
Investment	~\$20K		~ \$300K		~\$1M



MGI: DNB Seq technology



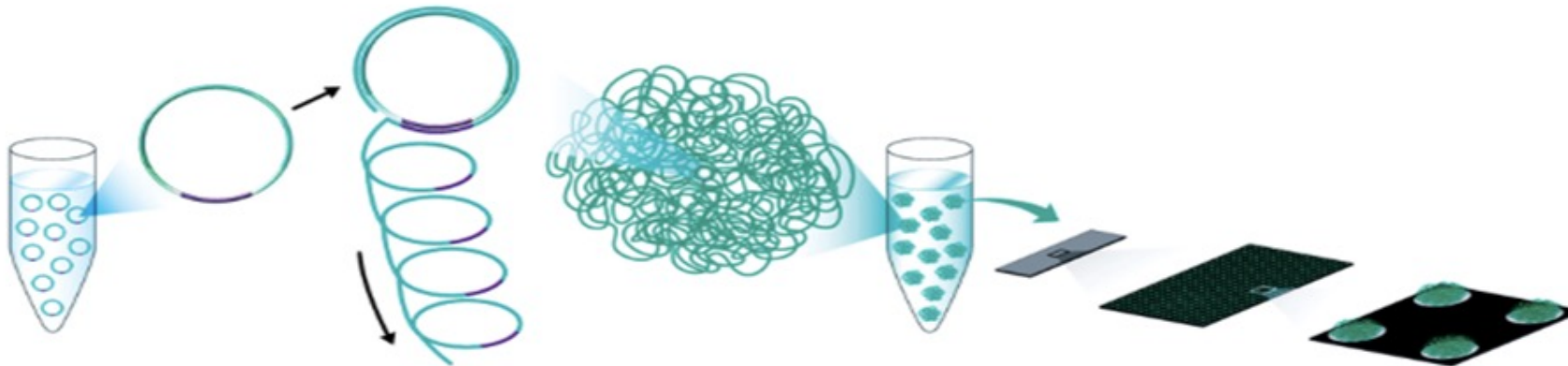
- Circularisation of DNA & Rolling Circle Amplification
- Sequencing of **DNA Nanoballs (DNB)**
- BGI - Republic of China
- **Entry in EU market** in 2022



DNBSeq-G50
150 GB



DNBSeq-T7
6 TB



- **Ion semiconductor, well established in the market**
- **Short-read** platform: 200 - 600 bp
- **Output** per flowcell: 0.3 to 25 GB
- **Seq duration:** 3 - 21.5 h
- High sequencing quality
- Up to two flowcells



Some (short-read) technologies to follow



Rapid SBS technology

US: San Diego



Benchtop sequencing

US: California



Short & long read seq

Avidity sequencing

US: San Diego



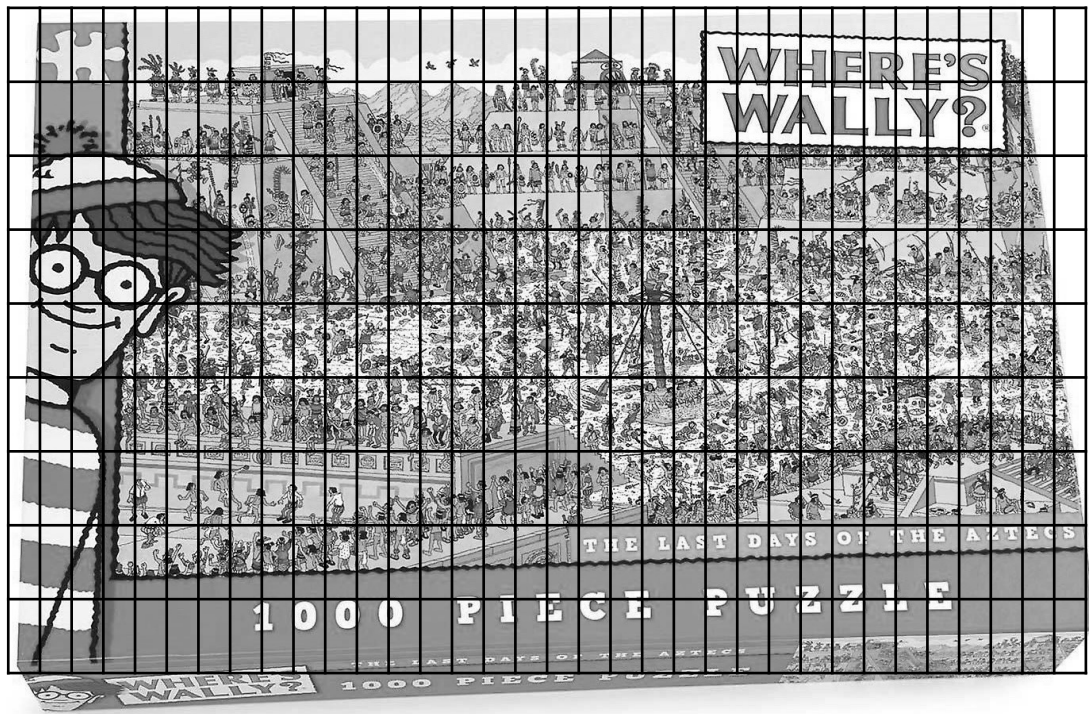
No flowcells, new chemistry

US: San Diego

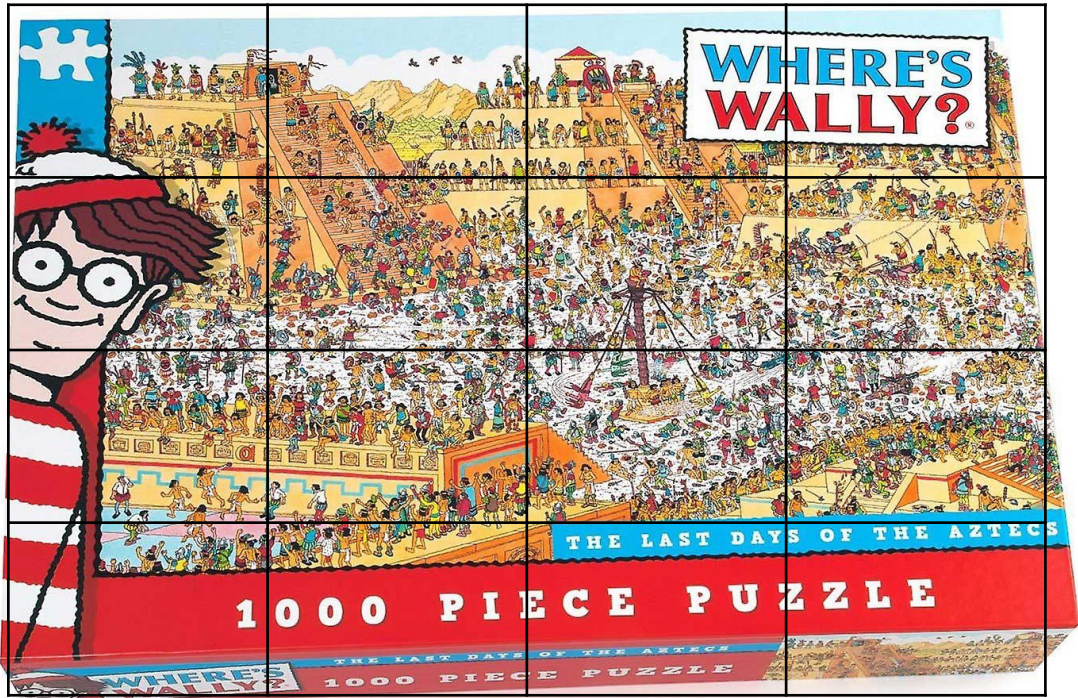


Read-length and genome assembly

Short read



Long read



illumina

iontorrent
by Thermo Fisher Scientific

MGI
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Oxford
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Technologies

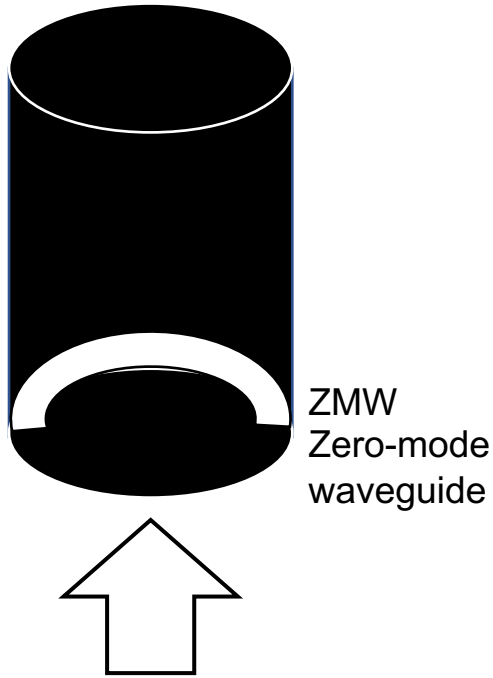
PacBio

GENAPSYS

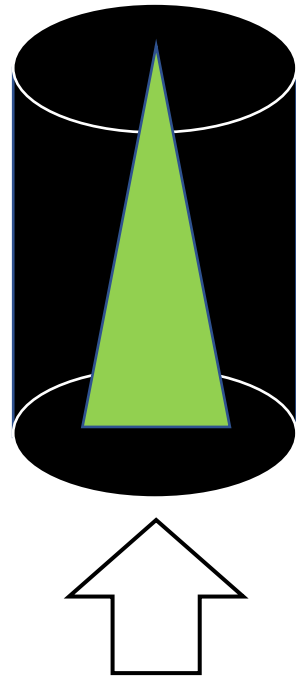
Element
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ULTIMA
GENOMICS

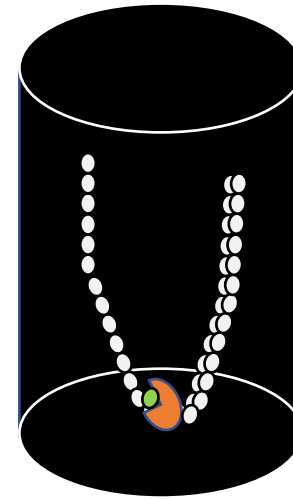
Pacific Biosciences (PacBio)



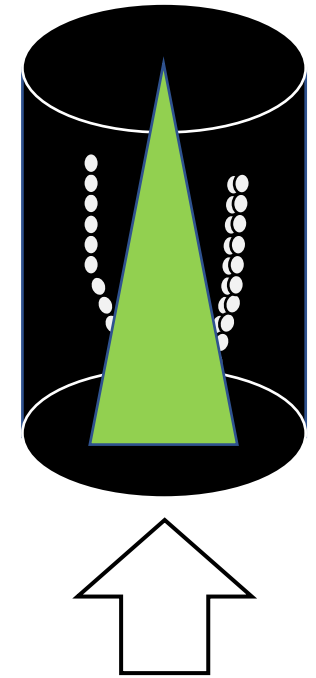
1. Light from below (wavelength too large, cannot pass through the waveguide)



2. Attenuated light from excited fluorophores can penetrate through the ZMW



3. DNA & polymerase complex is present in the ZMW
=> Incorporation of phospho-linked nucleotides containing a fluorophore



4. Attenuated light is >1000 stronger than light

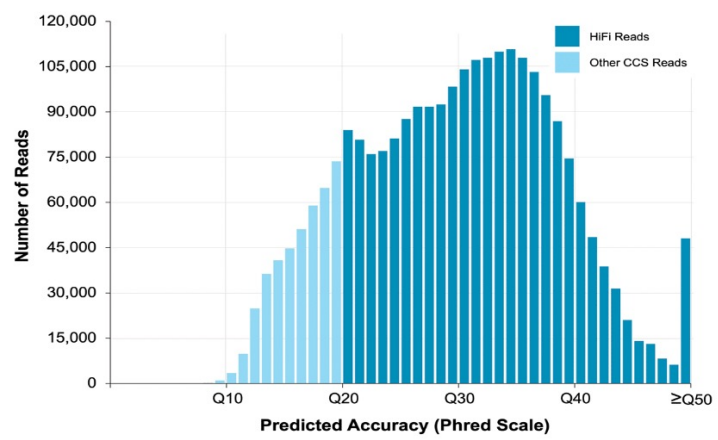
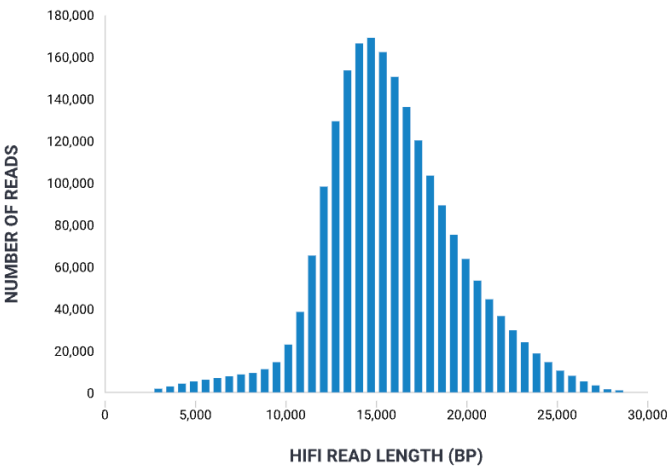
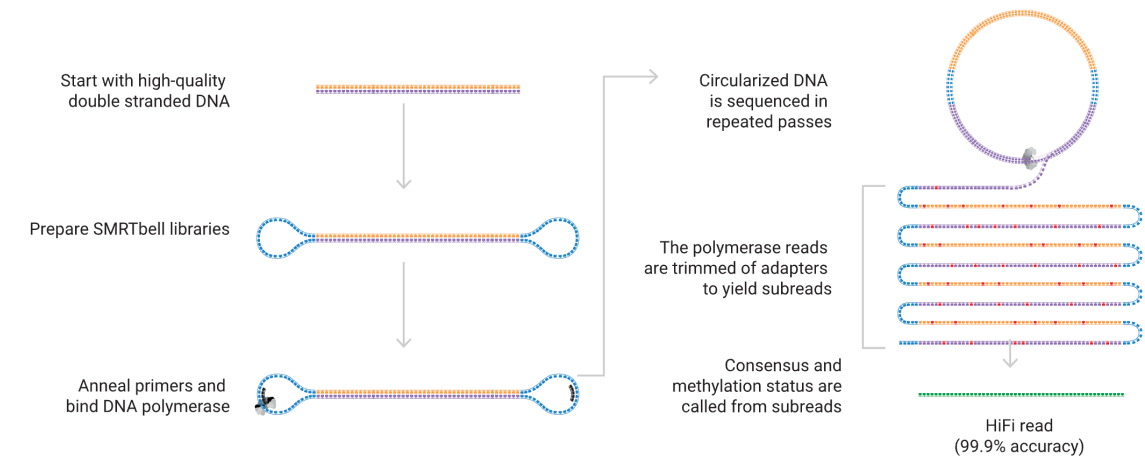


Single-molecular real-time sequencing (SMRT)

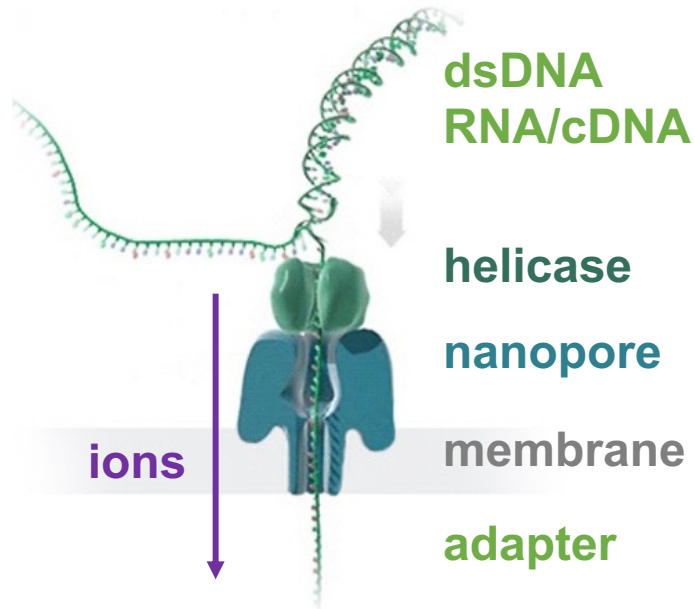
Sequel II

Hifi Sequencing

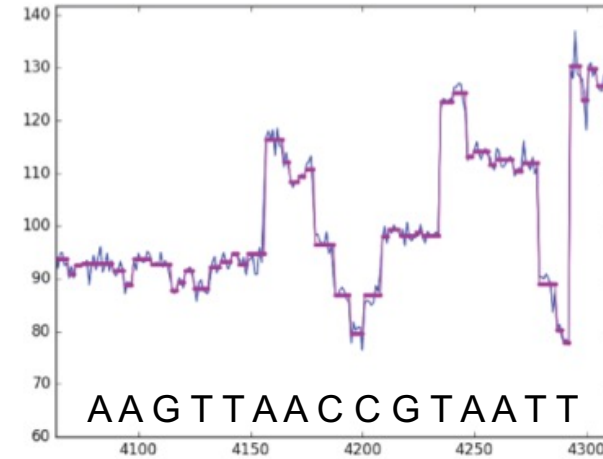
Max 8 million reads
Max 30h seq per cell
150 GB



Oxford Nanopore Technologies



Ion current (pA)



Time



Nanopore sequencing devices



Parameter/device	Flongle	MinION	GridION X5	PromethION 2	PromethION 24 or 48
Max output	2.8 GB	30GB	150GBP	0.5 TB	7 to 14 TB
Channels	~ 100	512	5 x 512	3000	24 or 48 x 3000
Read length	< - - - - -		Short to ultralong (4MB)		- - - - - >
Accuracy	< - - - - -		≥99% or Q20 with Kit 14		- - - - - >
Seq time	< - - - - -		1-72h, real-time		- - - - - >



Problem of classic pathogen/adv. agent testing

Analysis form

- ☒ Pathogen 1
- ☐ Pathogen 2
- ☒ Pathogen 3
- ☐ Pathogen 4
- ☐ Pathogen 5



Result

NEG Pathogen 1

NEG Pathogen 3

Problem: prior selection

- > Only known pathogens
- > No full overview
- > Pathogens change

Challenges of ad-random sequencing of pathogens

Virus (1.5 to 150 kb)

***Mycoplasma* (1 Mb)**

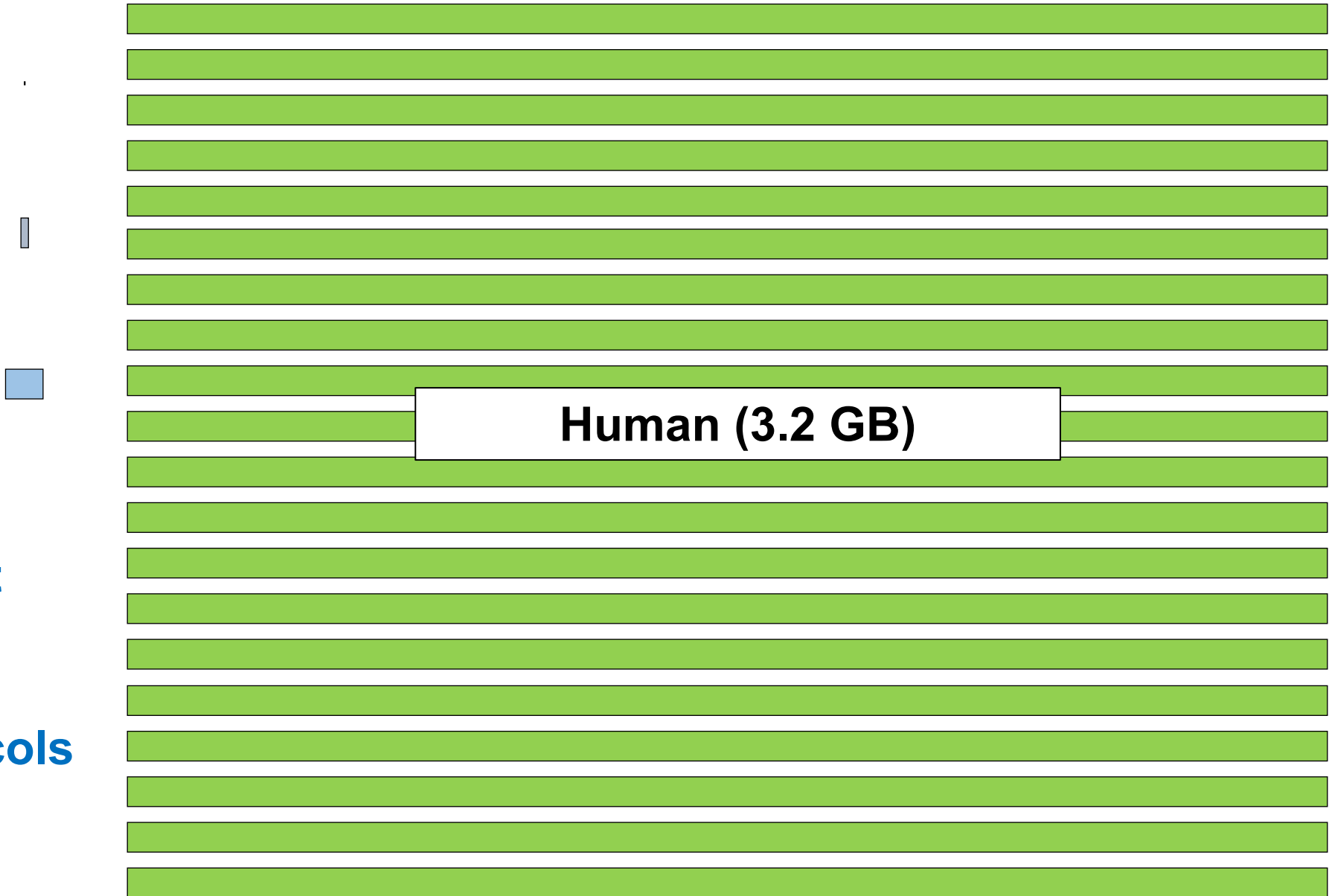
***E. coli* (5 Mb)**

**Pathogen enrichment
is crucial for good
detection**

> lab intensive protocols

vs

> patented swab





PathoSense



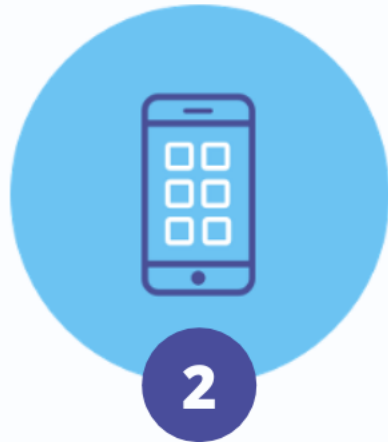
Spin-off of Ghent University

° Oct 2020

Co-founders Dr. Theuns & Prof. Nauwynck



**Good
samples**



**Fast
admin**



**No prior
selection**



**Better
advice**



**Become
smarter**



No selection, scalable to all species

Future perspectives

- NGS is an **innovative field** in continuous evolution
- Advances in technologies > **challenges** for regulatory bodies
- **NGS is available as a diagnostic tool** for pathogens in **veterinary medicine**
- **NGS has value in R&D and manufacturing of biologicals**
 - Lead selection - rapid mutation ID
 - Screening for adventitious agents from early passages till production
 - Pharmacovigilance: rapid detection of mutations, recombination, reassortments...



PathoSense

dcvmn

Developing Countries Vaccine
Manufacturers Network



VLAAMS
SUPERCOMPUTER
CENTRUM



Vlaanderen
is supercomputing



federal public service
HEALTH, FOOD CHAIN SAFETY
AND ENVIRONMENT

AGENTSCHAP
INNOVEREN &
ONDERNEMEN



Vlaanderen
is ondernemen

UGhent Industrial Research Fund



START IT
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Thank you