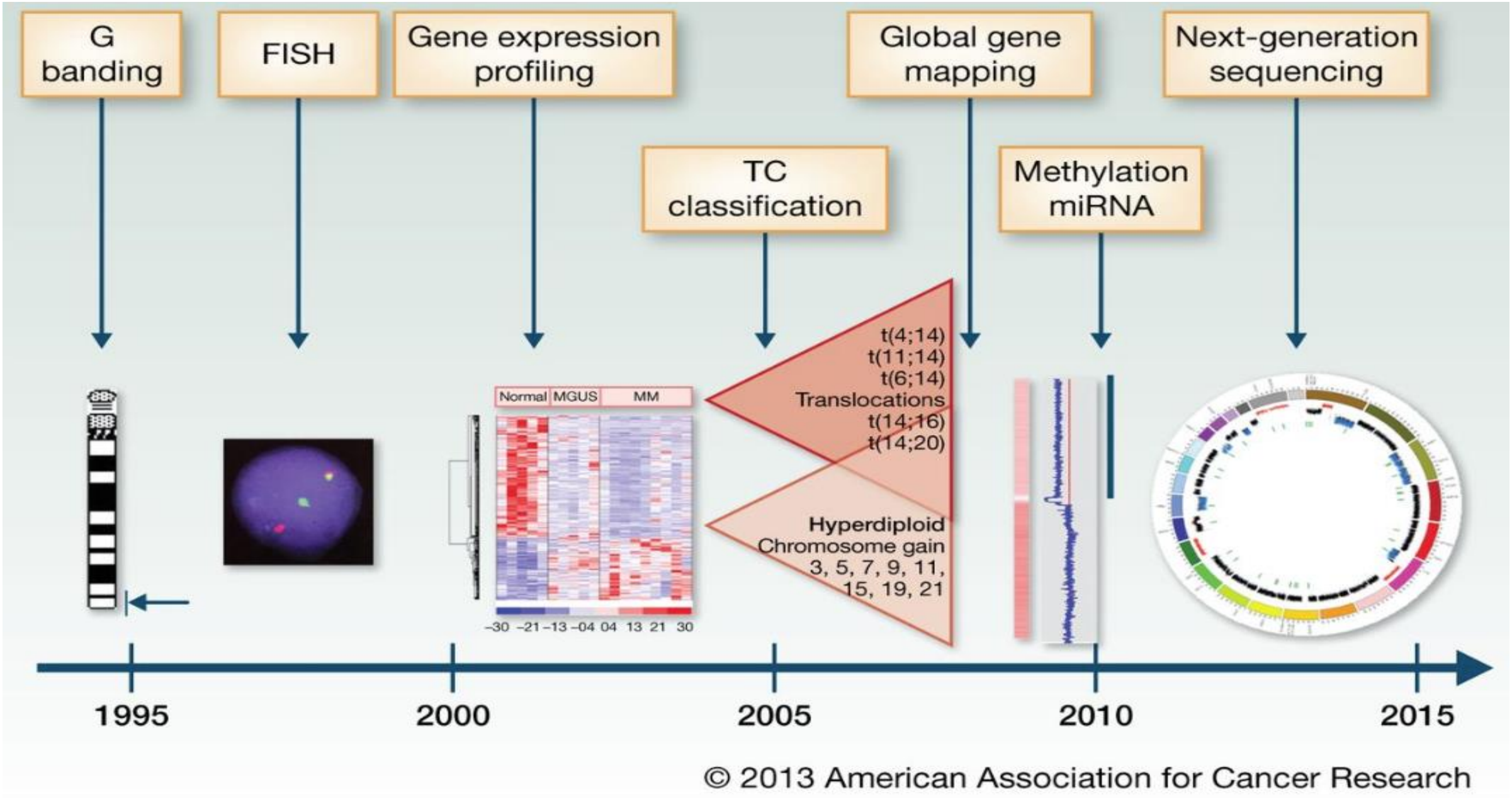
From Cytogenetics to Cytogenomics

Comprehensive Genetic Test

Siraprapa Tongkobpetch

Technological advances in detecting biomarkers in Multiple myeloma



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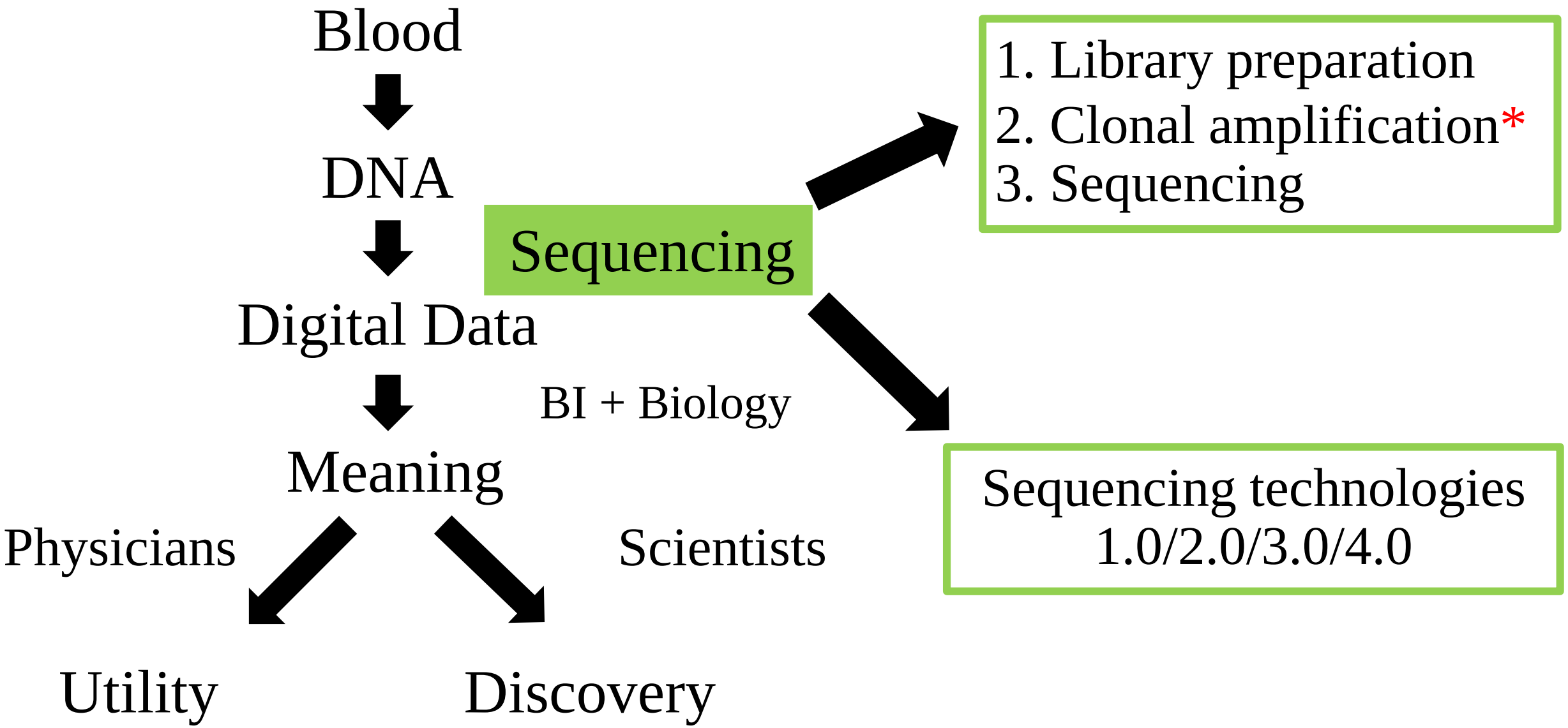
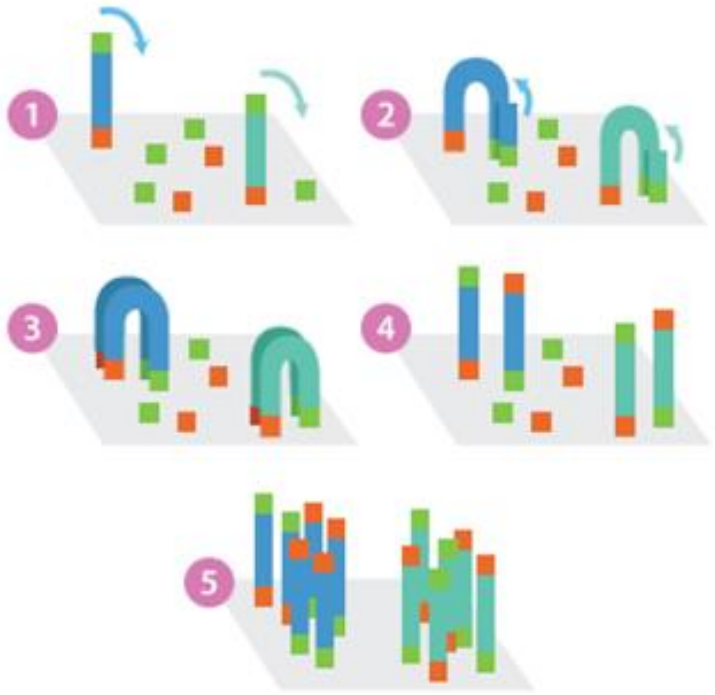
Process of Next generation sequencing (NGS)



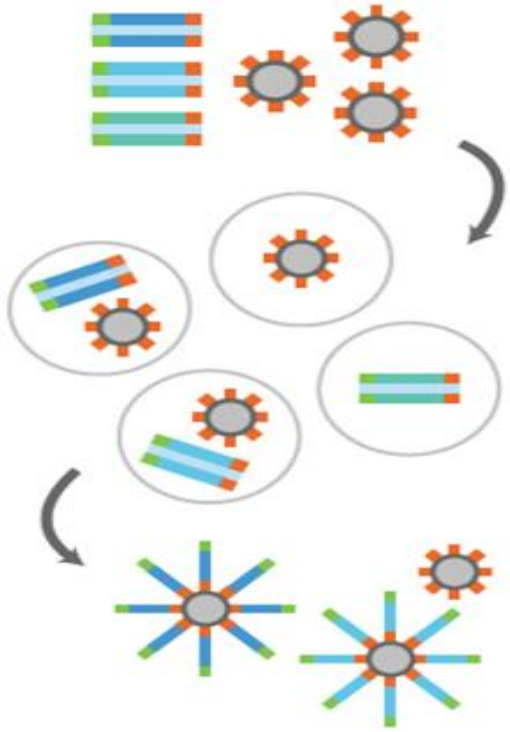
1. Library preparation

- gene panel
- exome
- customize
- pool samples

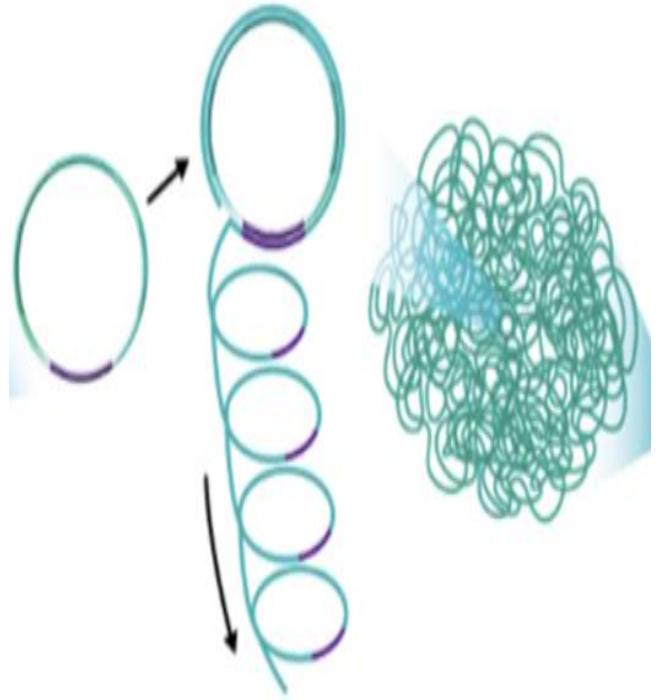
2. Clonal amplification*



Bridge PCR

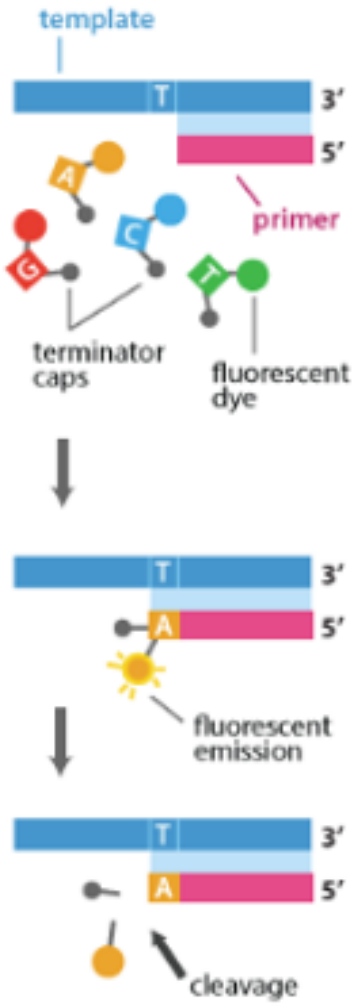


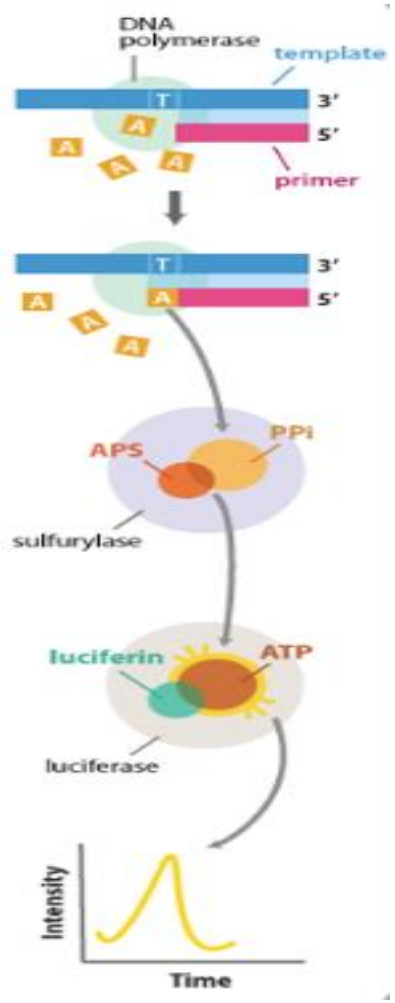
Emulsion PCR

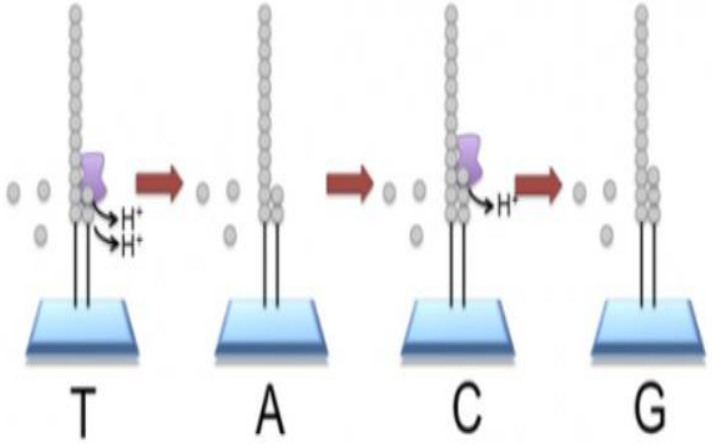


Rolling circle replication

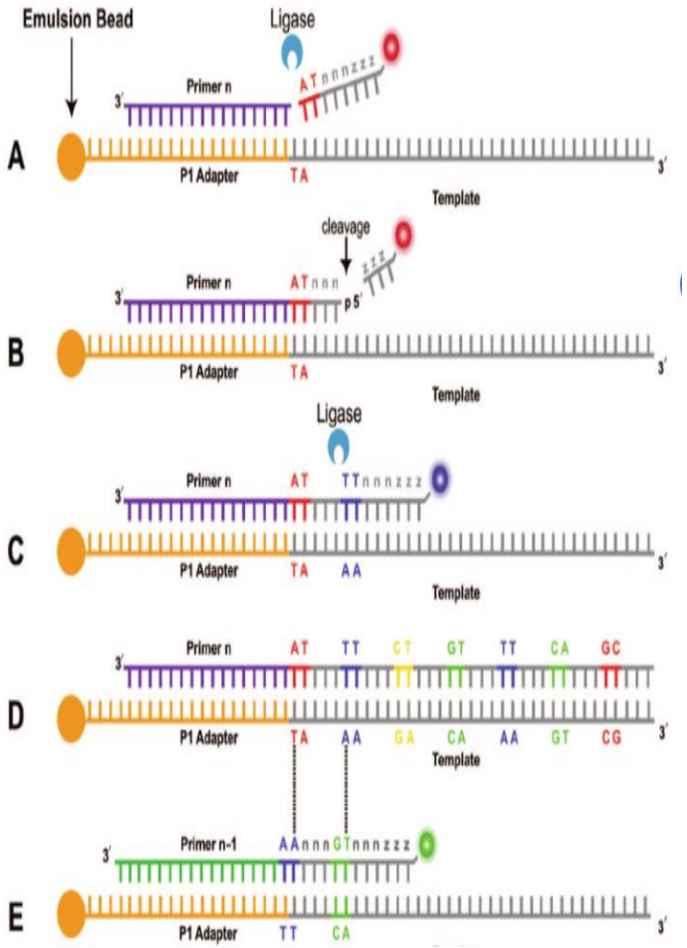
3. Sequencing





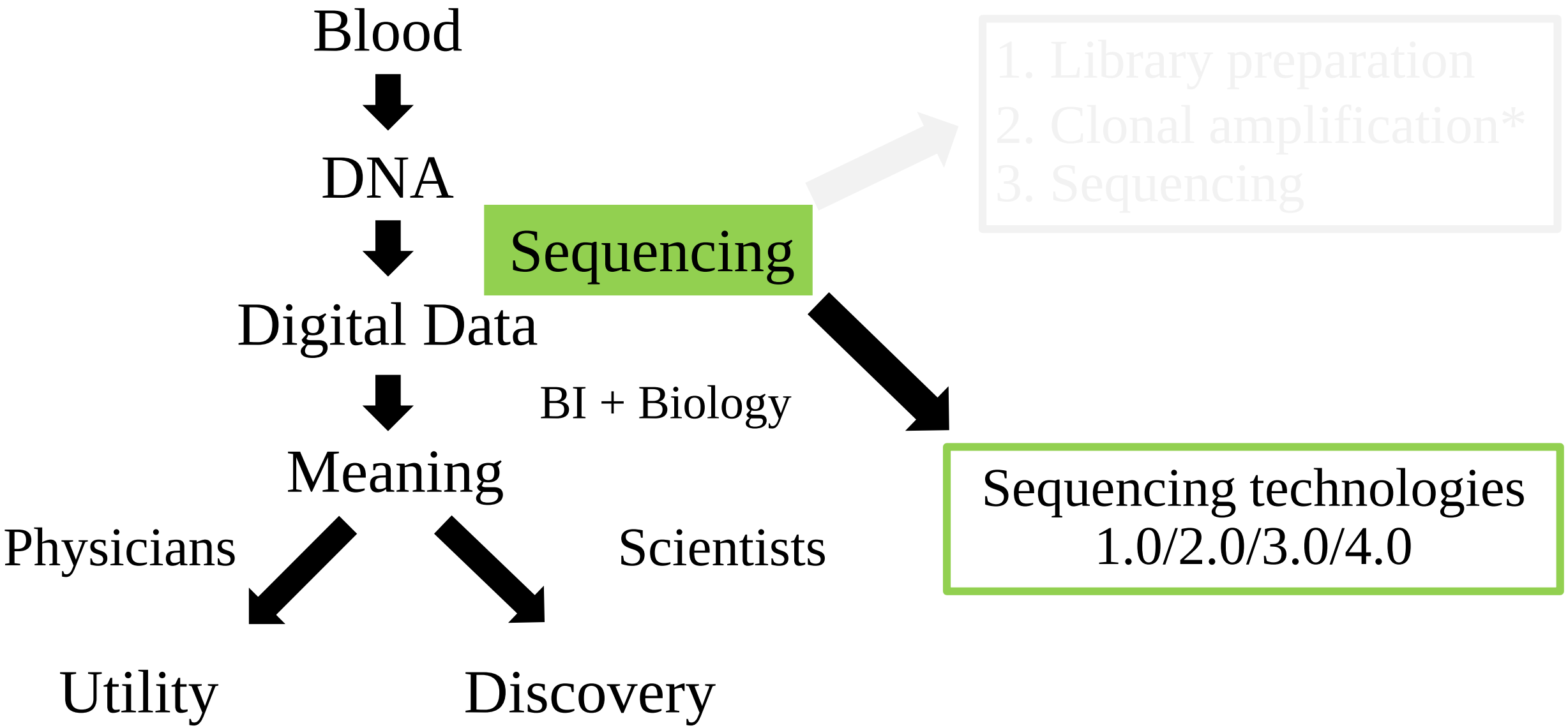


by proton
(pH changing)



by synthesis by pyrosequencing by ligation

Process of Next generation sequencing (NGS)



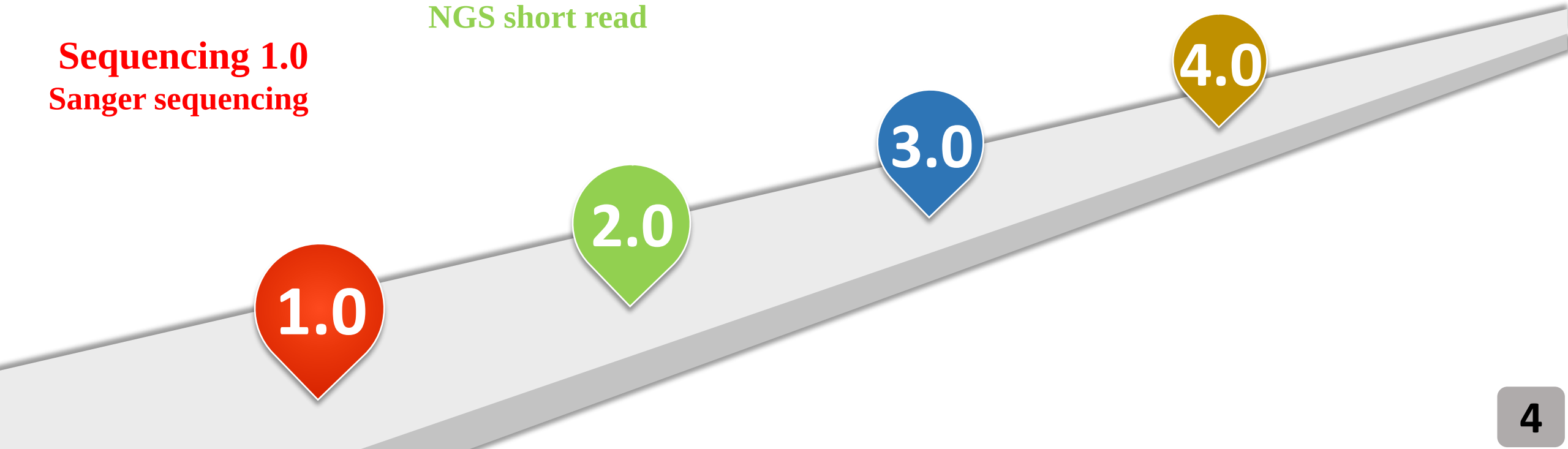
Sequencing technology

Sequencing 4.0
Genome mapping technology

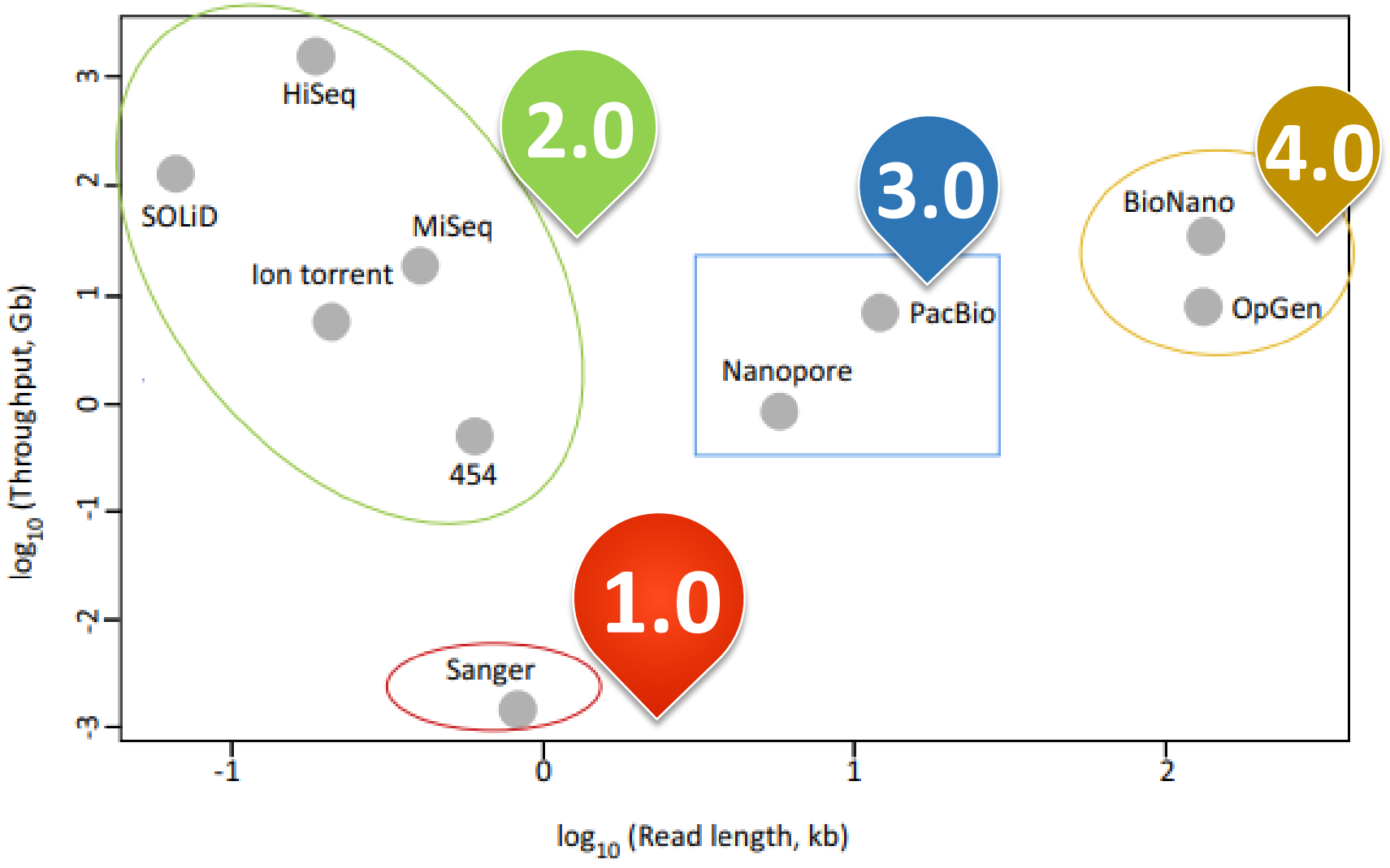
Sequencing 3.0
NGS long read

Sequencing 2.0
NGS short read

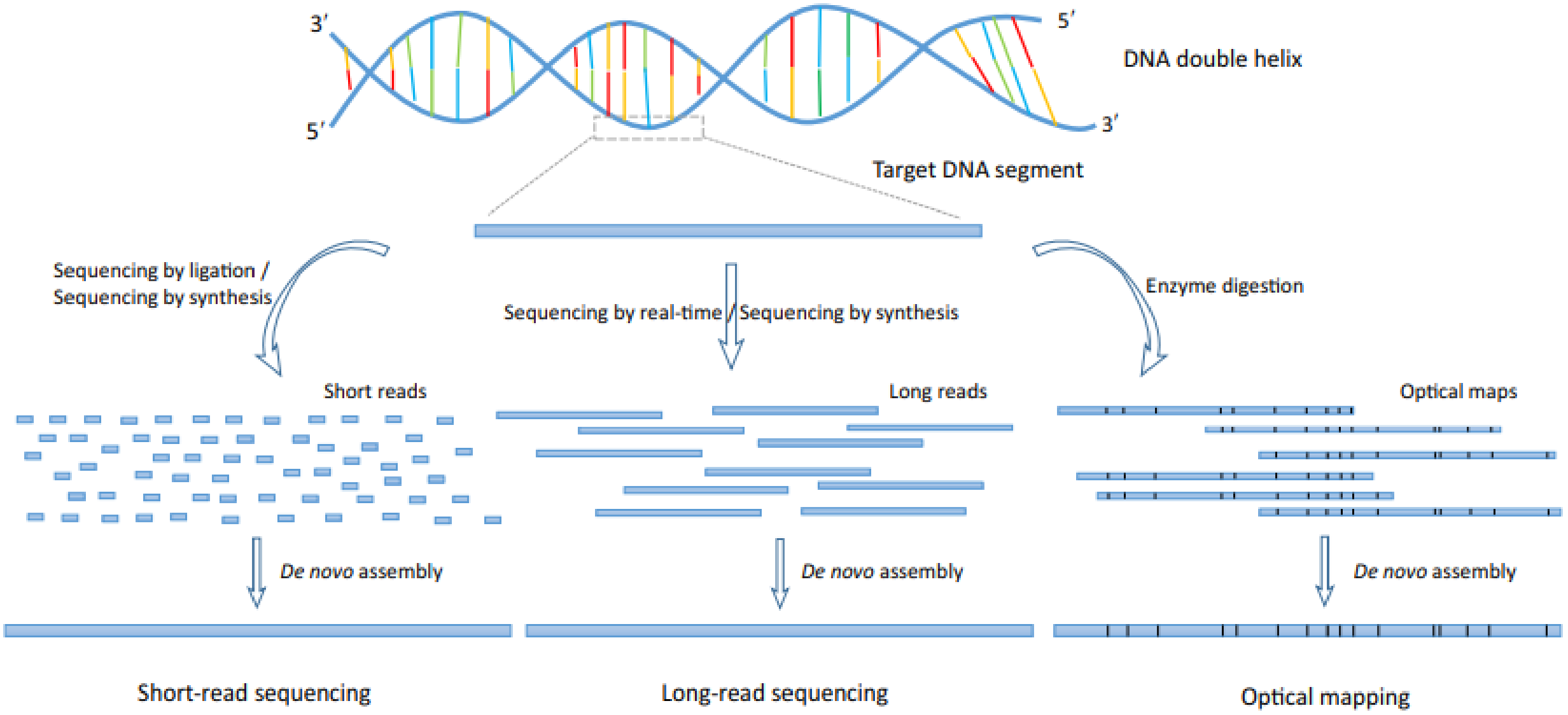
Sequencing 1.0
Sanger sequencing



Sequencing technology

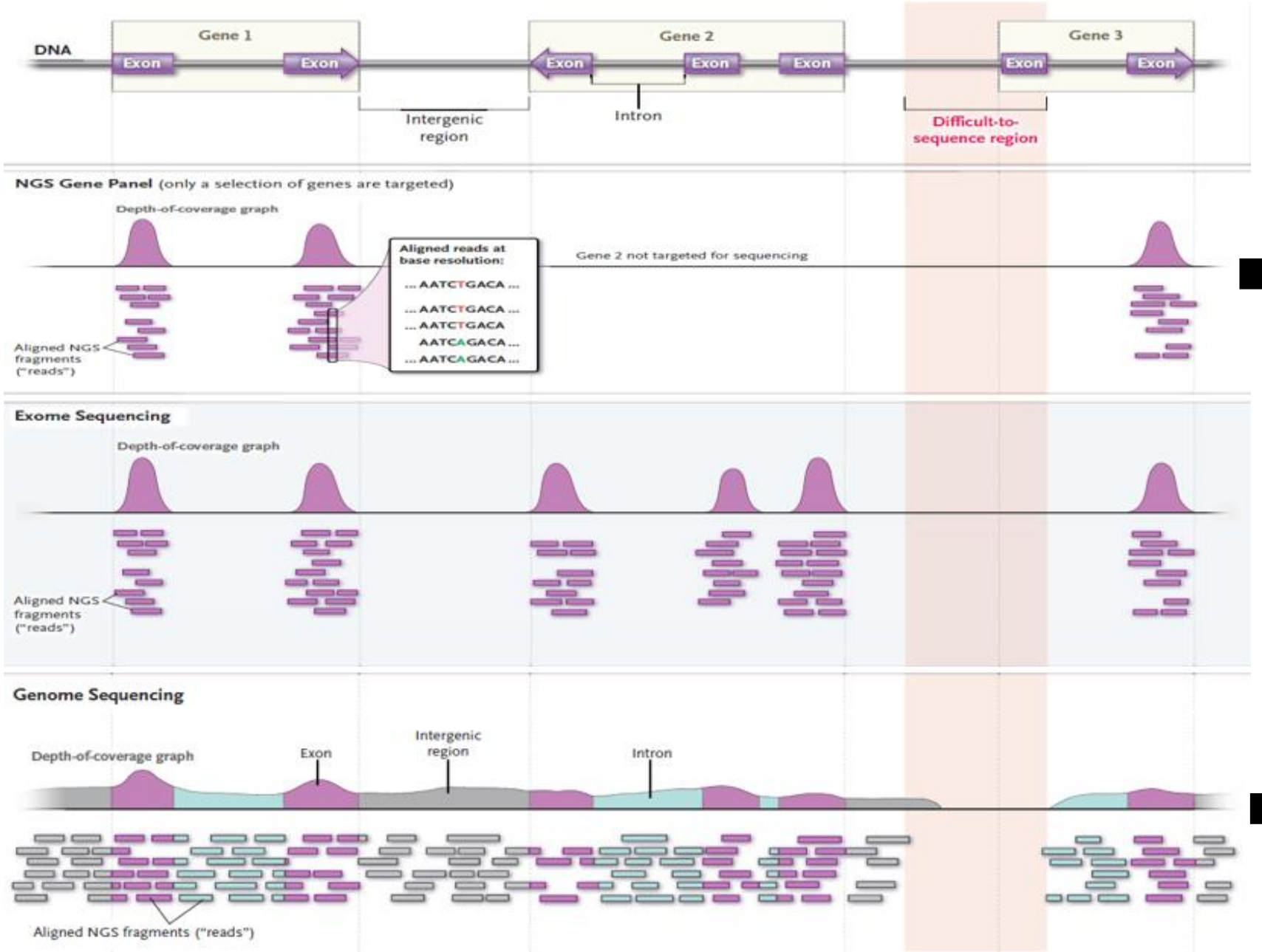


Short-read/Long-read/Optical mapping



Trends in Biotechnology

Sequencing 2.0: NGS short-read sequencing



- cancers
- known mutations

Methods

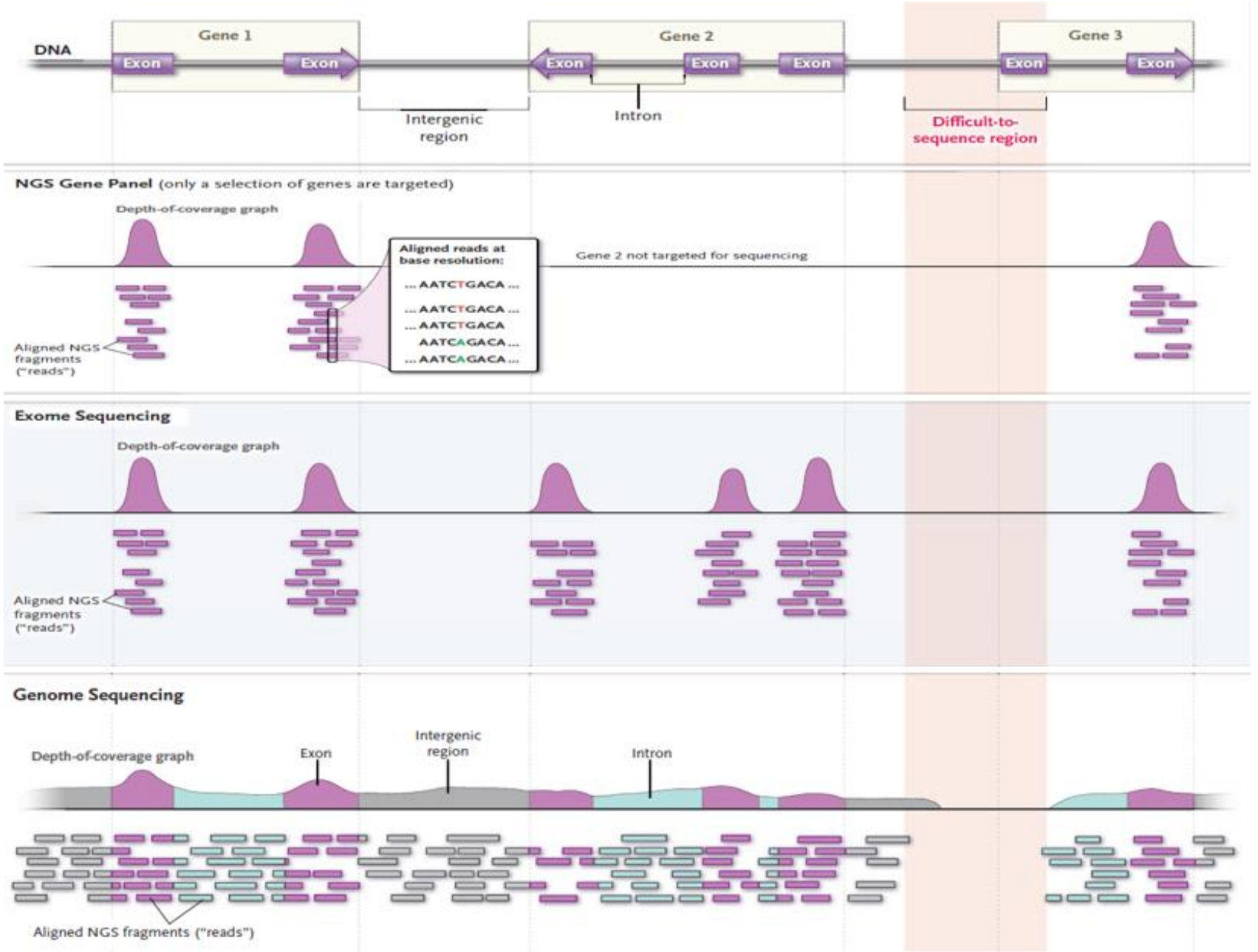
- Sanger sequencing
- Real-time PCR

- SV/CNV*

Methods

- Chromosome banding analysis (CBA)
- FISH
- Microarray

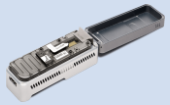
Sequencing 2.0: NGS short-read sequencing



- More read depth
- Less price

- Unknown variants
- Incidental finding

Sequencing 3.0: NGS long-read sequencing

	Platform	Application
	Oxford Nanopore	- Pathogen detection
	Pacific Biosciences	- *de novo* genome assembly - SV and CNV detection - Complex gene sequencing - Expanded repeat detection - RNA isoform sequencing
	10X genomics	- *de novo* genome assembly - SV and CNA detection - Haplotype phasing - Complex gene sequencing

Methods

- Karyotyping
- FISH
- Array

Bridging the Gaps: Short/Long/Mapping

Sequencing lengths available

NGS Single End (50–300,Illumina)

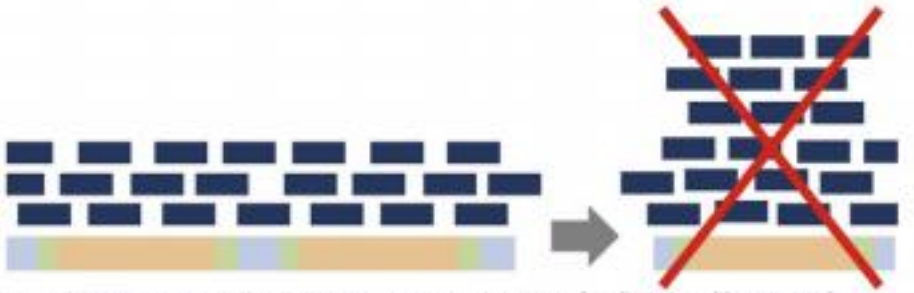
NGS Paired End (2*75–300, total 150–600bp, Illumina)

Long Read (>10 000, no fixed upper limit)

Assembling genome *de novo*

(A) Correct assembly with short reads

(B) Uneven coverage results in missing regions



(C) Repetitive regions can erroneously be collapsed

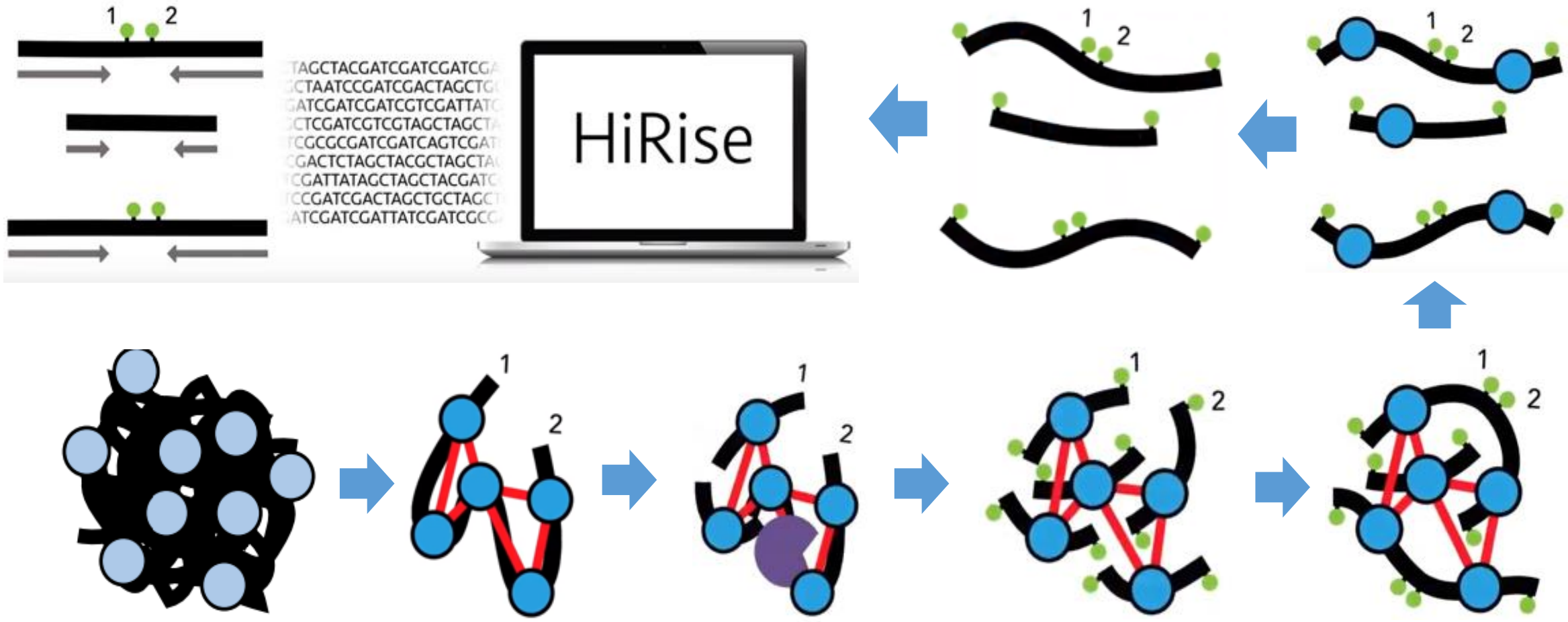
(D) Paired-end reads anchor repetitive sequences in unique flanking regions, providing the insert size is sufficient insert

(E) Long reads bridge regions, high error-rates

(F) Short reads can correct base calls of long reads

Sequencing 4.0: Genome mapping technology

Dovetail Genomics



- Hi-c technique + NGS short-read PE

Sequencing 4.0: Genome mapping technology

Bionano system

Structural variants (SVs): CBA

Unbalanced translocation (insertions, inversions and translocations):

Chromosomal microarray analysis (CMA)

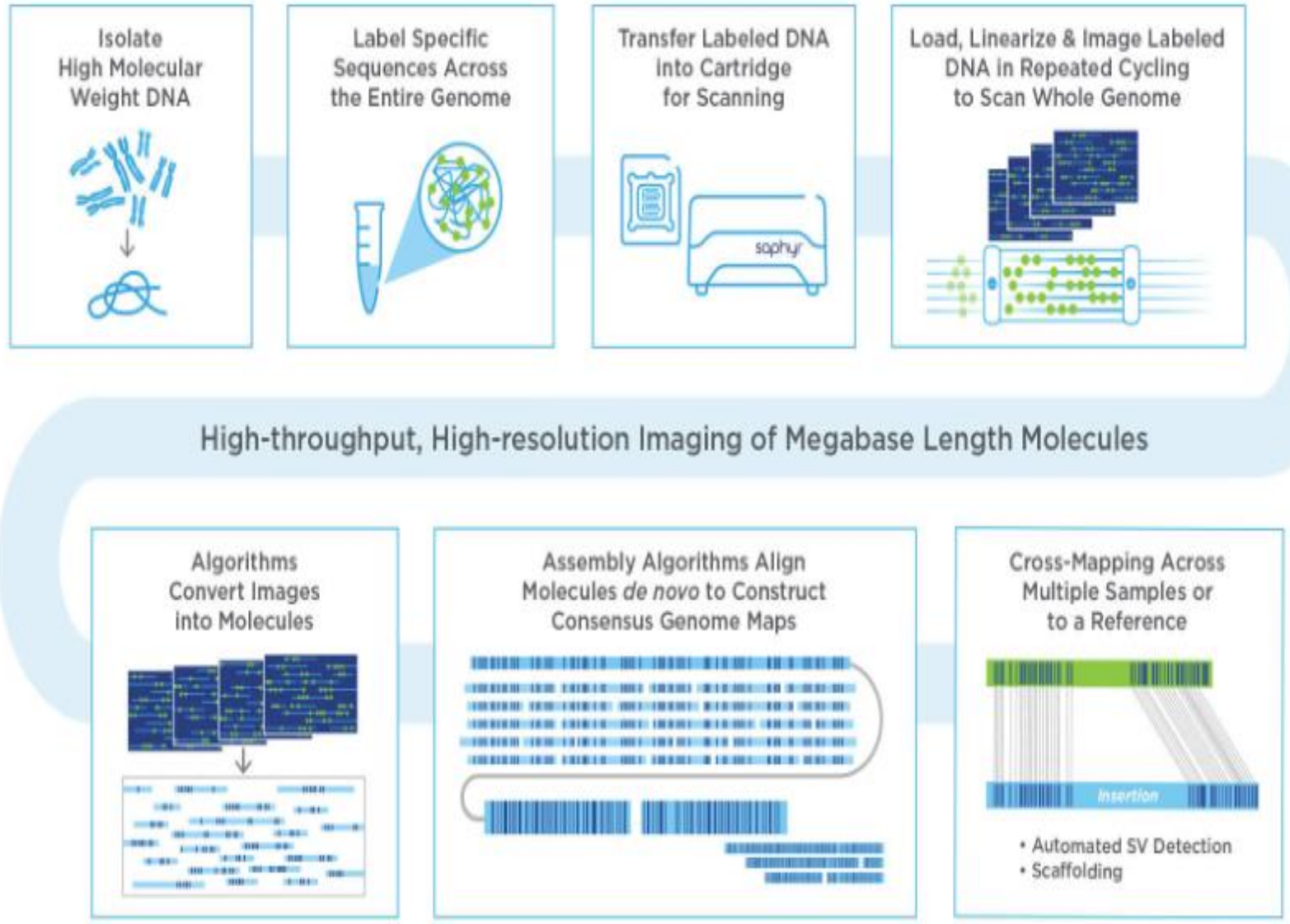
Whole genome sequencing (WGS)

limitation: technical/computational challenges

Balanced translocation (CNV):

Whole genome sequencing (WGS)

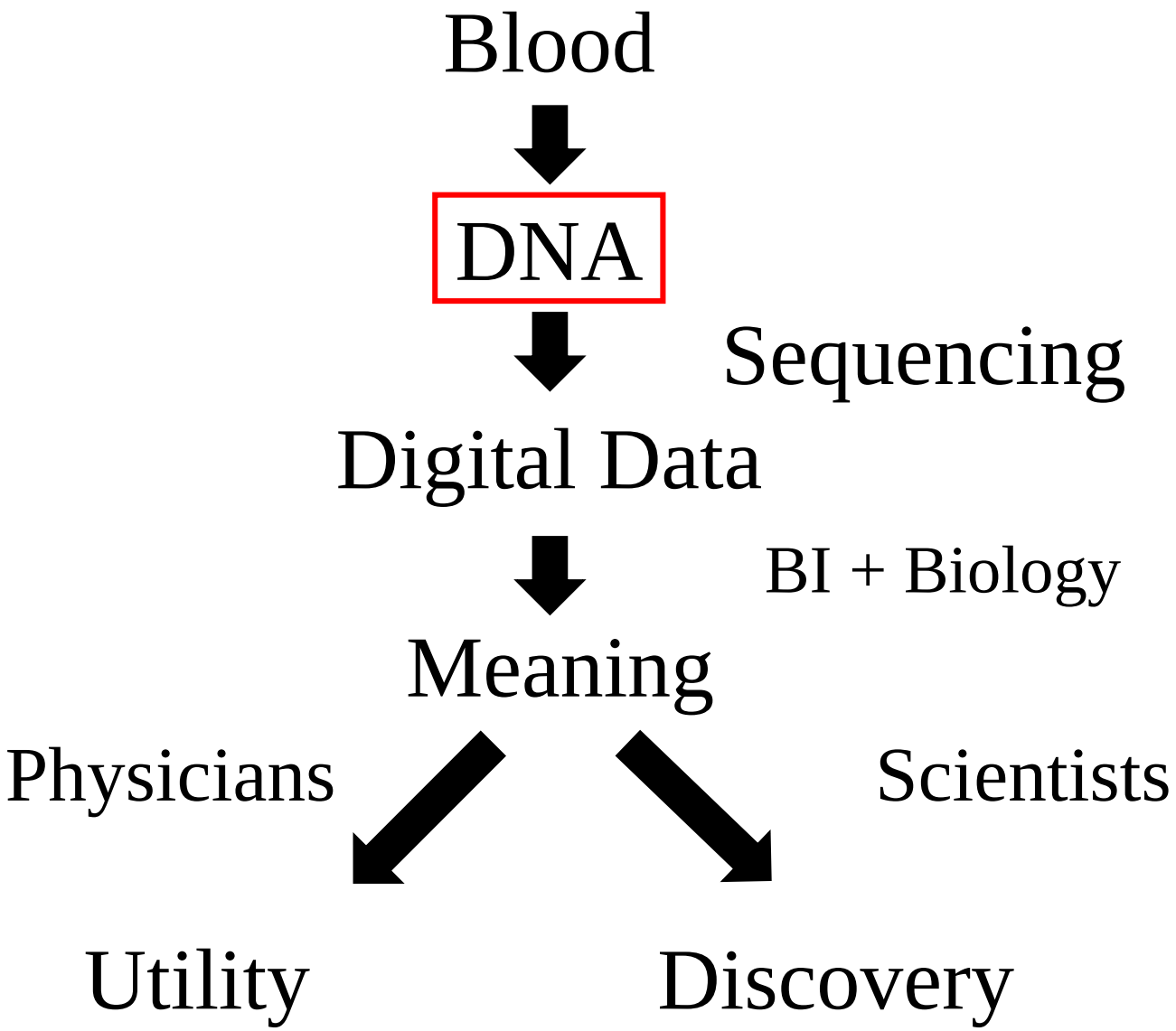
limitation: technical/computational challenges



Genomic Technologies for Cytogenetics

Parameter	FISH	Karyotyping	Arrays	Exome/ Gene panel	Genome
Whole-genome view	No	Yes	Yes	Yes	Yes
Resolution	>100 kb	5-10 Mb	20-200 Kb	1 b	1 b
Gains and losses	Yes	Yes	Yes	Yes	Yes
Balanced translocation/ inversion	Yes	Yes	No	No	Yes
Unbalanced translocation	Yes	Yes	Yes	Yes	Yes
Uniparental disomy (UPD)	No	No	Yes (SNP array)	Yes (Trio)	Yes
Copy-neutral LOH (CN-LOH)	No	No	Yes (SNP array)	No	Yes
Gene fusions	Yes	No	Yes (unbalanced)	Yes	Yes

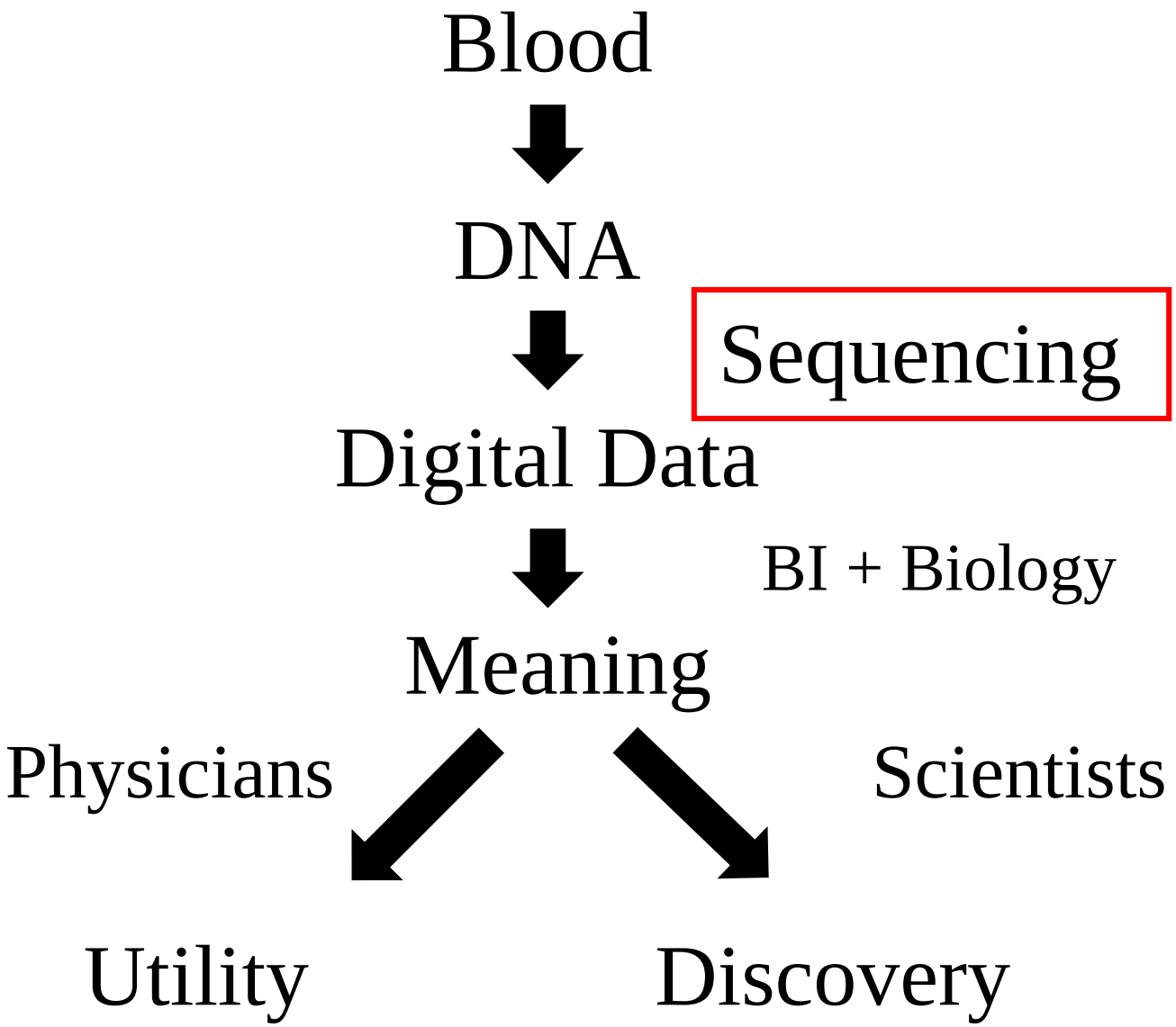
Considerations: NGS



Sample preparation

- FFPE
- CVS
- ICM

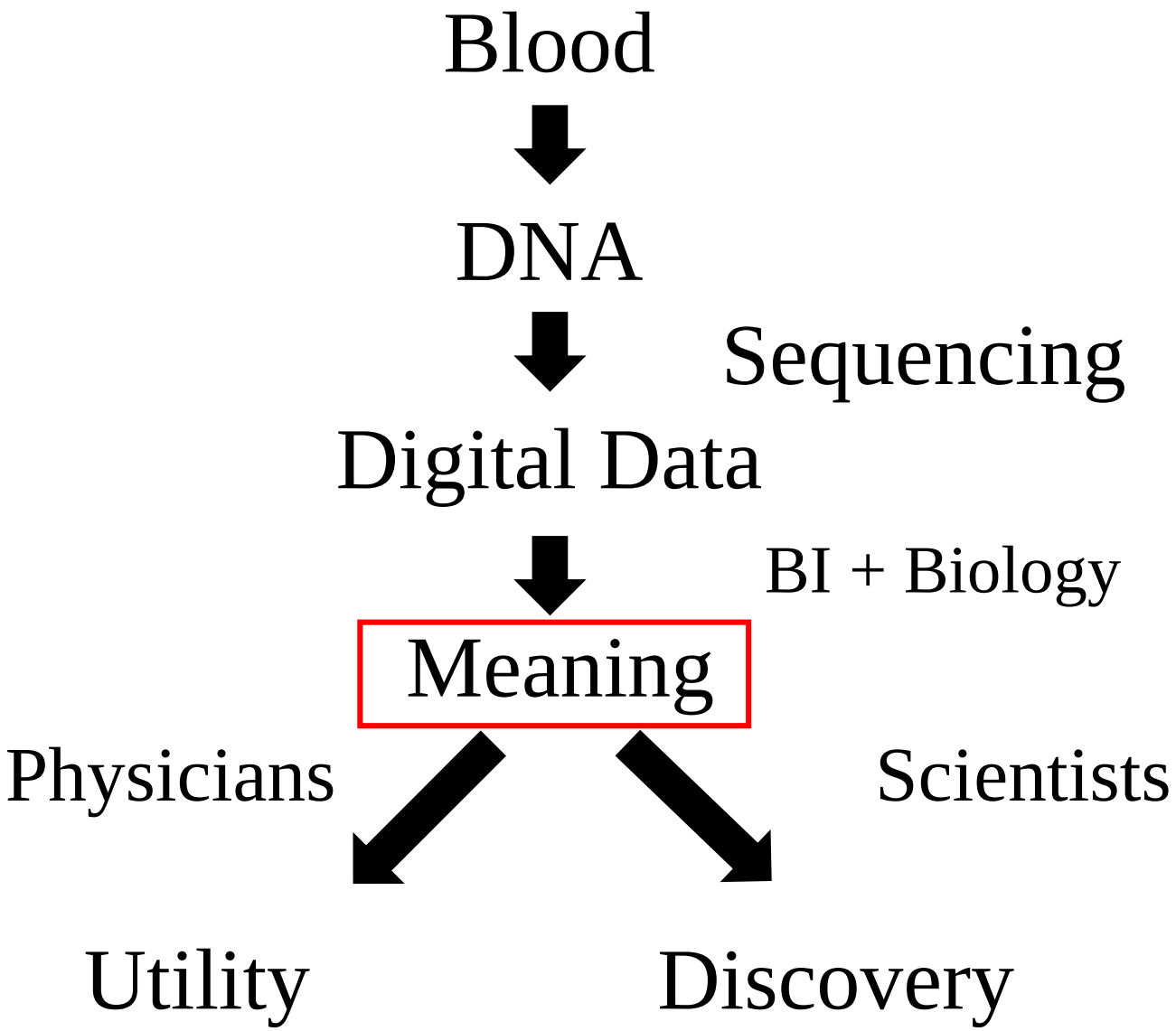
Considerations: NGS



Price

- Sequencing technology
- Read depth
- Interpretation

Considerations: NGS



Interpretation

- Incidental finding
- Variant of uncertain significance (VoUS)

Thank you

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