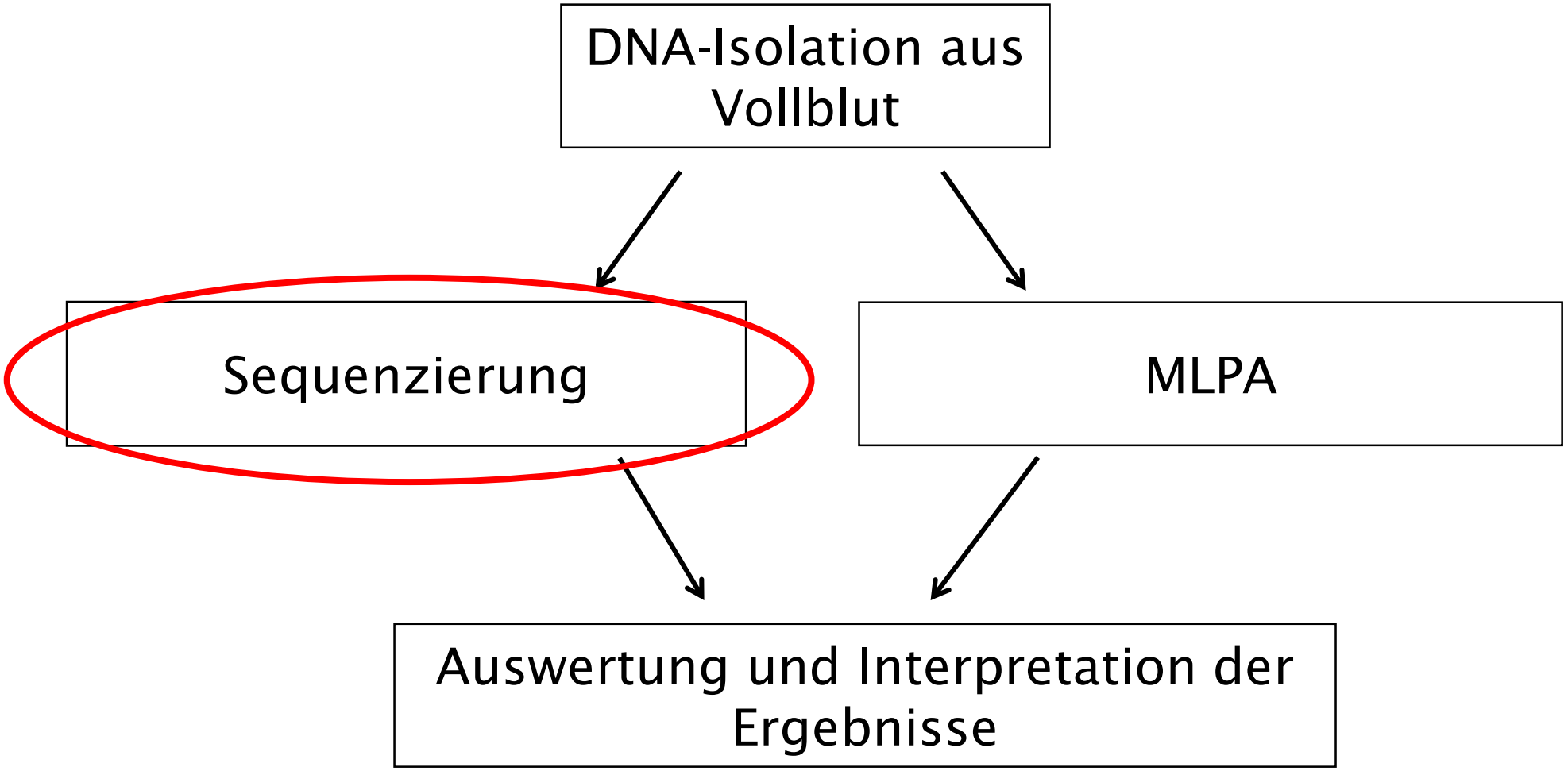
Genetische Analyse

Gabriel Wagner, PhD

Oncolab Diagnostics GmbH

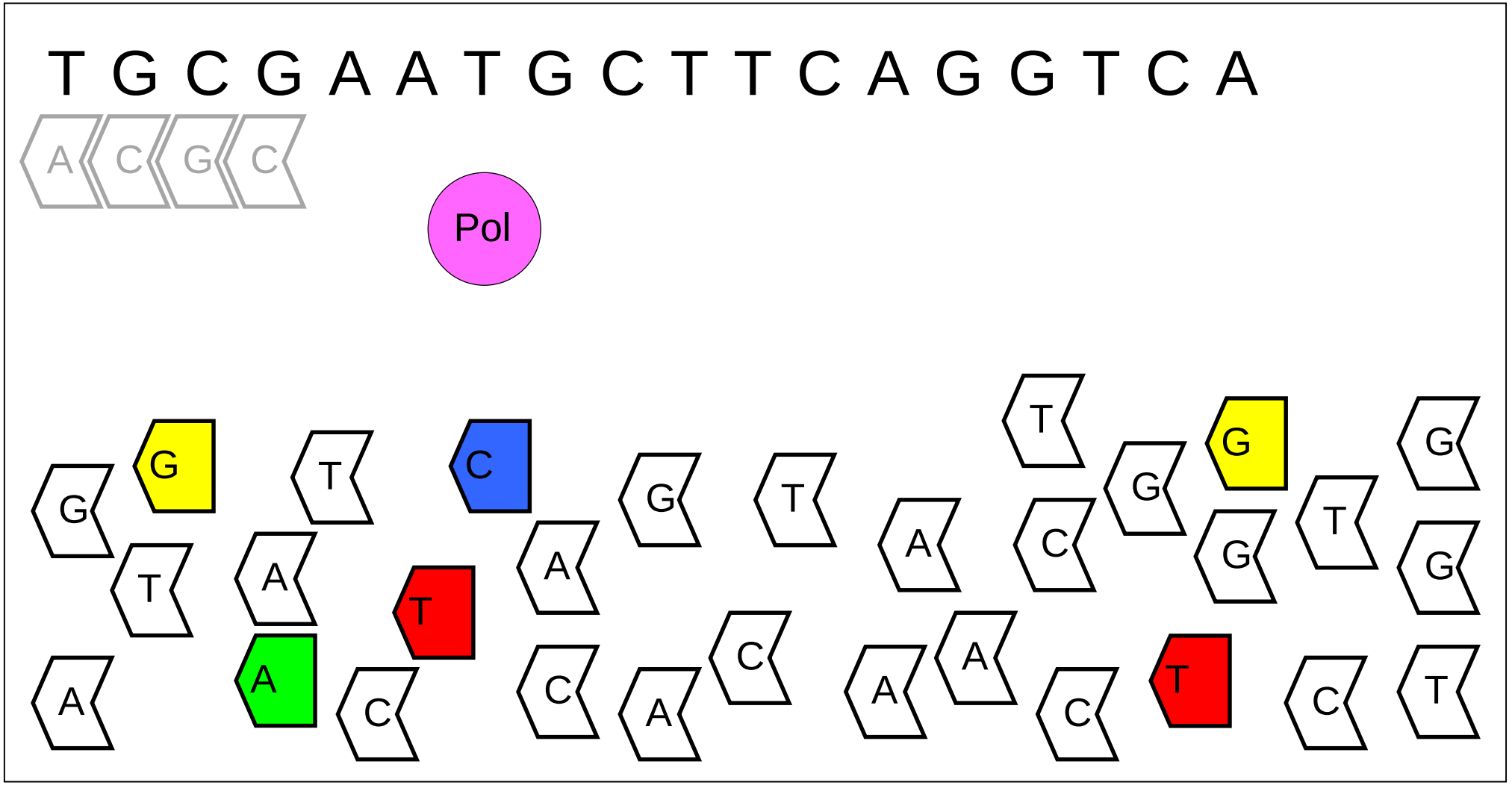
Genetische Analyse



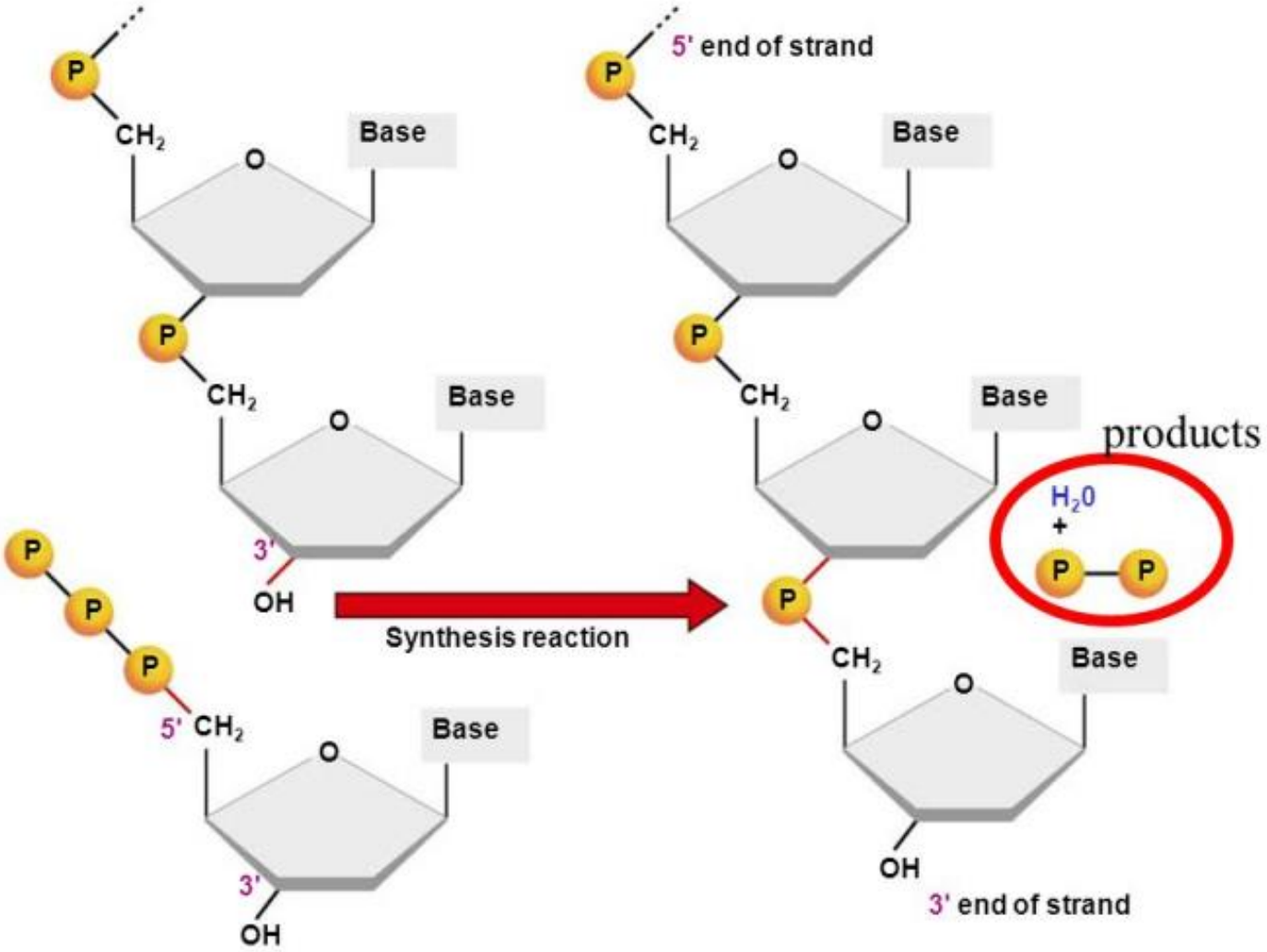
Kapillar-/Sangersequenzierung

- Frederick Sanger
- Britischer Biochemiker
- Zweifacher Nobelpreisträger
  - 1958 Proteinstruktur von Insulin
  - 1980 Basensequenzierung

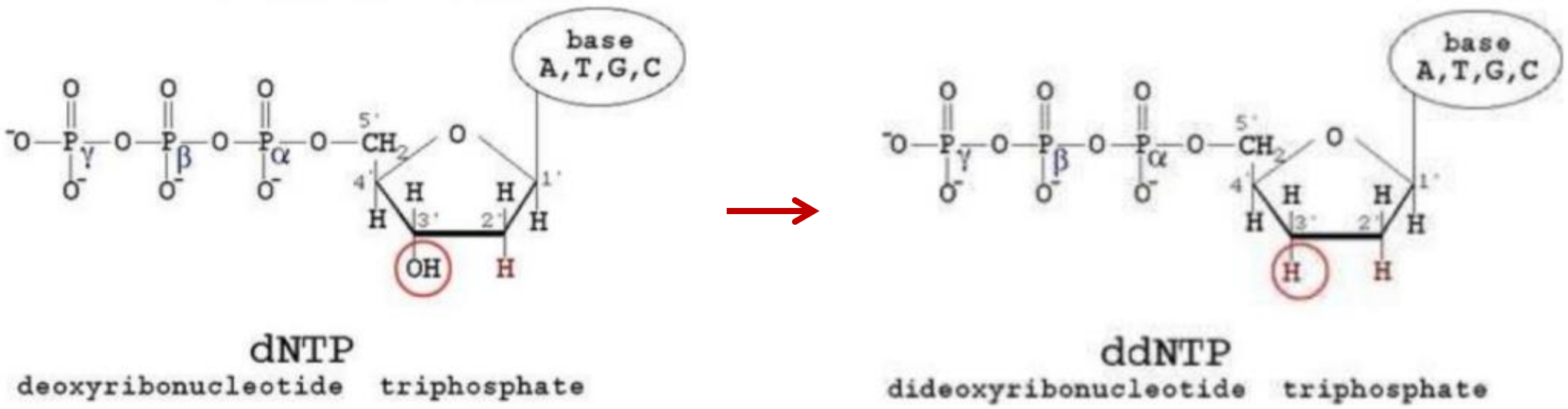
Sequenzierung



Sequenzierung

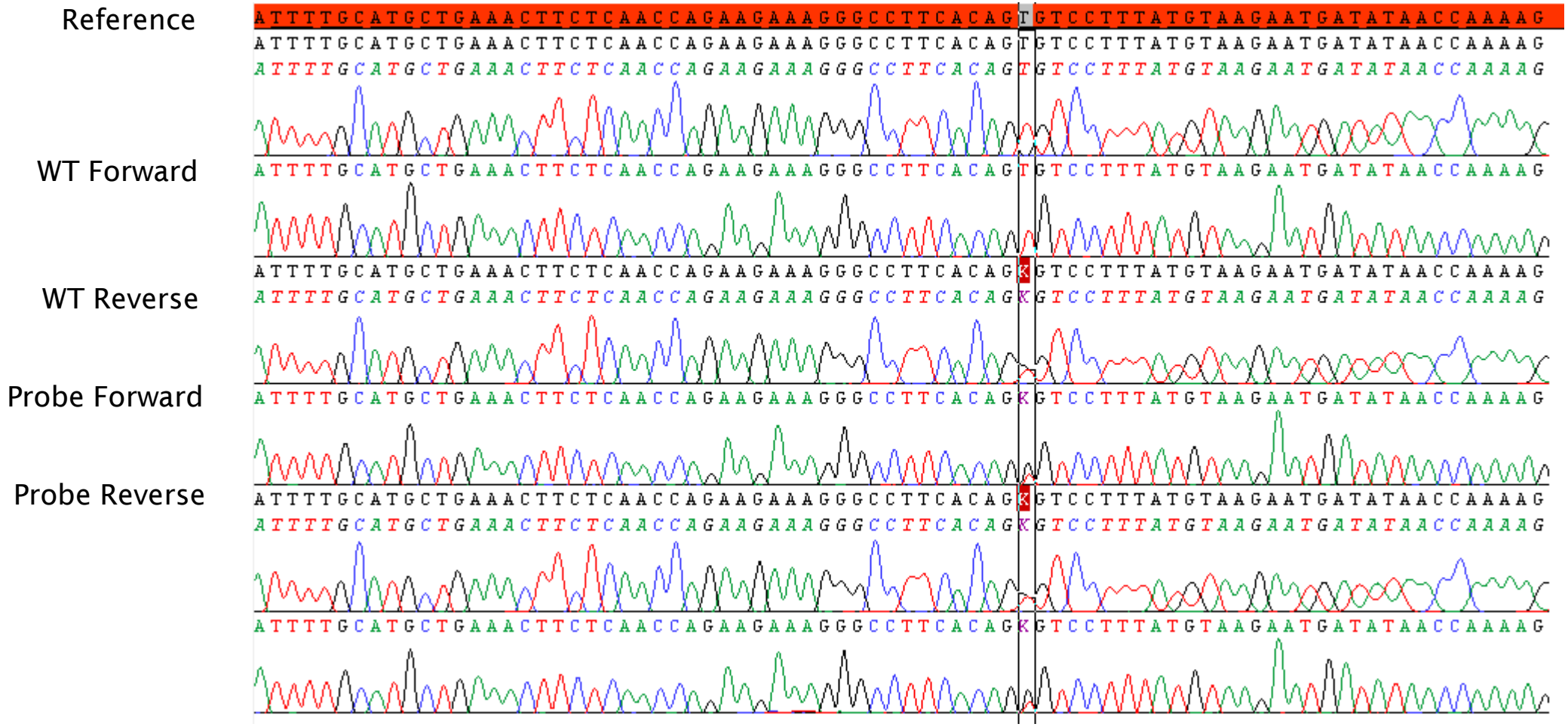


Sequenzierung



Ratio 100:1

Punktmutation



c.181T>G, p.Cys61Gly

Punktmutation

Austausch eines Nukleotids

1. Missense Mutation

TTA TCA CAA GCC GTA GGA

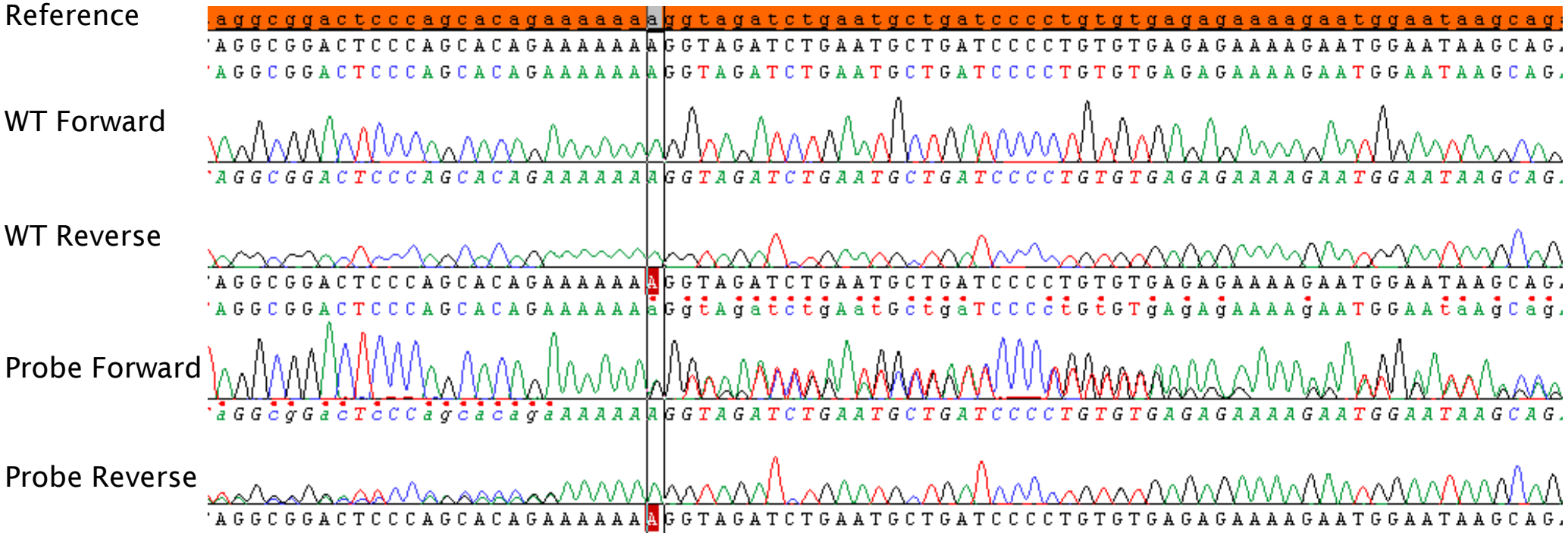
Leucin Serin Glutamine Alanin Valin Glycin

2. Nonsense Mutation

TTA TCA TAA

Leucin Serin Stop

Frameshift Mutation



c.1016delA, p.Lys339Argfs*2

Framshift Mutationen

Deletion oder Insertion

1. In frame

TTA TCA GTA GGA

Leucin Serin Valin Glycin

2. Frameshift

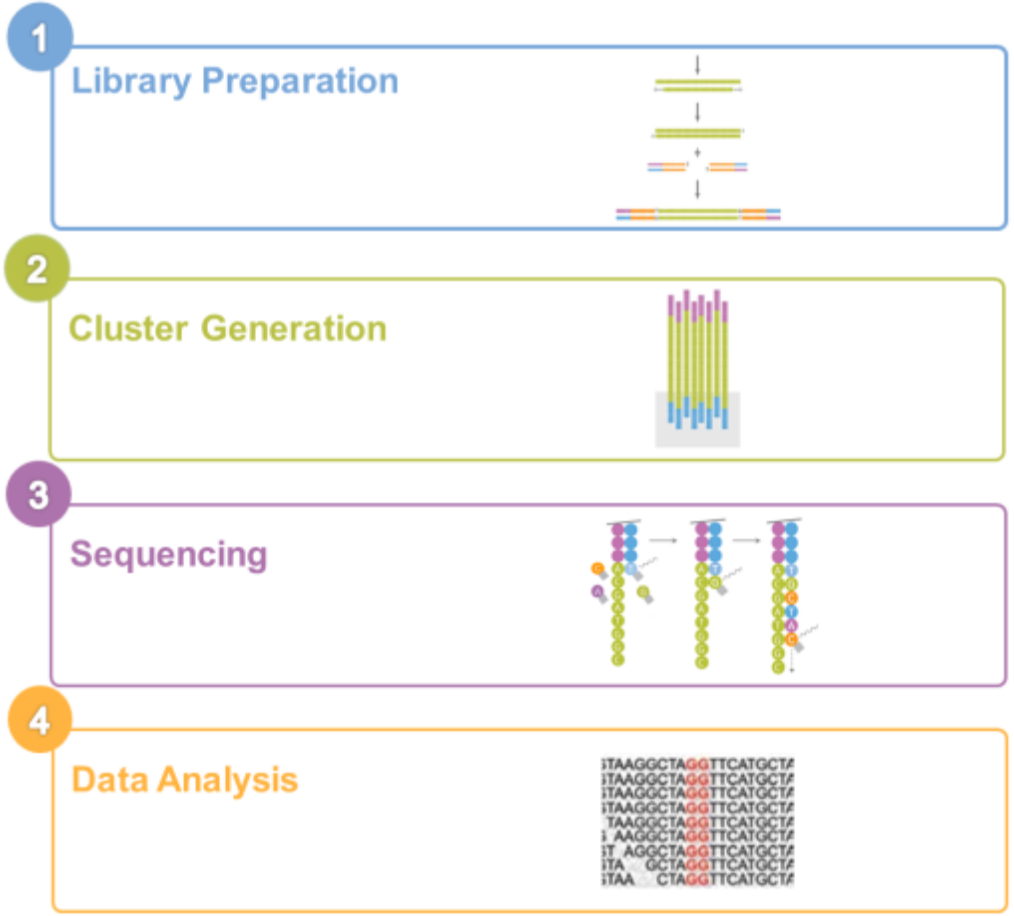
TTA TCA AAG CCG TAG

Leucin Serin Lysin Prolin Stop

Next Generation Sequencing

Illumina MiniSeq

Illumina Sequencing Workflow



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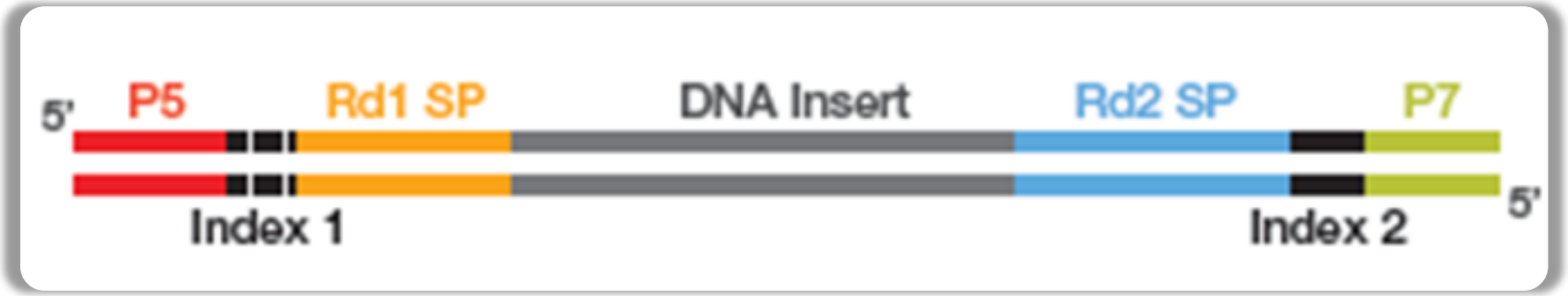
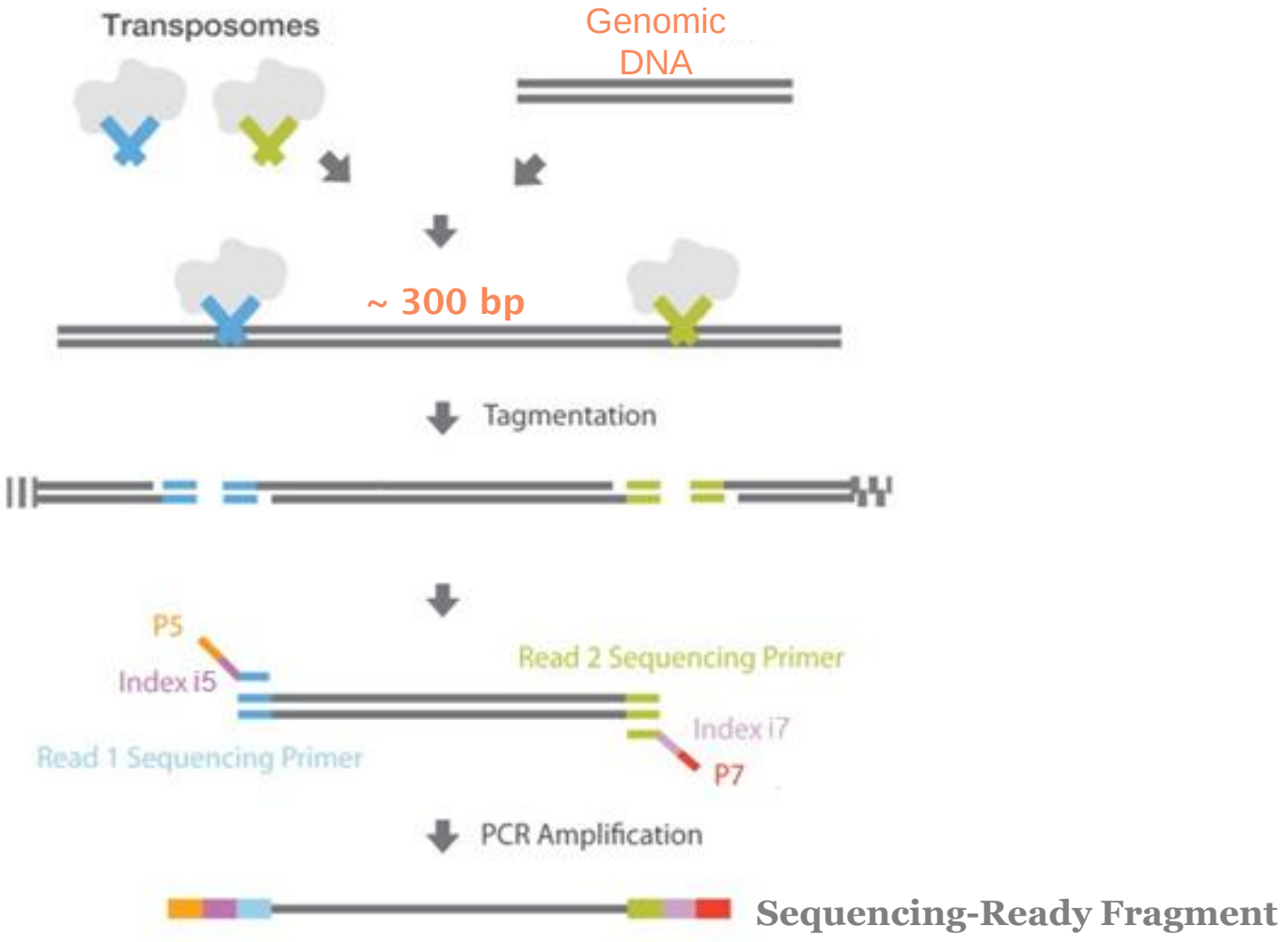
Library Preparation

Illumina TruSight Cancer V2 Panel

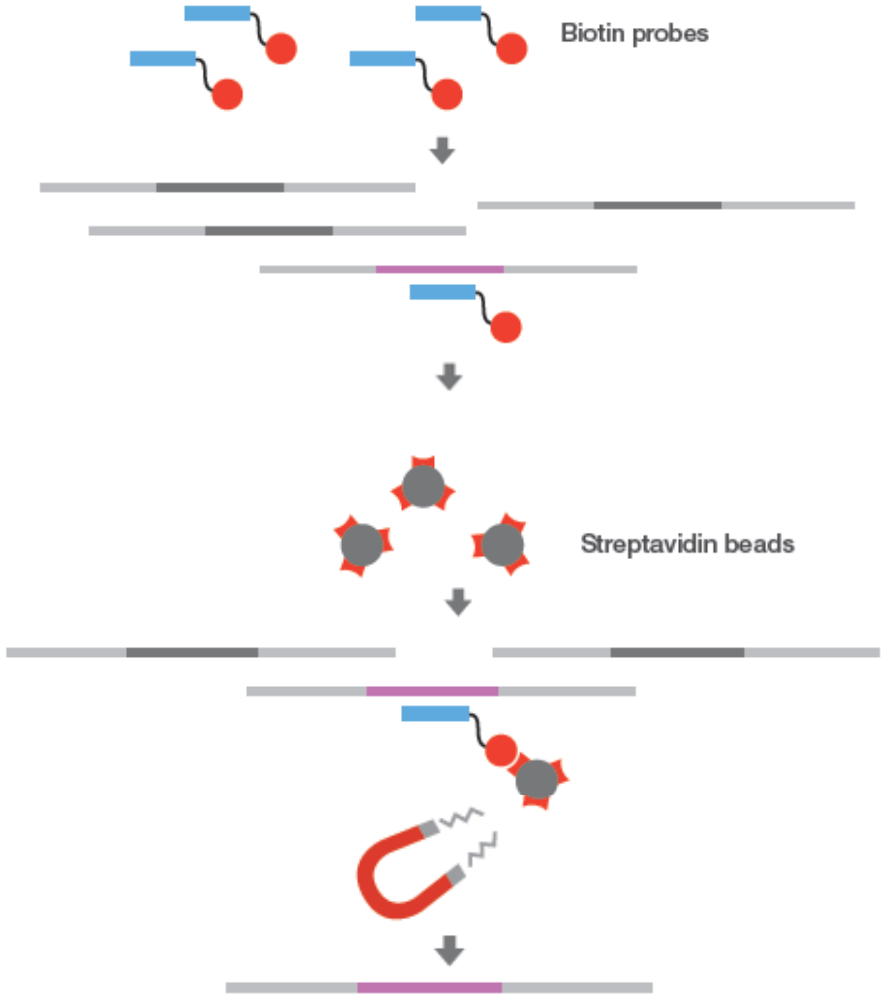
Probenvorbereitung bis die Proben so aussehen:



Library Preparation



Library Preparation – Target Enrichment



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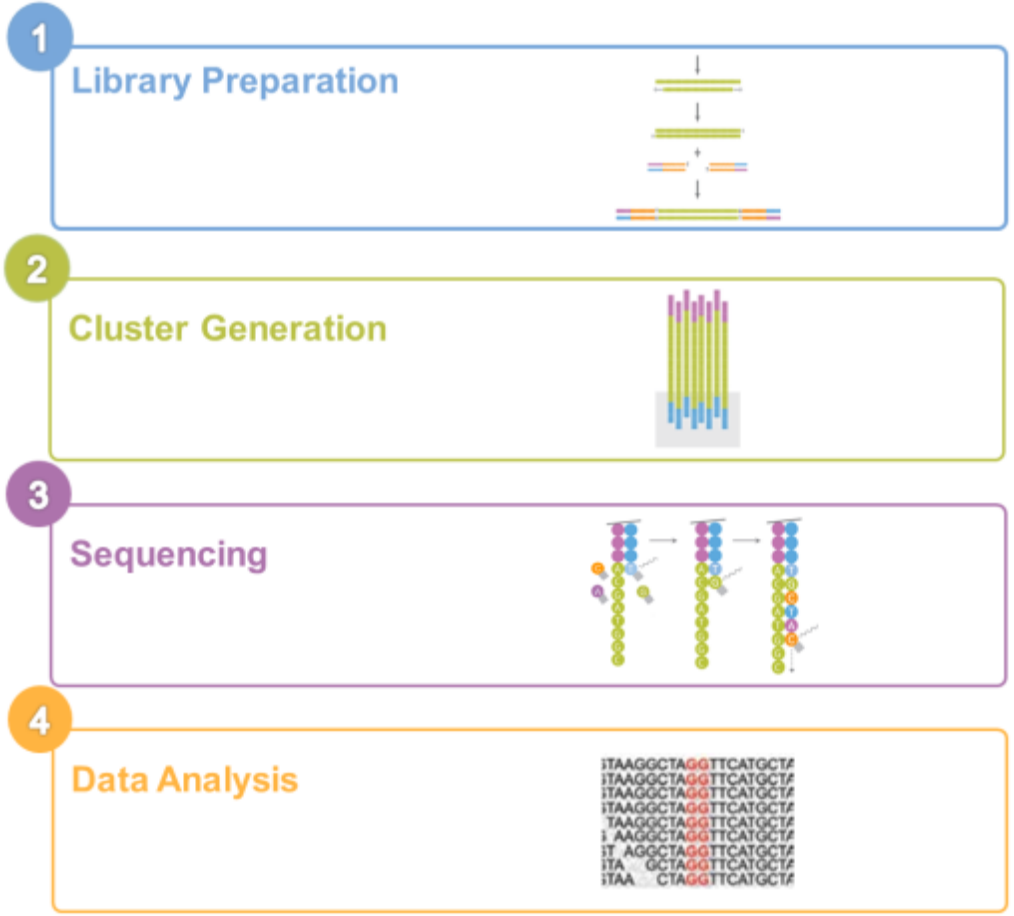
Illumina Sequencing Workflow



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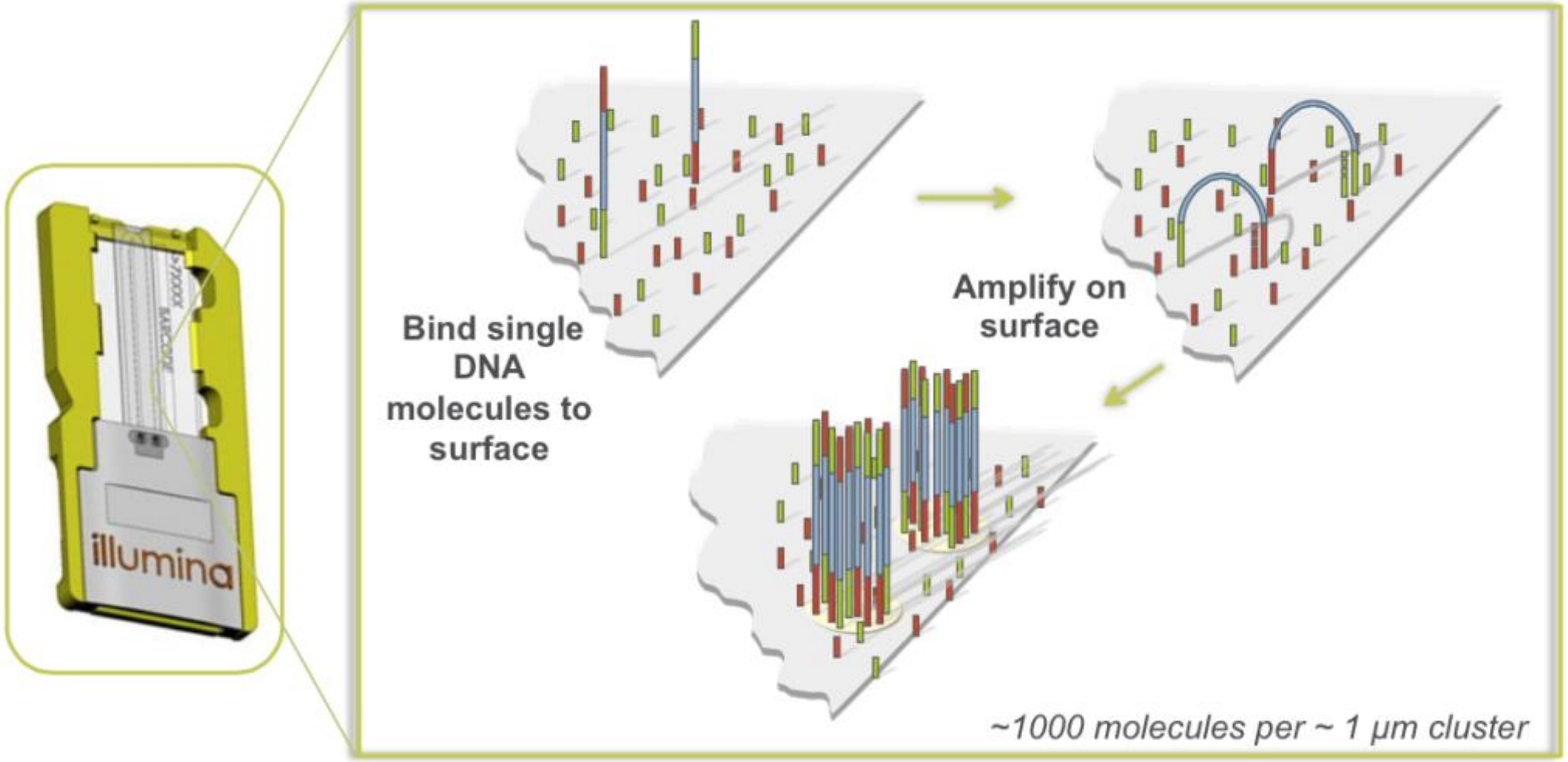
Illumina MiniSeq - Technologie



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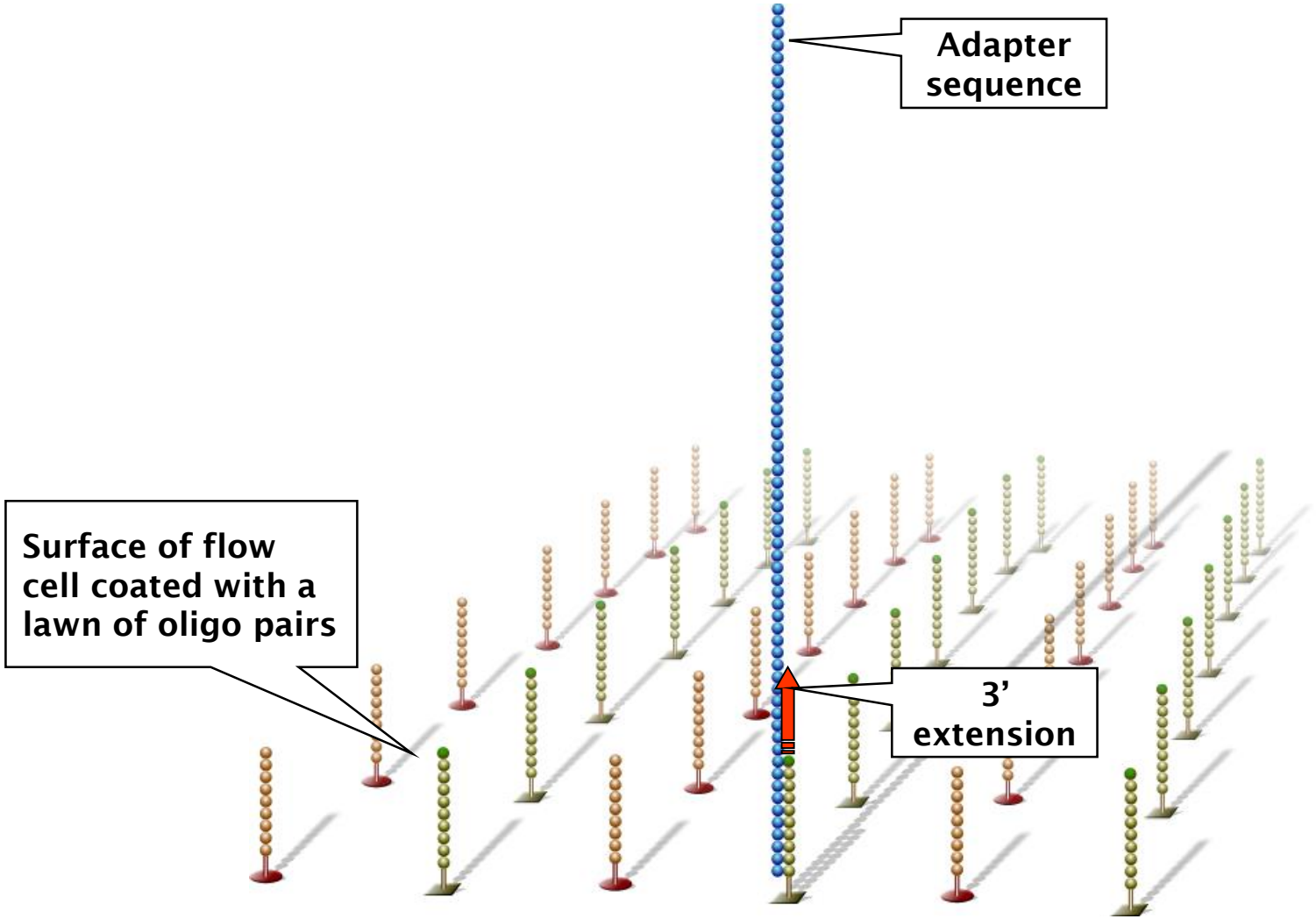
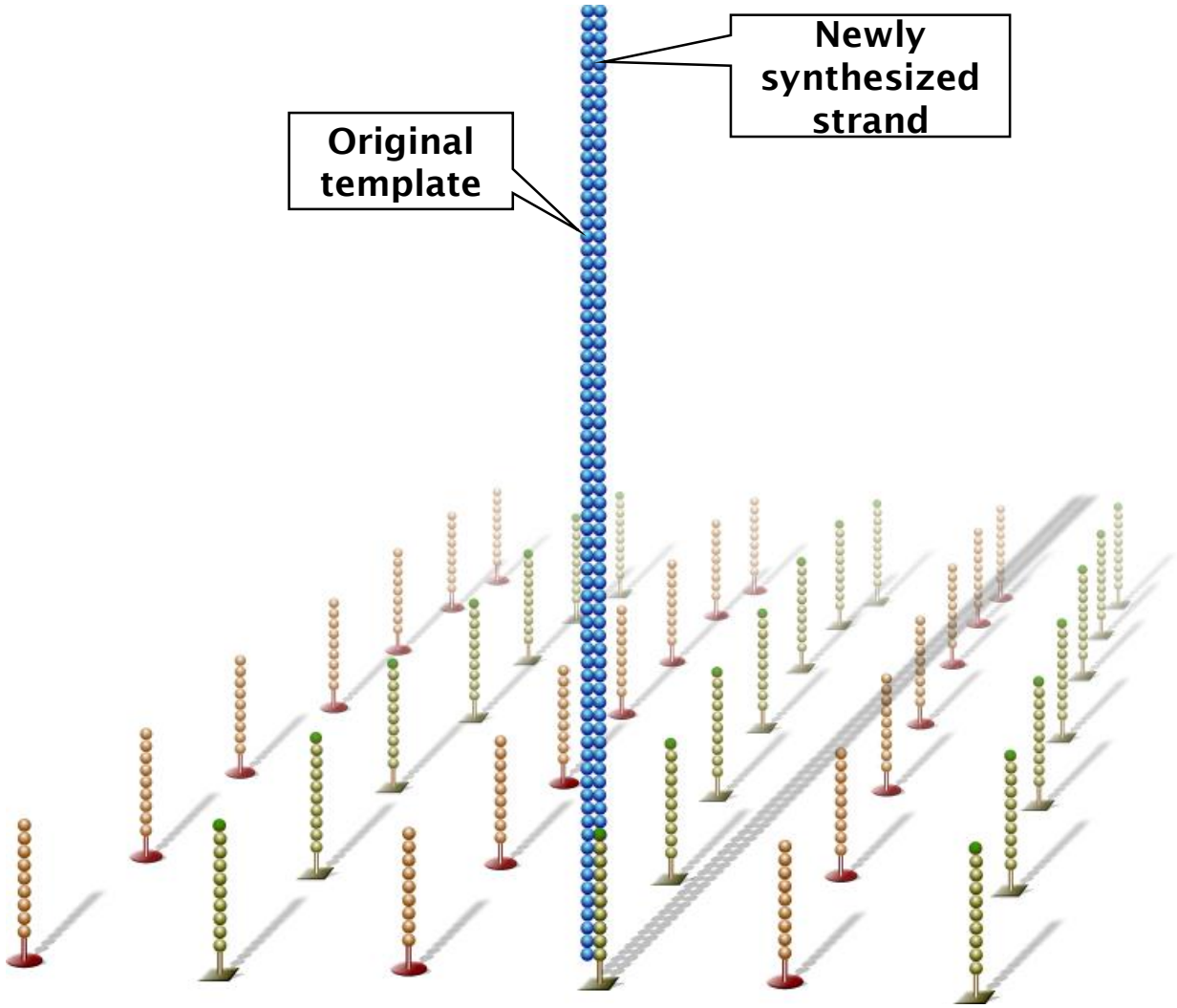
Cluster Formation



Cluster Formation



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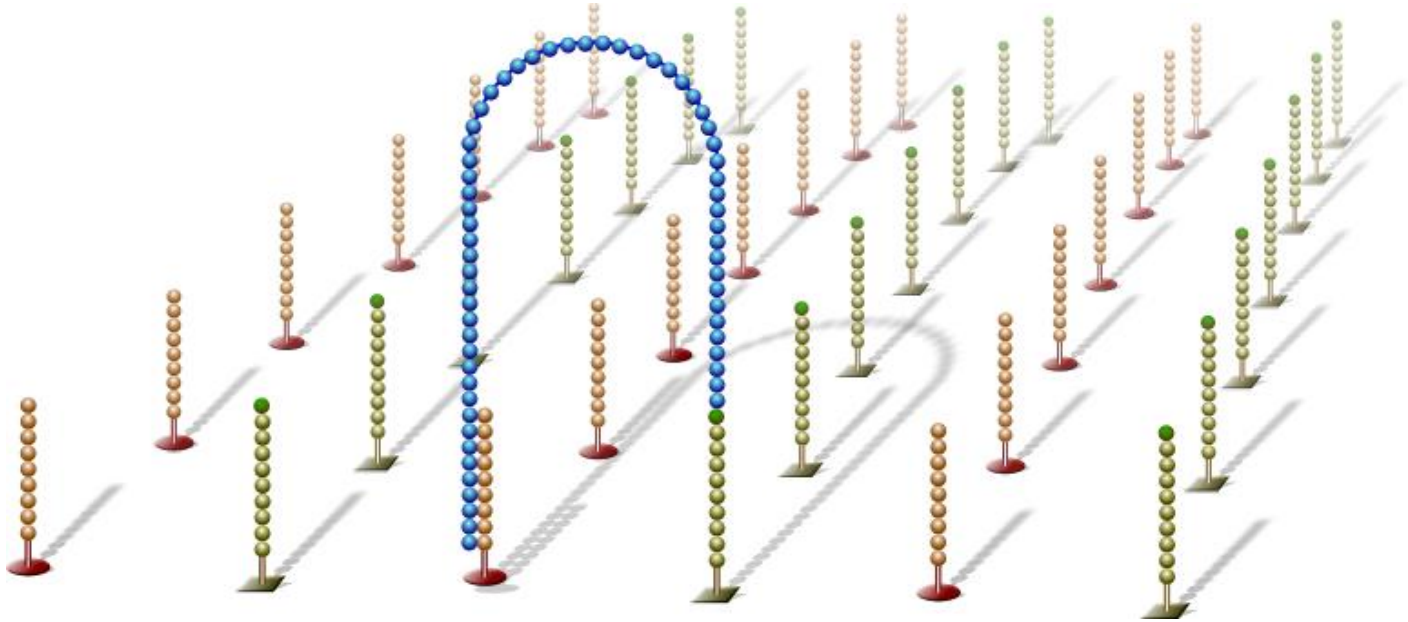
Cluster Formation

Bridge Amplification



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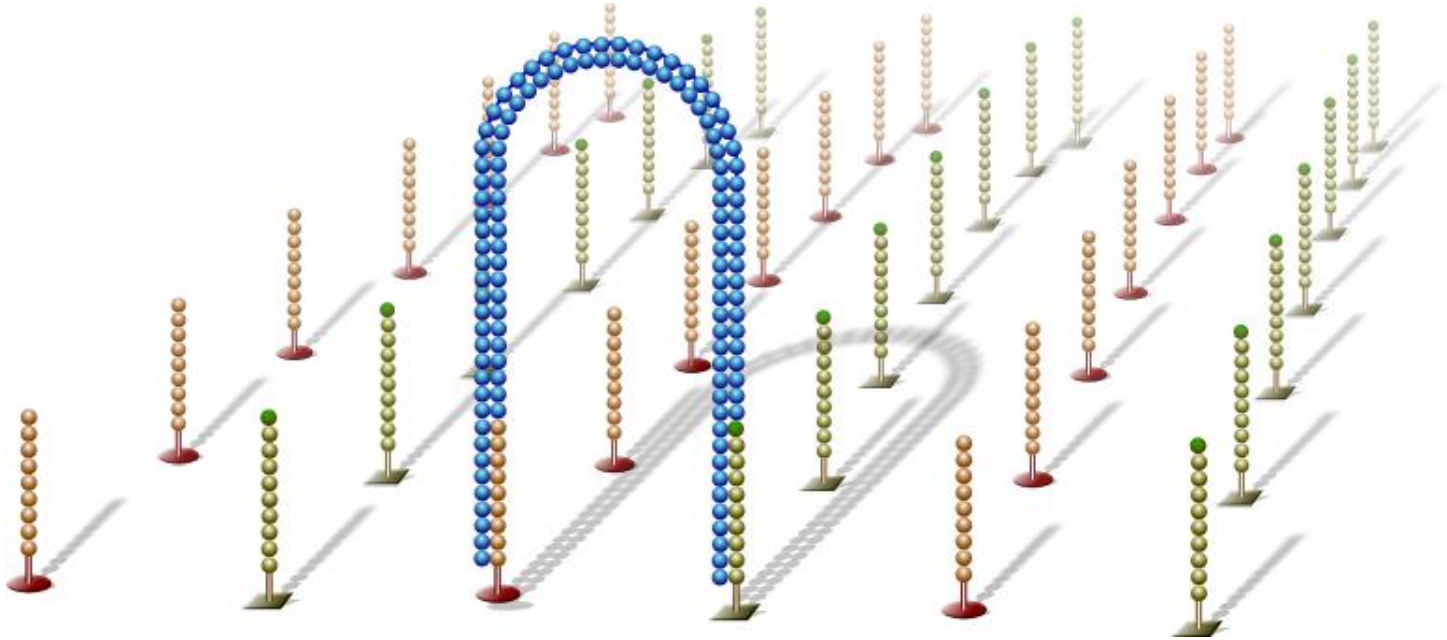
Cluster Formation

Bridge Amplification



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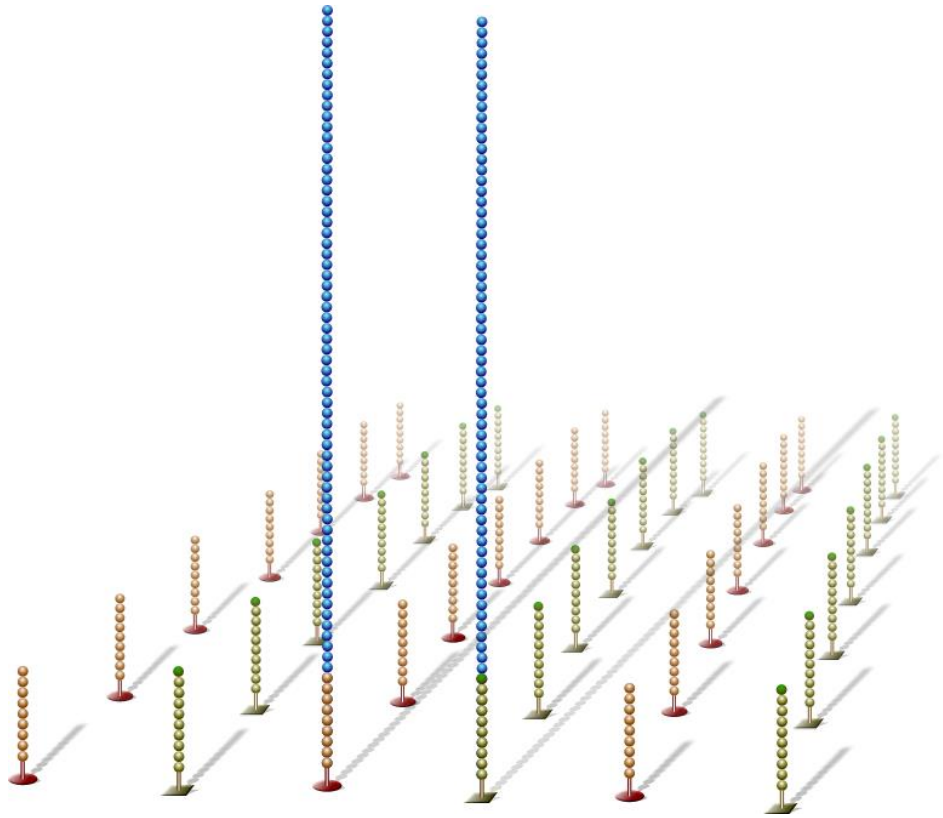
Cluster Formation

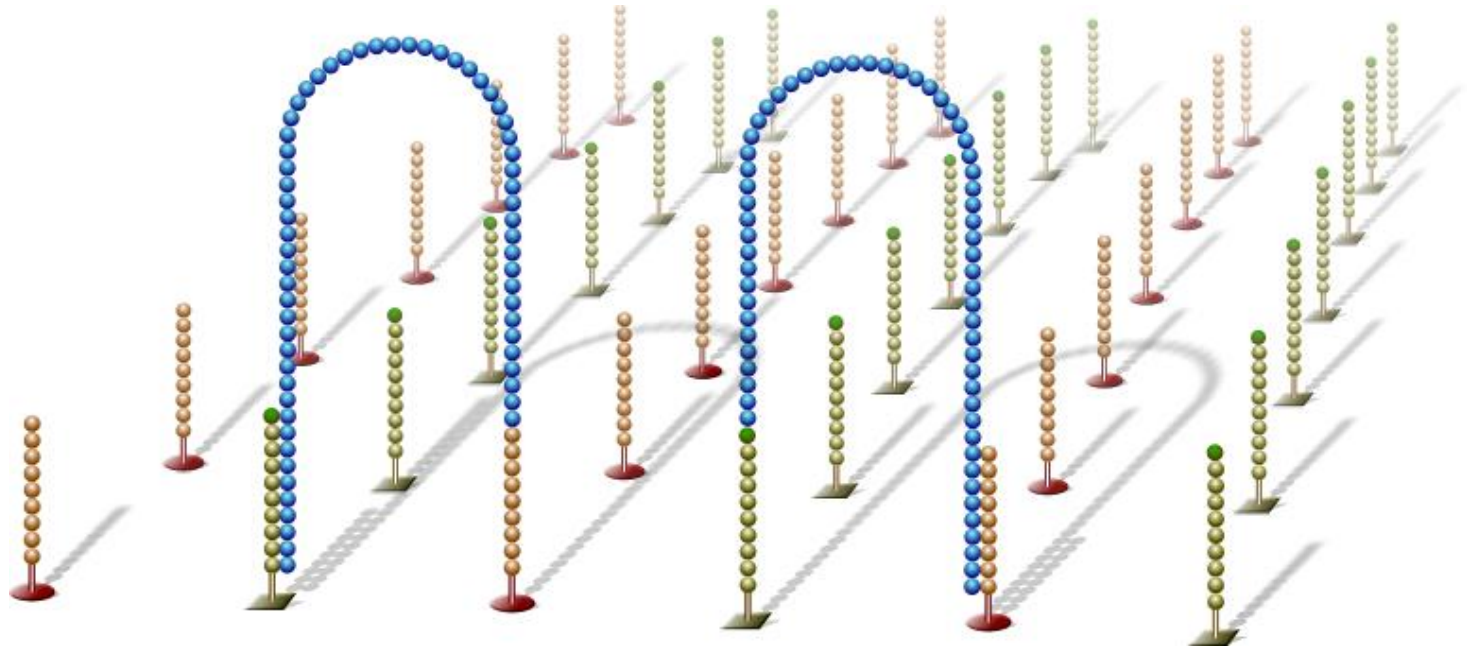
Linearisierung



© Illumina

Cluster Formation

Bridge Amplification



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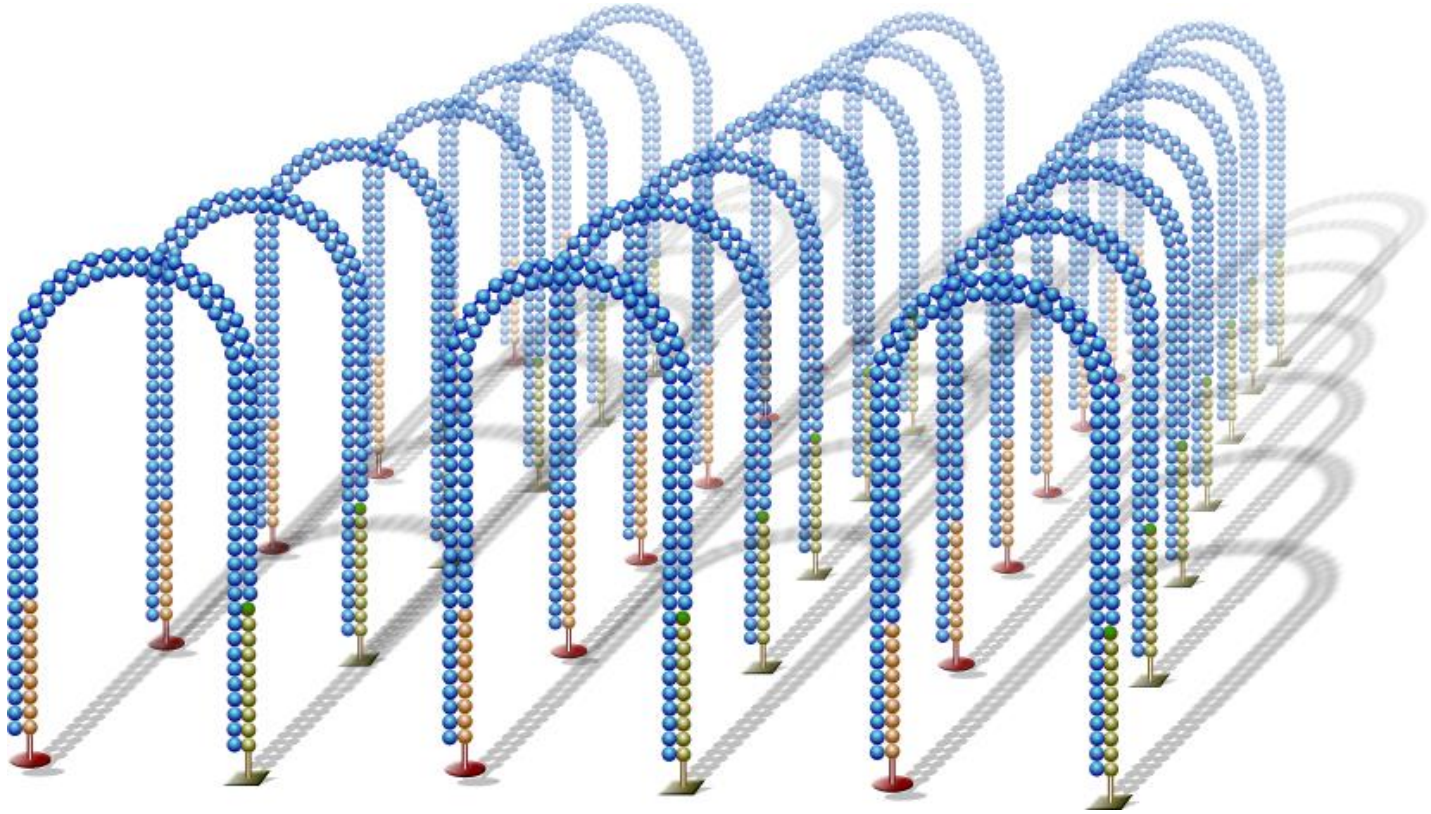
Cluster Formation

Bridge Amplification



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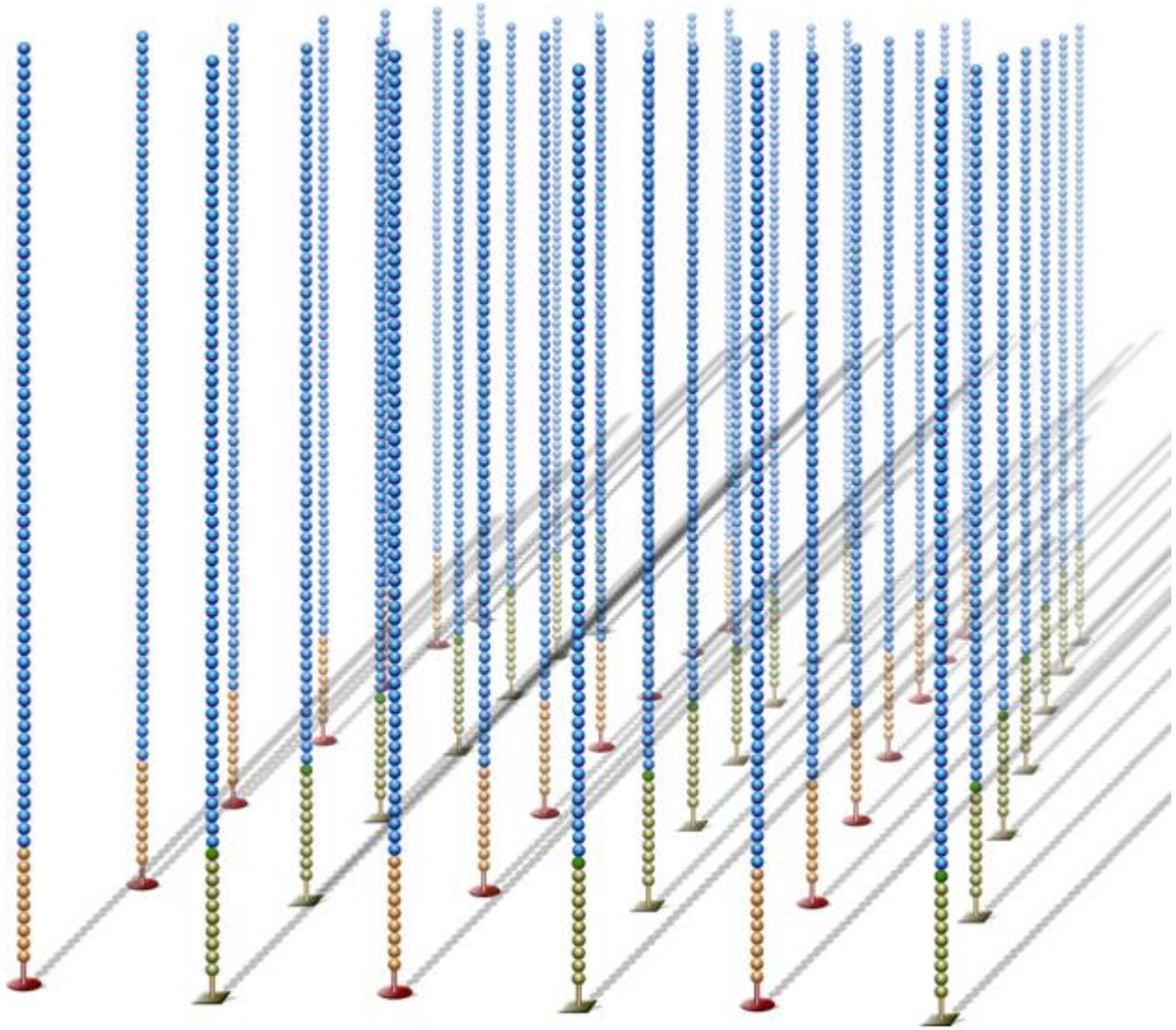
Cluster Formation

Linearisierung

Verwerfen der

R-Stränge



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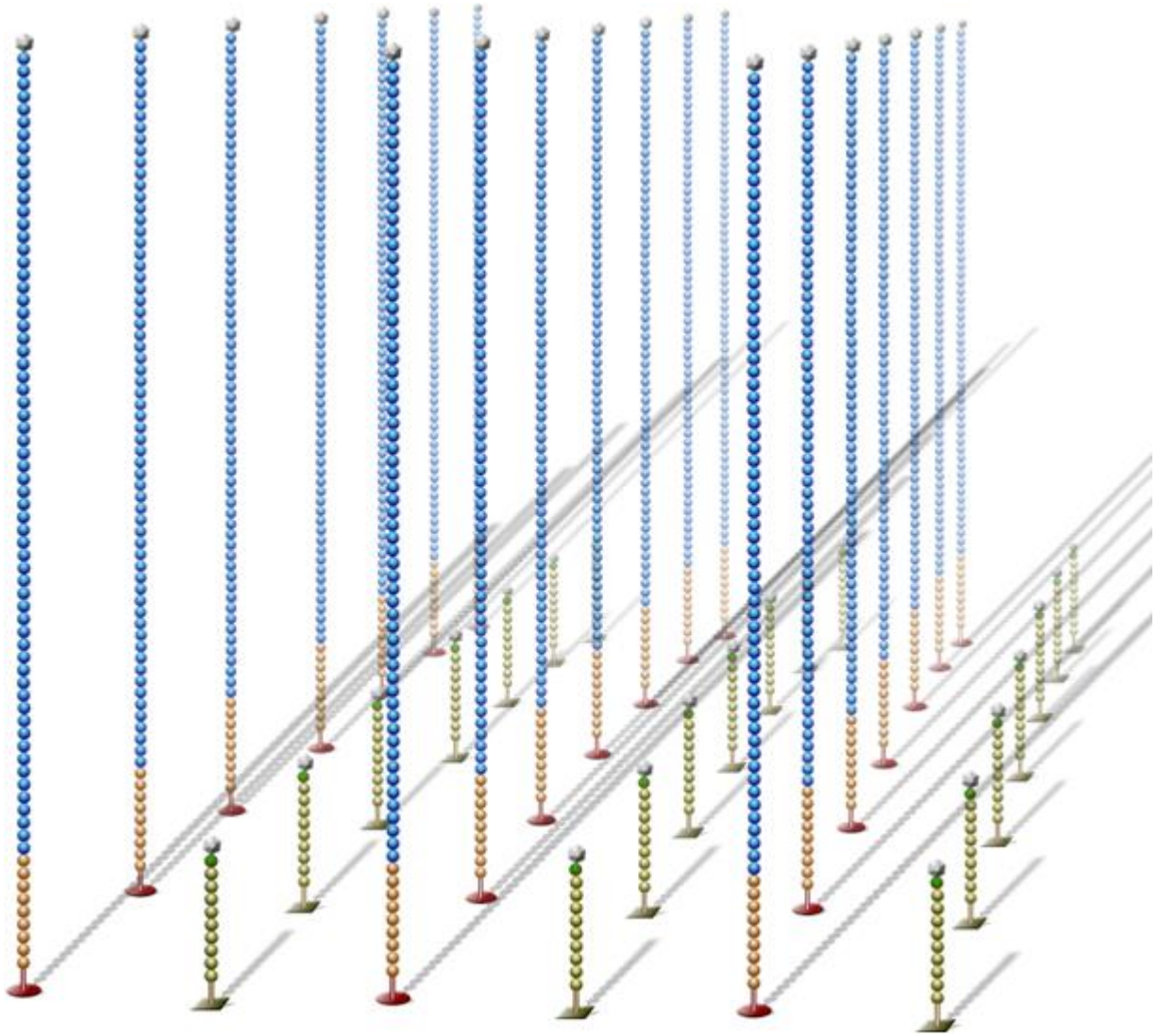
Cluster Formation

freie 3´Enden
werden geblockt



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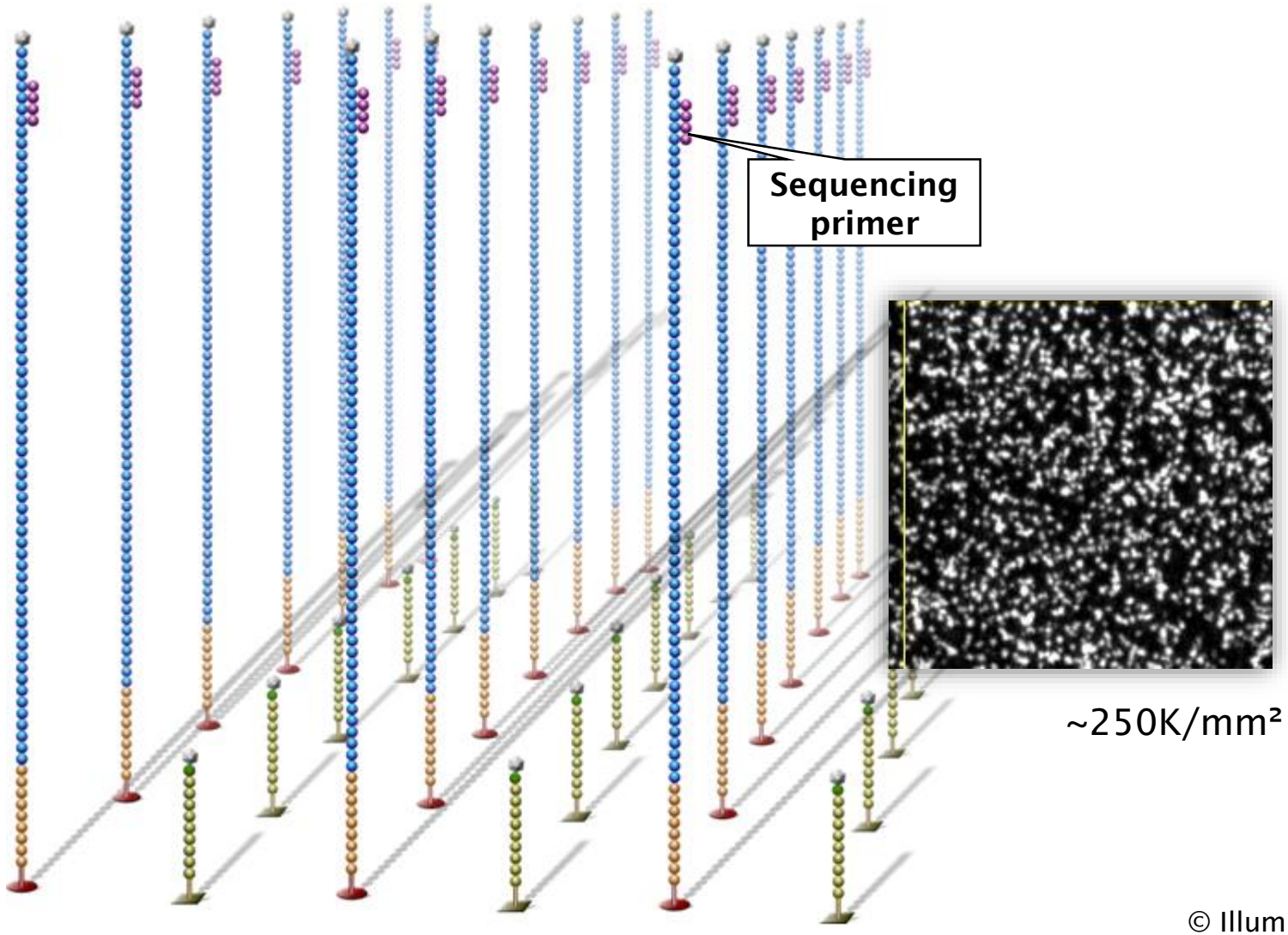
Cluster Formation

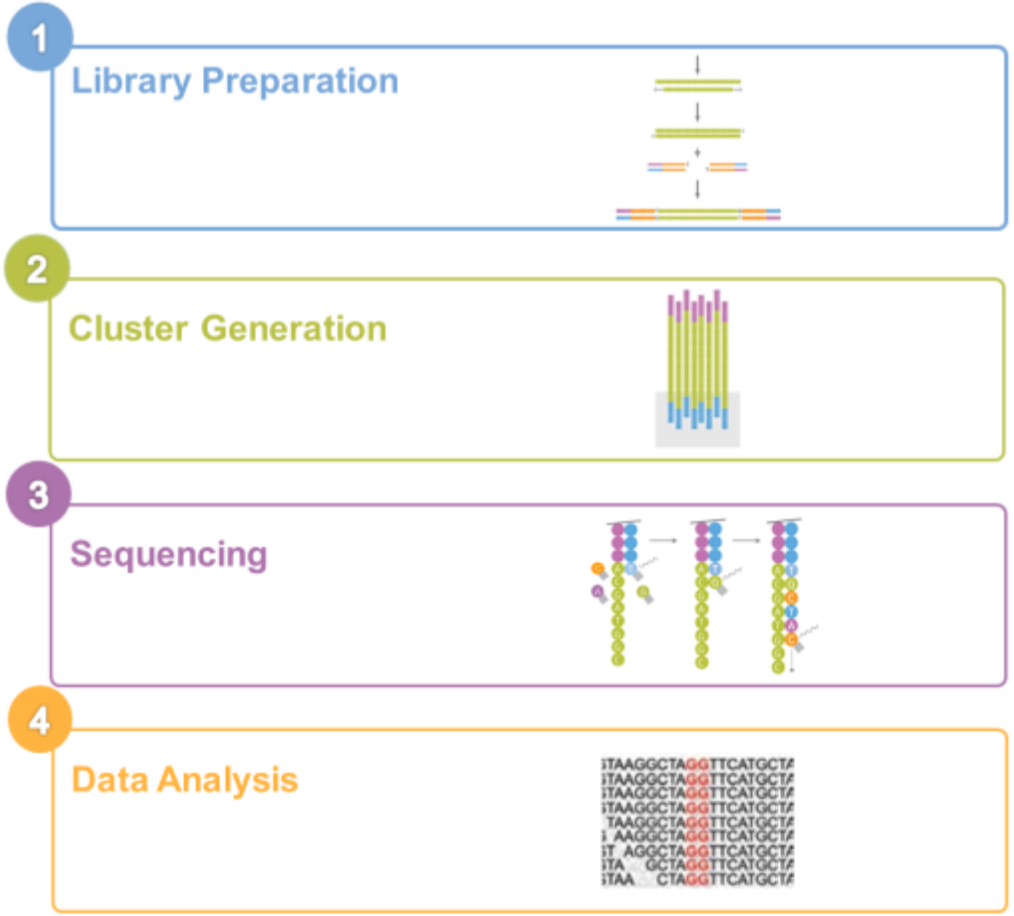
Primer Hybridisierung



~250K/mm²

© Illumina

Illumina Sequencing Workflow



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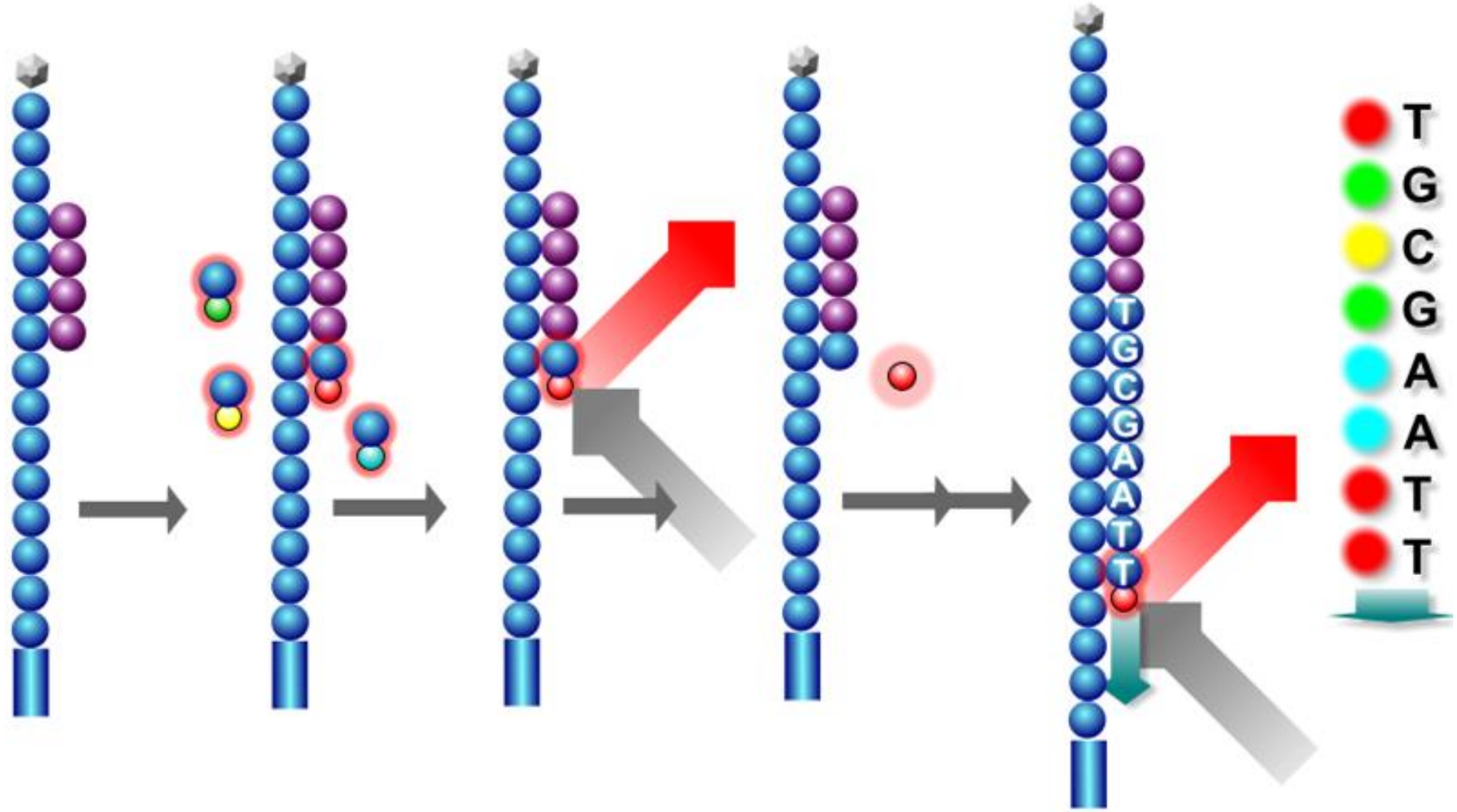
Sequencing by Synthesis



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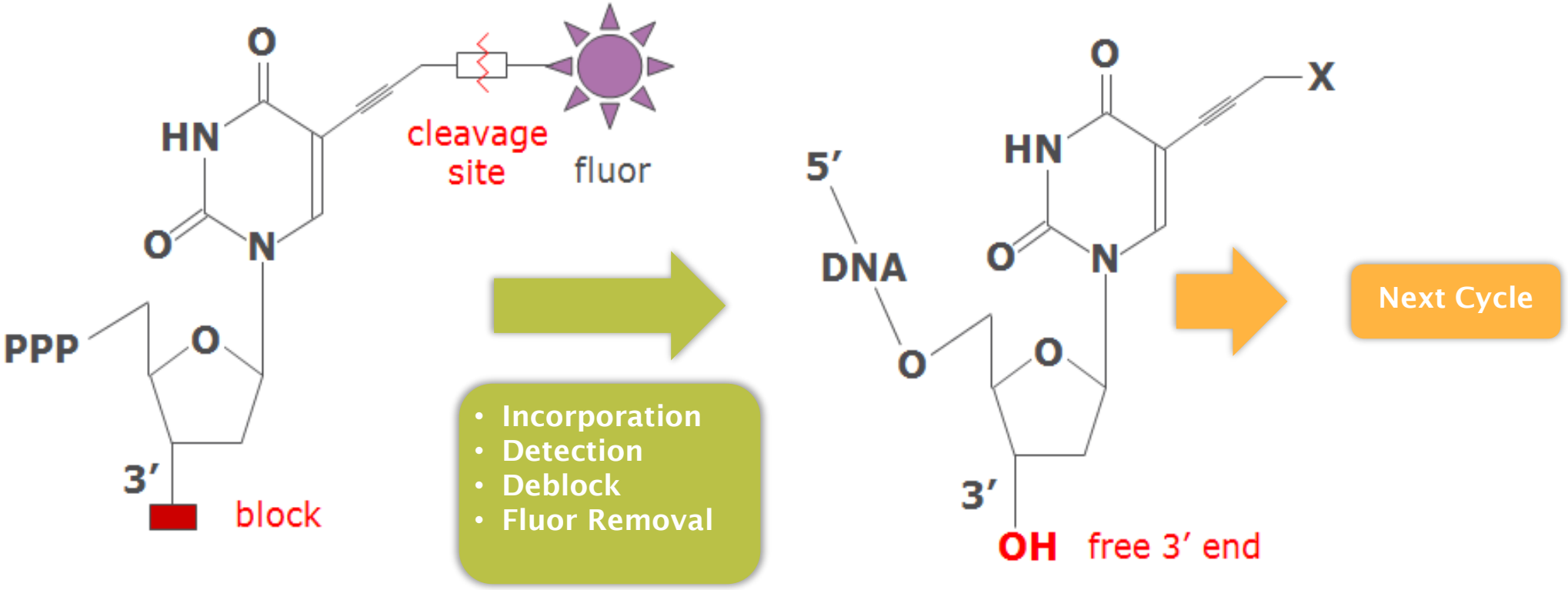
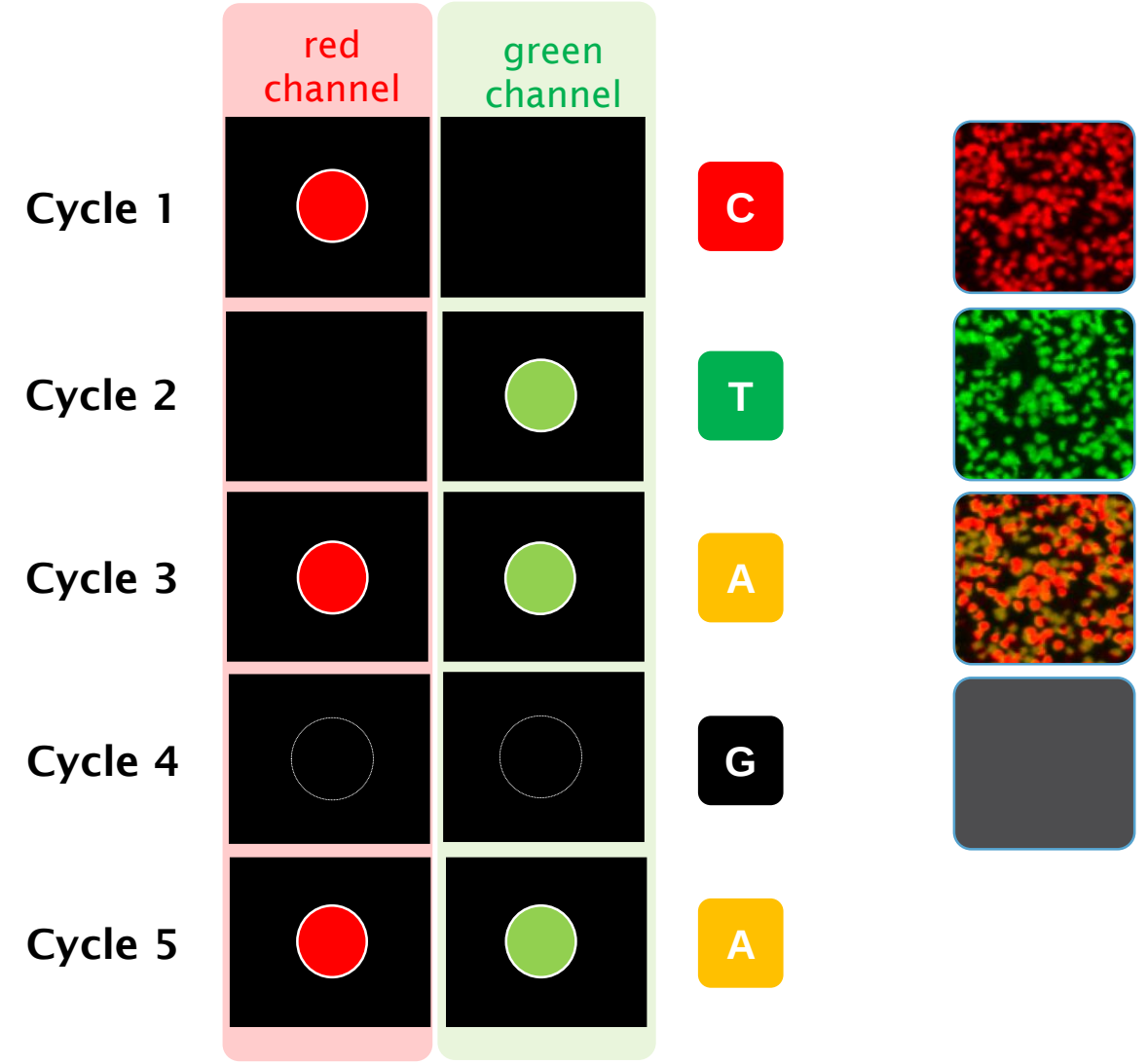
Sequencing by Synthesis



Sequencing by Synthesis

MiniSeq – 2 Kanal SBS



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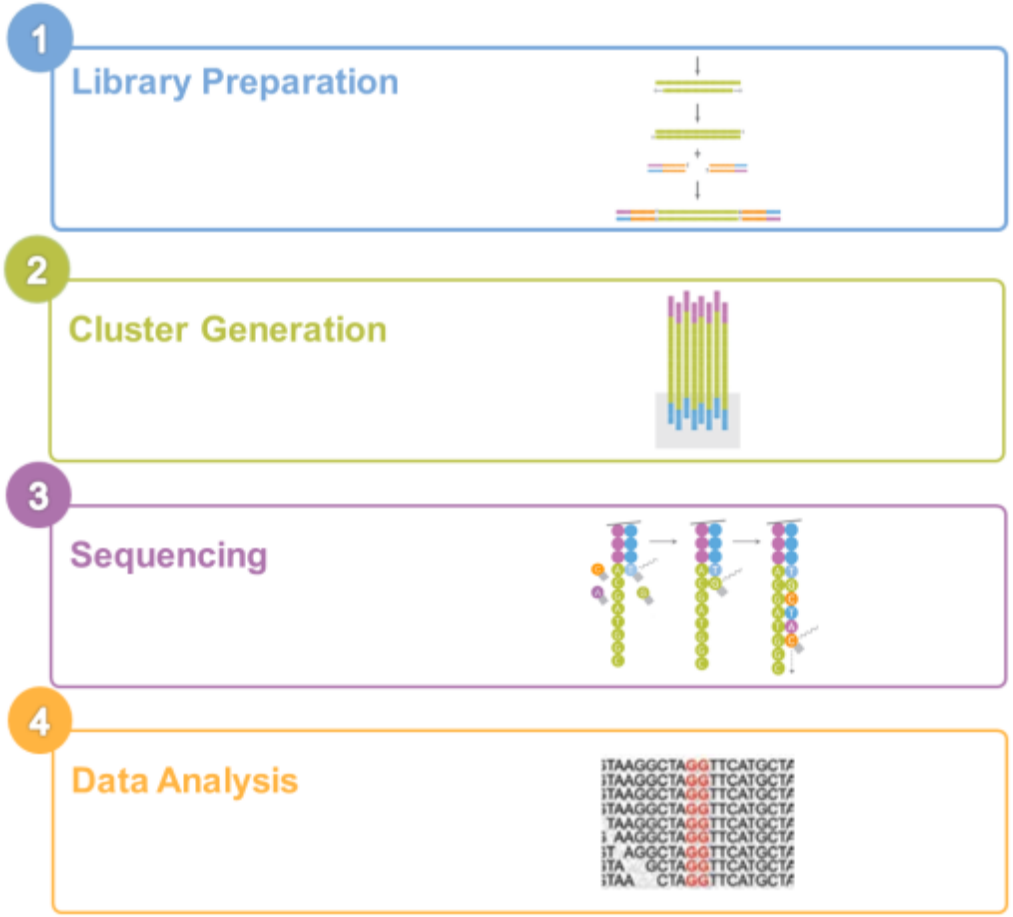
Illumina Sequencing Workflow



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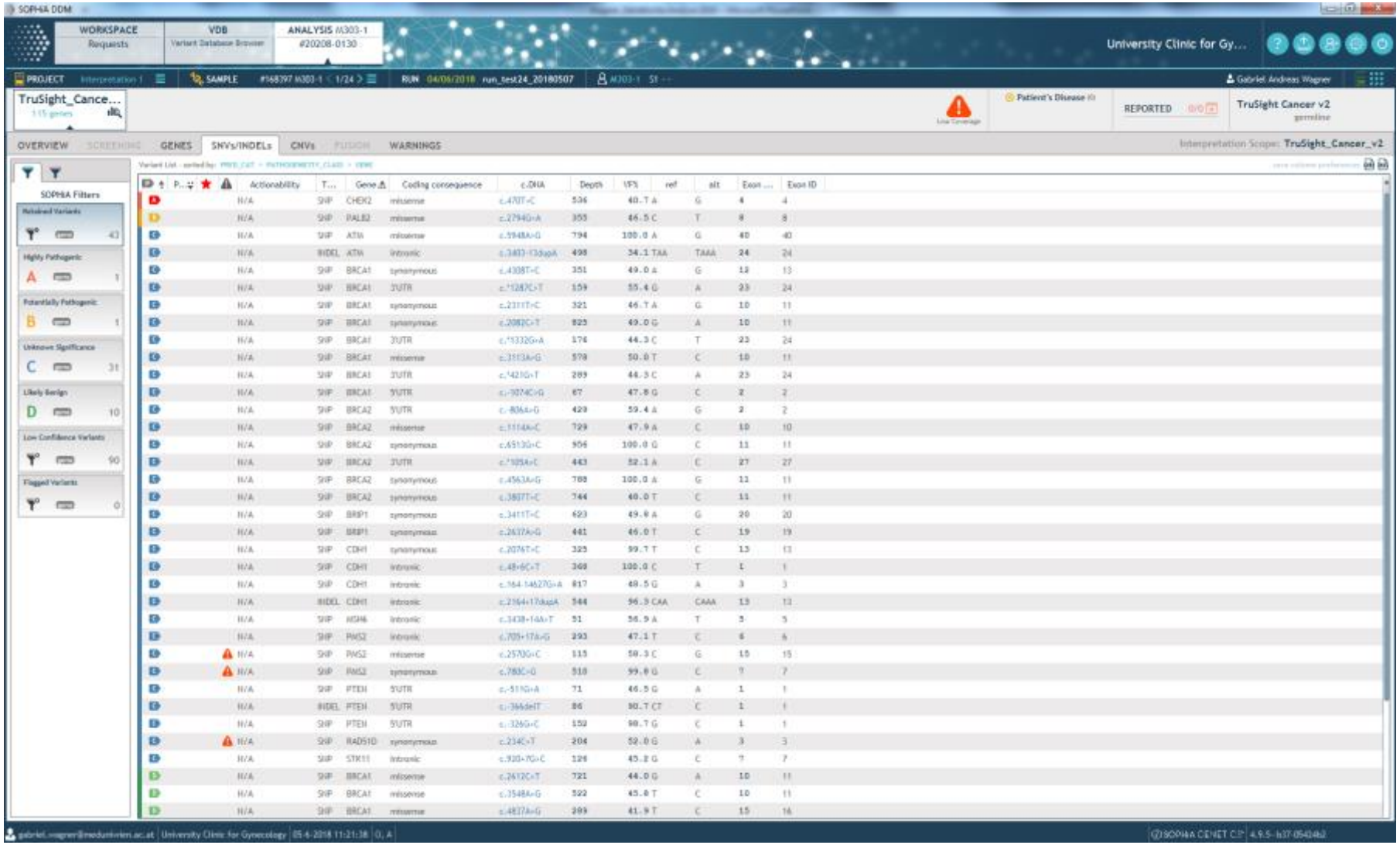
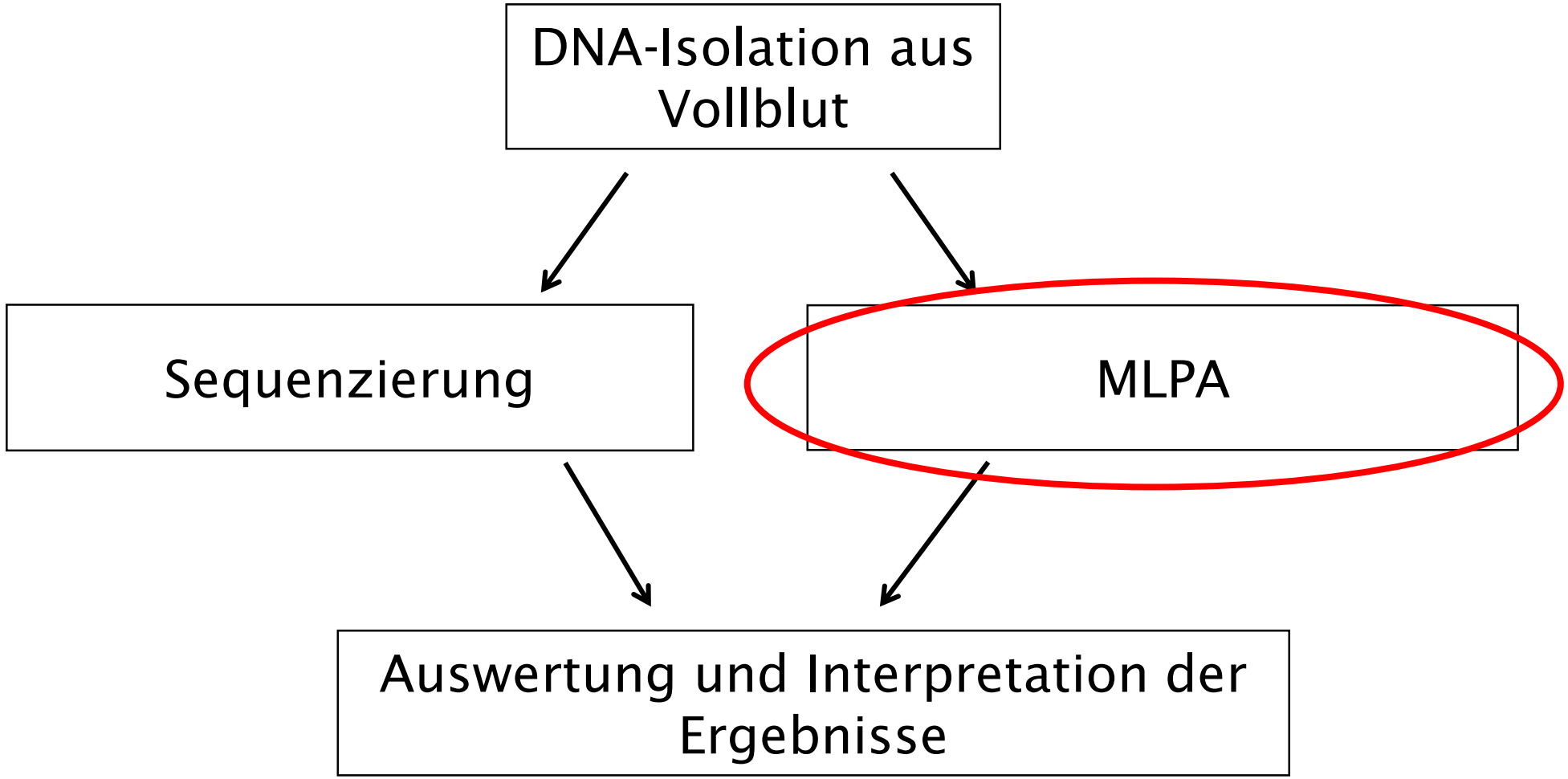
Data Analysis – Sophia DDM



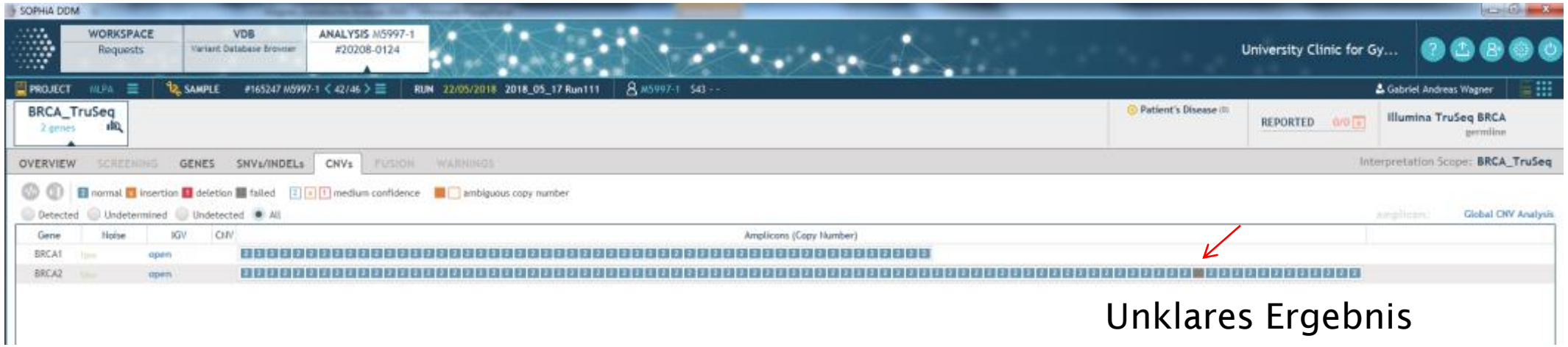
Genetische Analyse

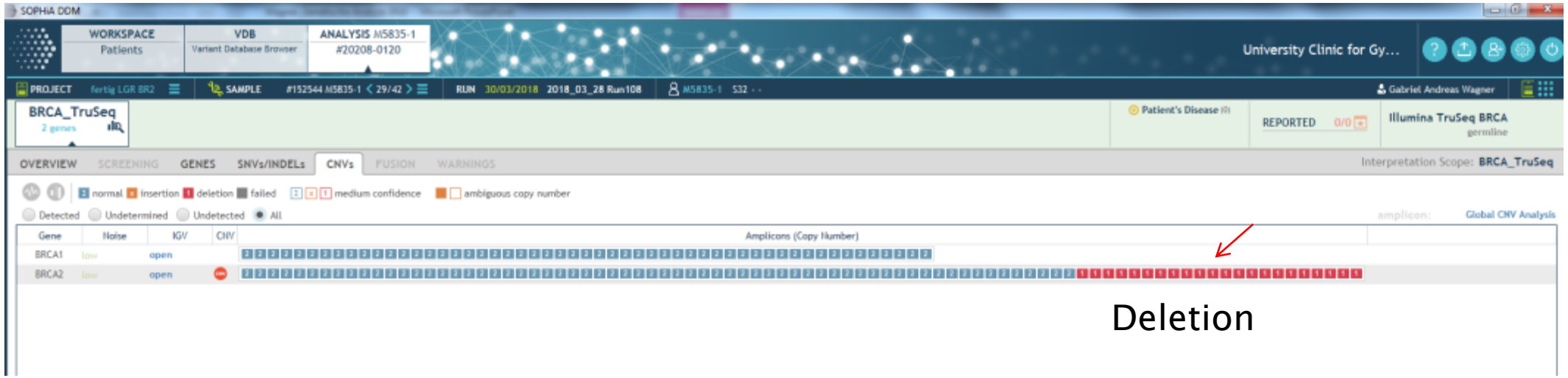


MLPA

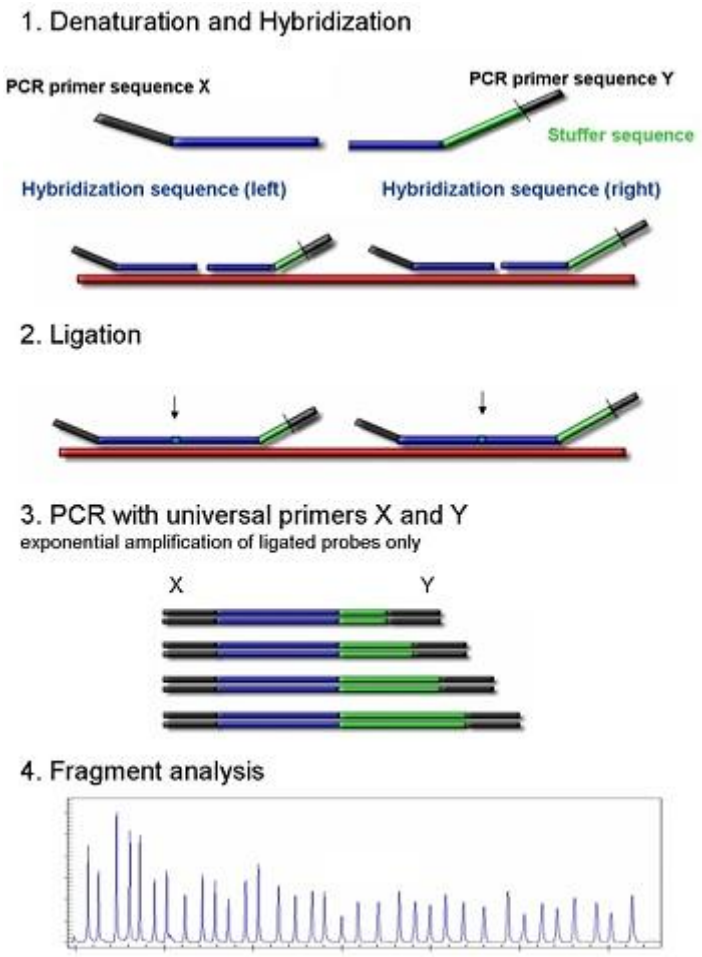
- Multiplex Ligation-Dependent Probe Amplification
- Nur zur Ergänzung bzw. Bestätigung von Ergebnissen
- Zur Feststellung von Deletionen oder Multiplikationen von ganzen Exons – CNVs (Copy Number Variants)/LGRs (Large Genomic Rearrangements)

Data Analysis – Sophia DDM





MLPA



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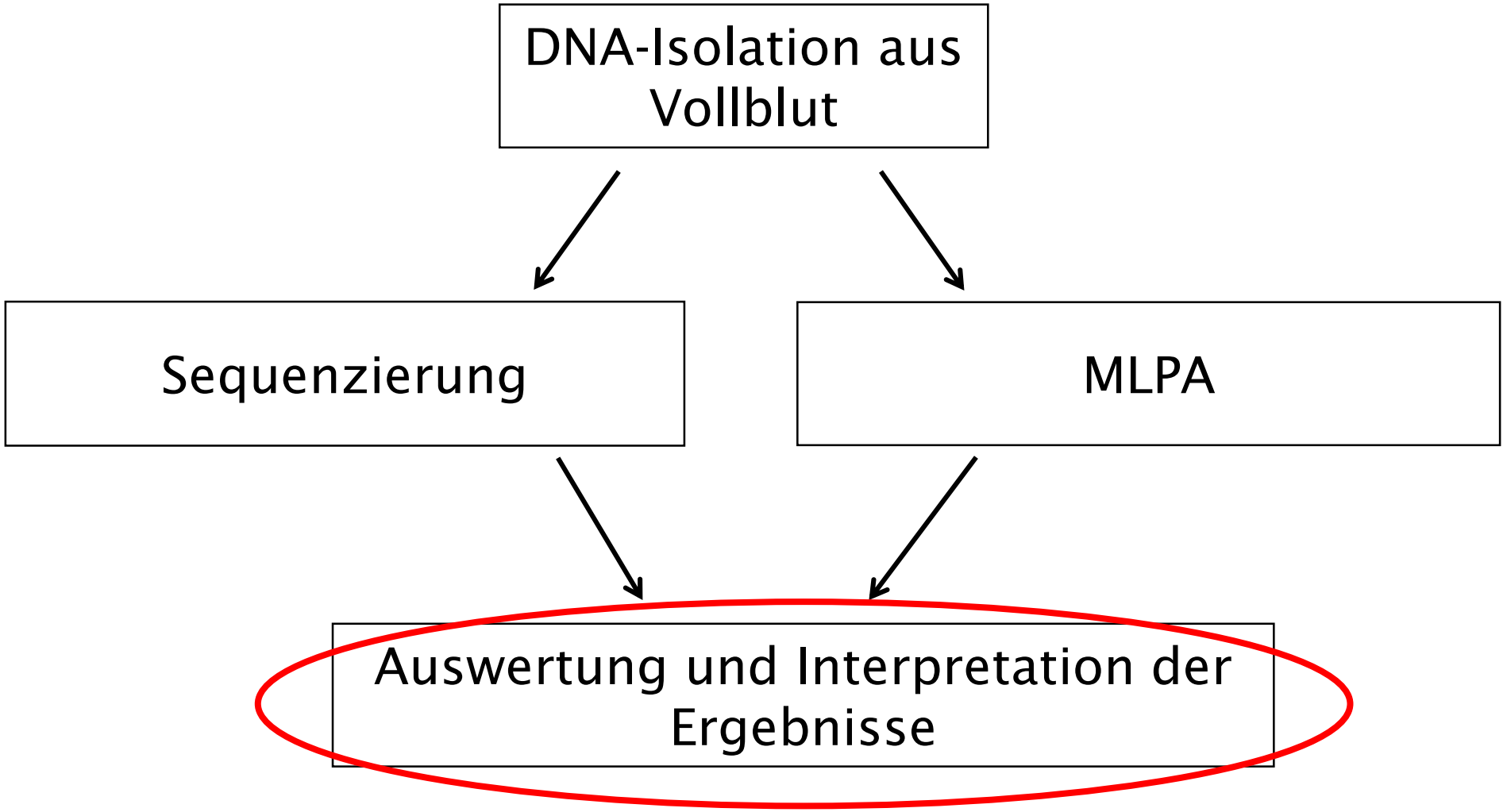
Genetische Analyse



Interpretation der Veränderung

Klasse	Veränderung ist	Proteinfunktion	Klinische Relevanz	Risiko
1	Polymorphismus	nicht beeinträchtigt	nein	Normalbevölkerung
2				
3	unklassifizierte Variante	unbekannt	unbekannt	unbekannt
4	Mutation	beeinträchtigt	ja	BRCA1: 85% BC 53% OC BRCA2: 84% BC 27% OC
5				

Befunde

- Polymorphismen der Klasse 1 & 2 werden nicht gelistet
- UVs werden nur für BRCA1 & 2 berichtet
- Primer für Mutationsbestätigungen werden erst bei Bedarf bestellt
- Alle ein bis zwei Jahre wird eine Reklassifizierung durchgeführt

Vielen Dank für die Aufmerksamkeit