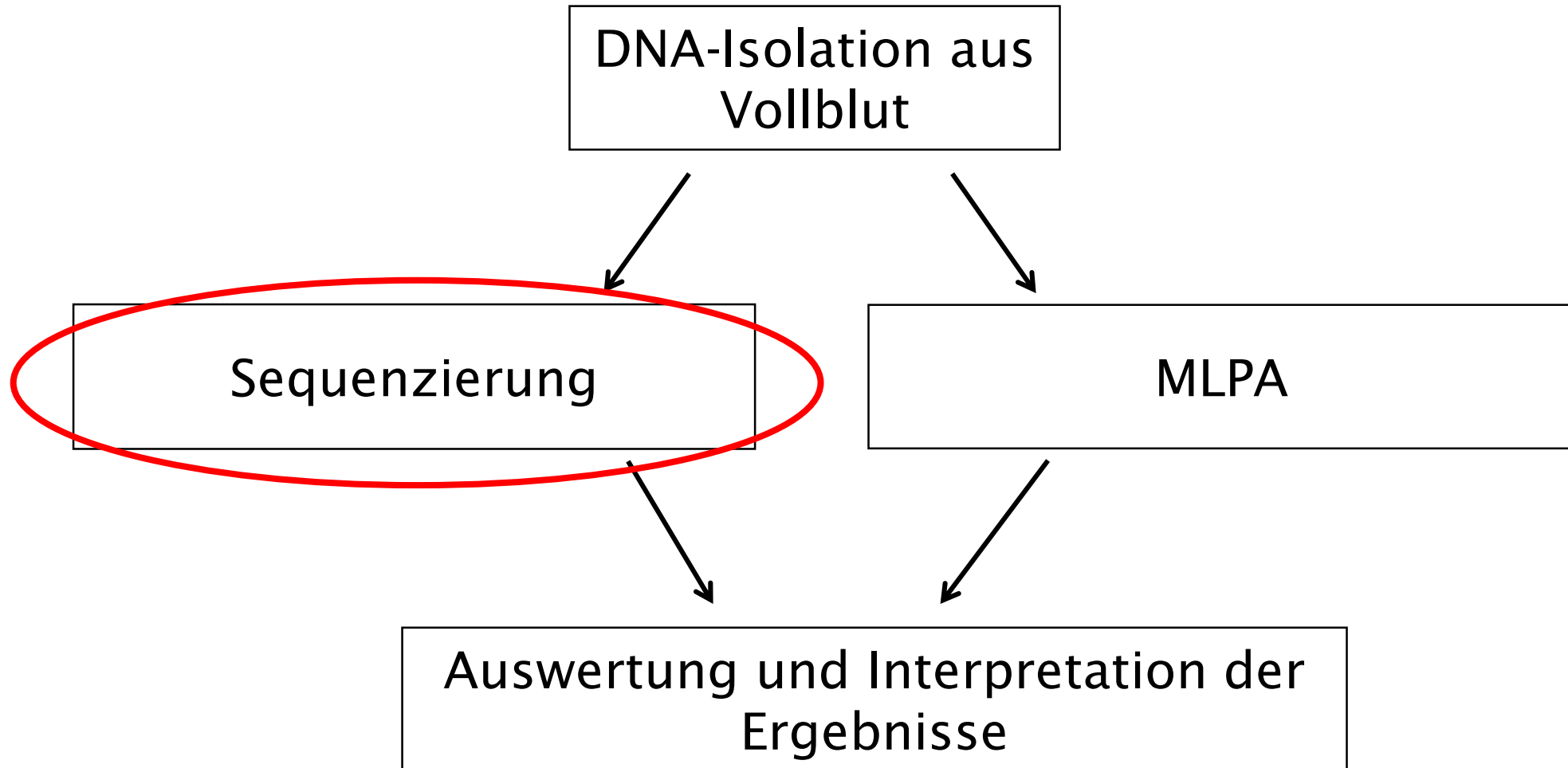


# Genetische Analyse

Gabriel Wagner, PhD

Oncolab Diagnostics GmbH

# Genetische Analyse

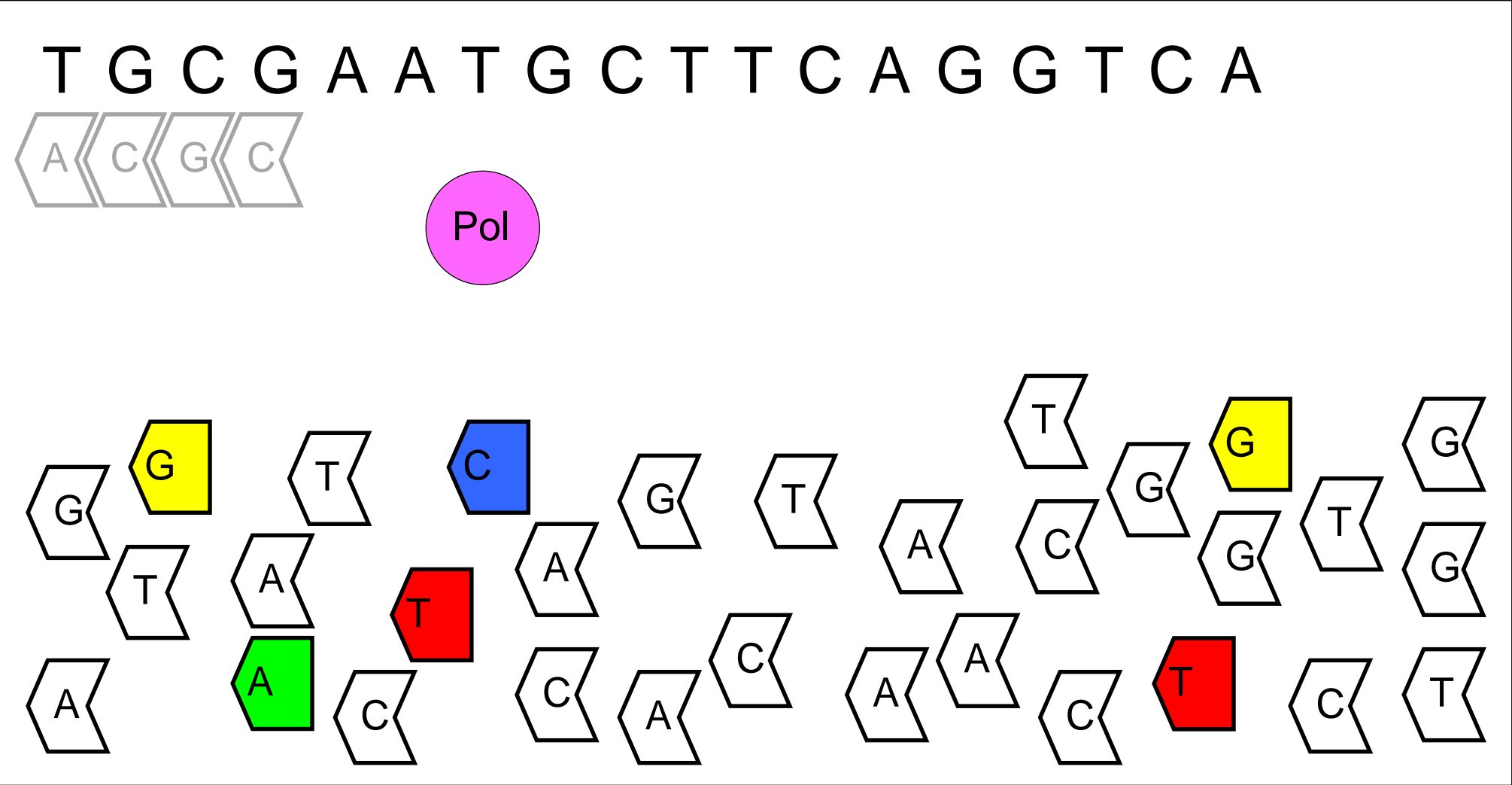


# Kapillar-/Sangersequenzierung

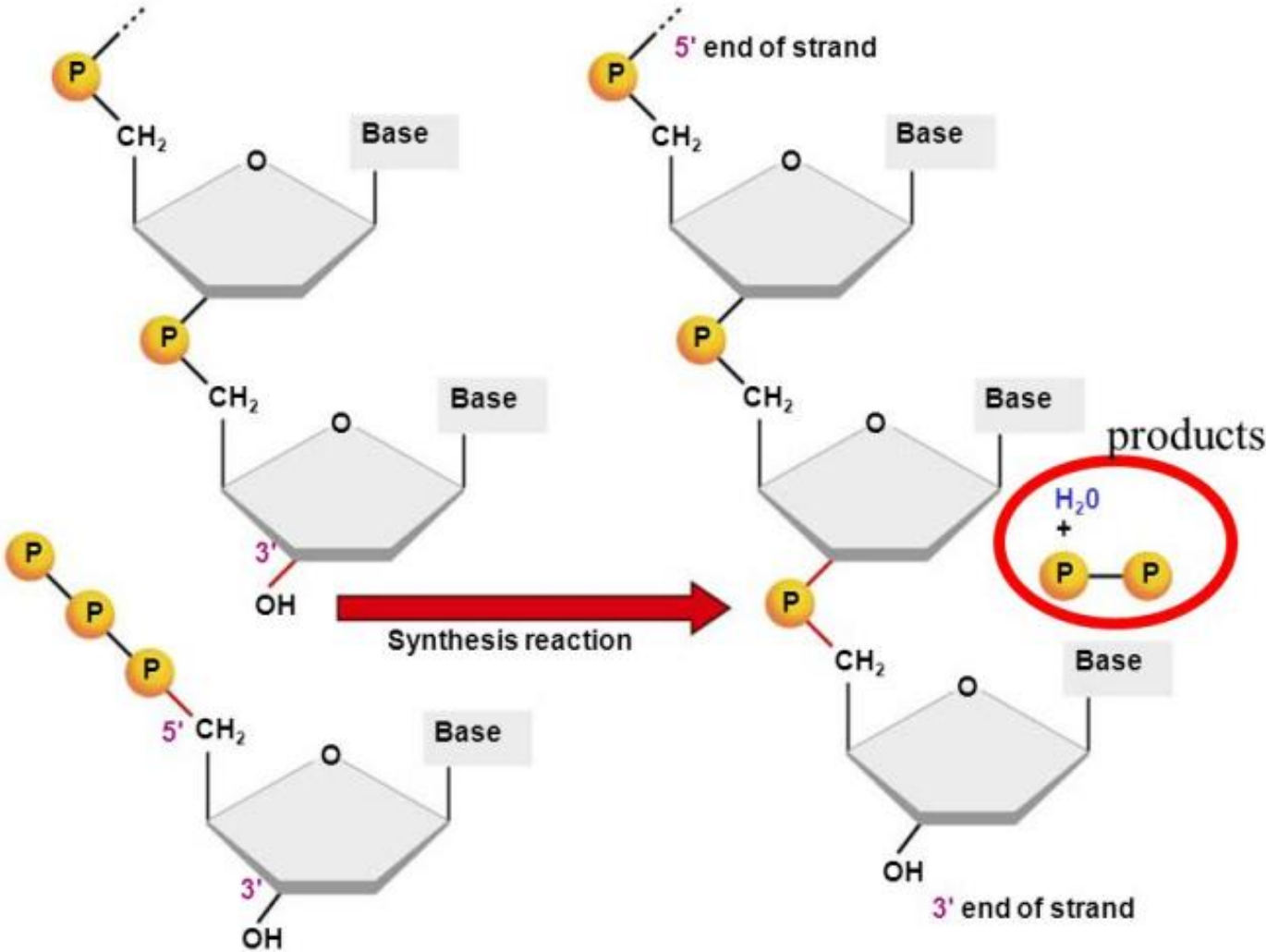


- Frederick Sanger
- Britischer Biochemiker
- Zweifacher Nobelpreisträger
  - 1958 Proteinstruktur von Insulin
  - 1980 Basensequenzierung

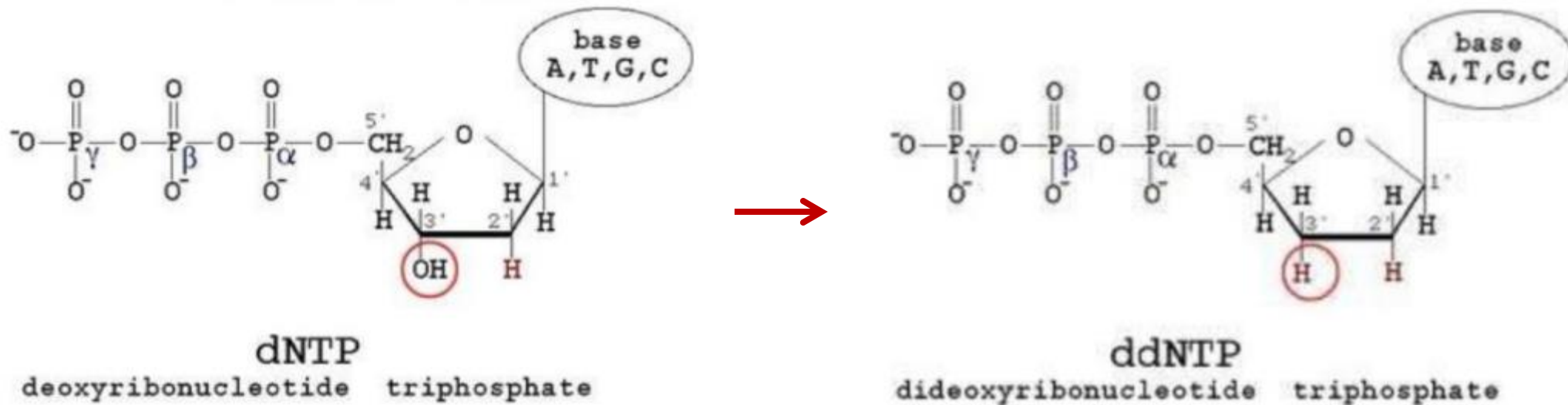
# Sequenzierung



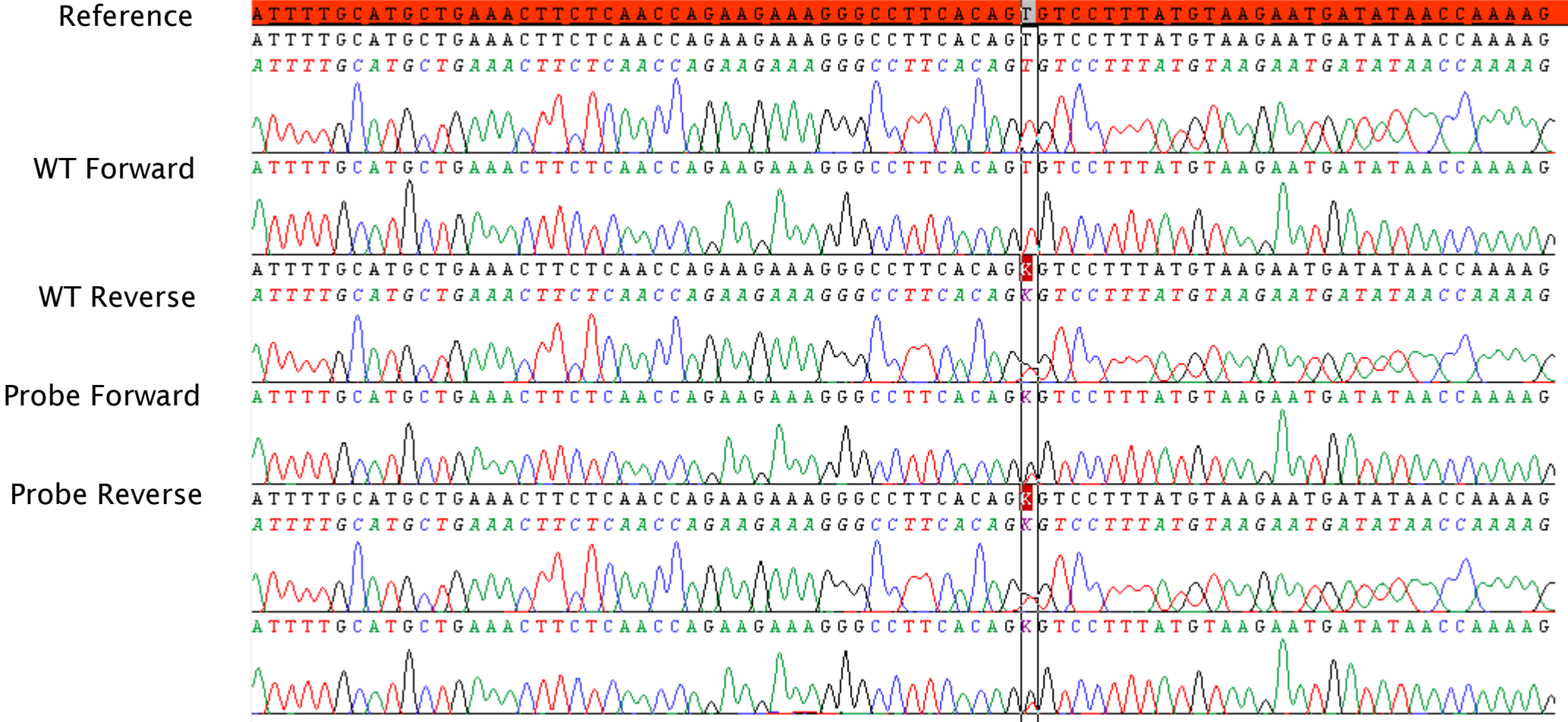
# Sequenzierung



# Sequenzierung



# 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

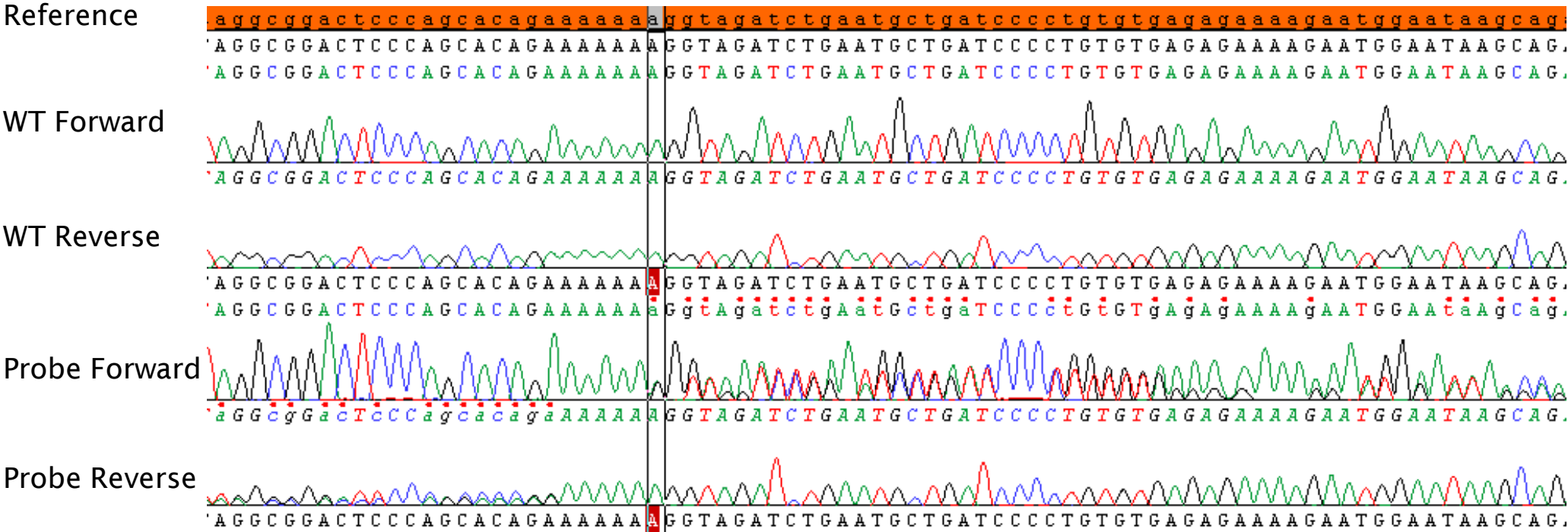
### 2. Nonsense Mutation

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**

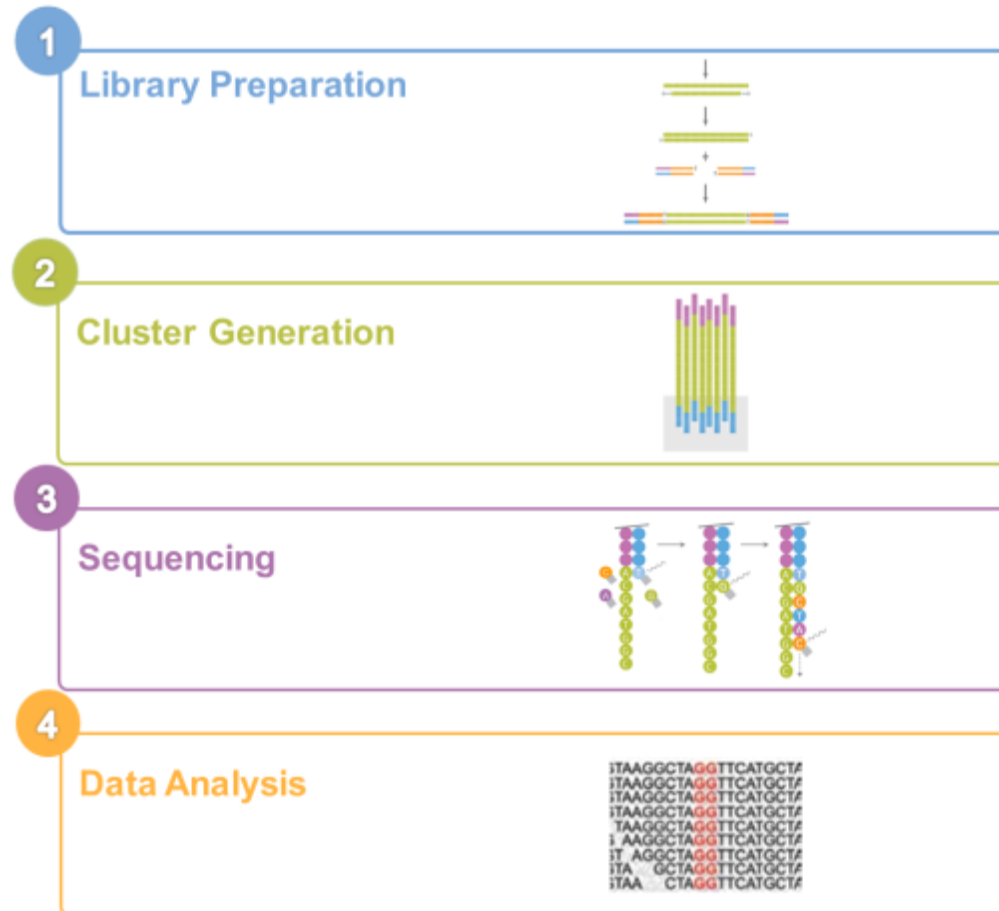
Leucin Serin **Lysin** **Prolin** **Stop**

# Next Generation Sequencing

## Illumina MiniSeq



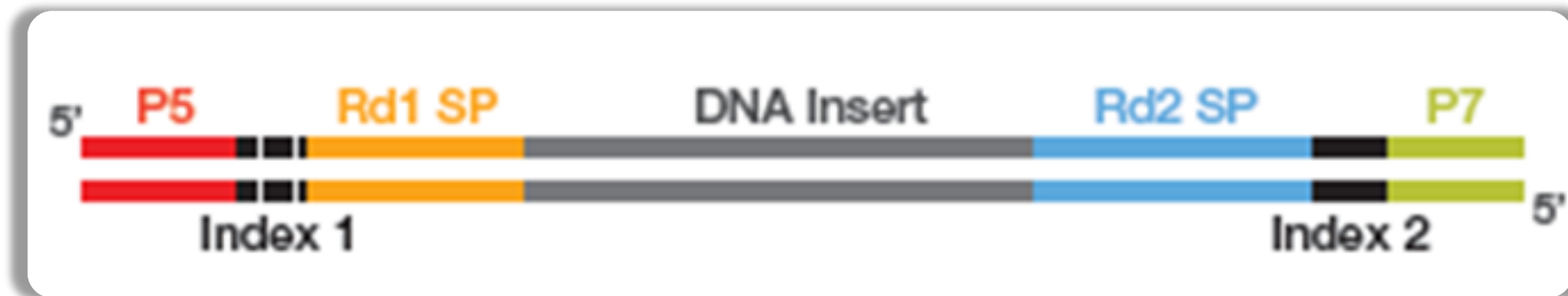
# Illumina Sequencing Workflow



# Library Preparation

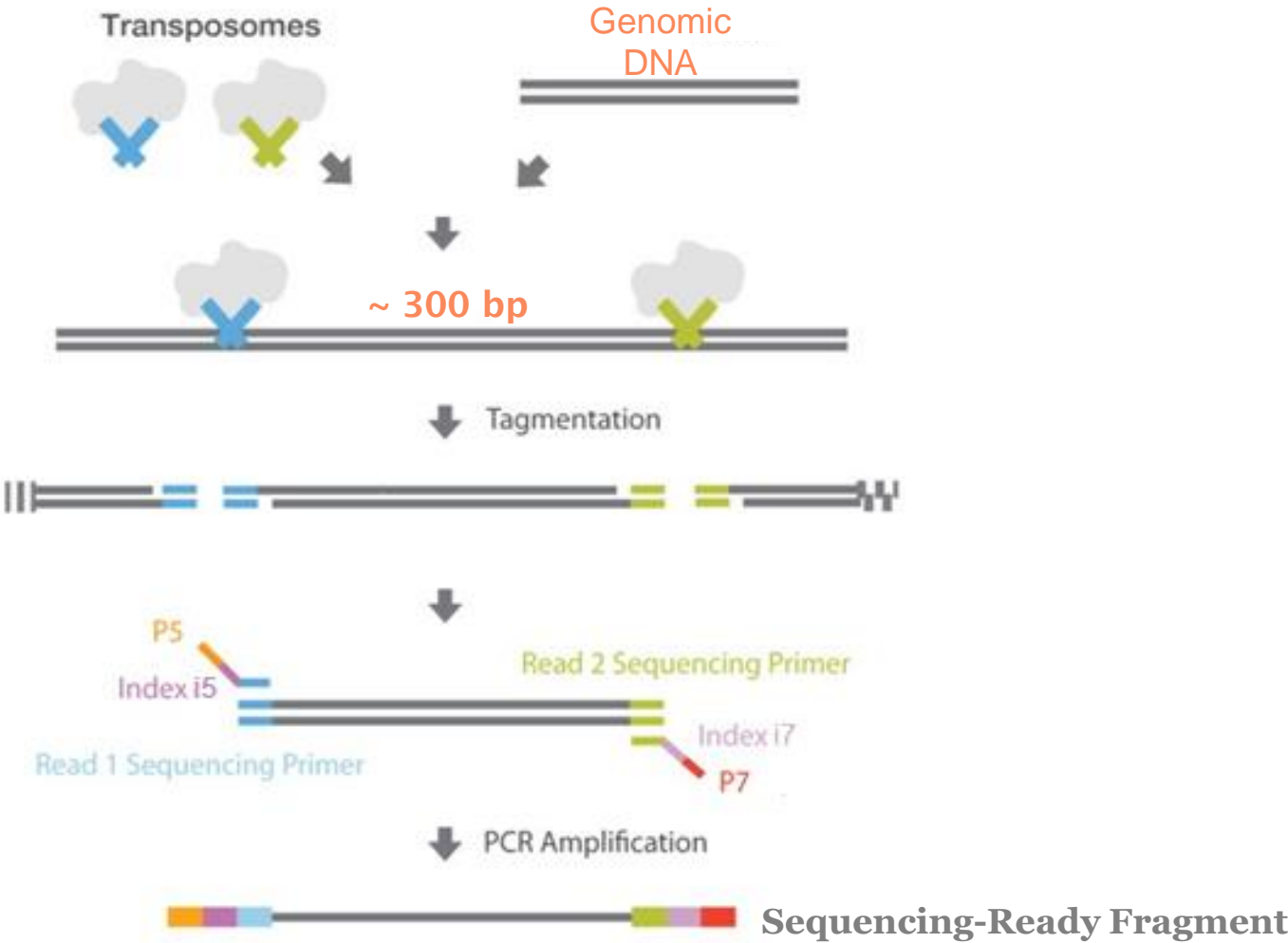
## Illumina TruSight Cancer V2 Panel

Probenvorbereitung bis die Proben so aussehen:

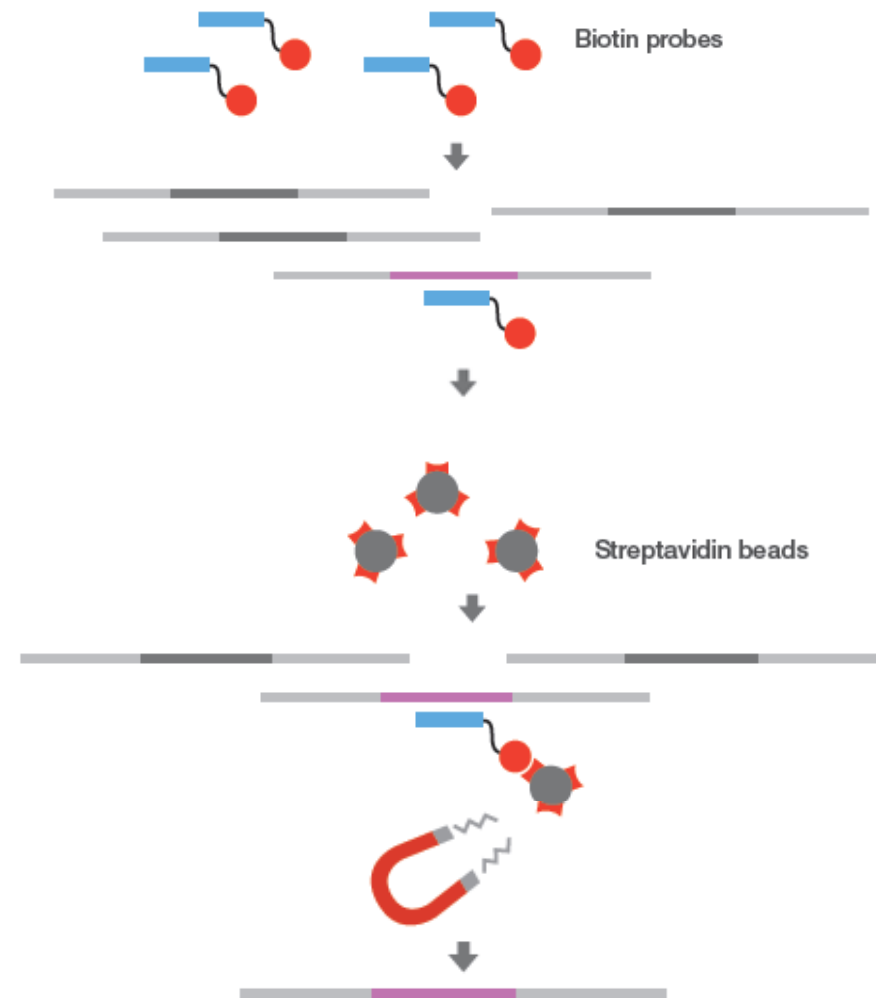


© Illumina

# Library Preparation

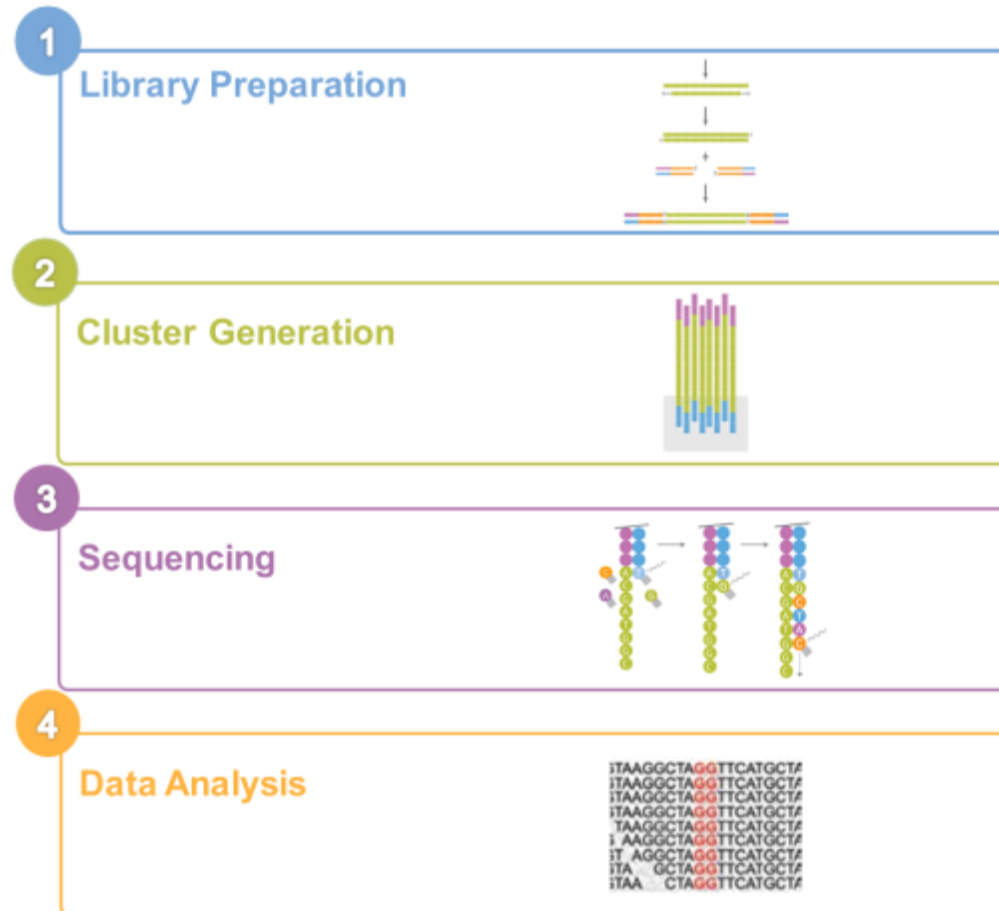


# Library Preparation – Target Enrichment



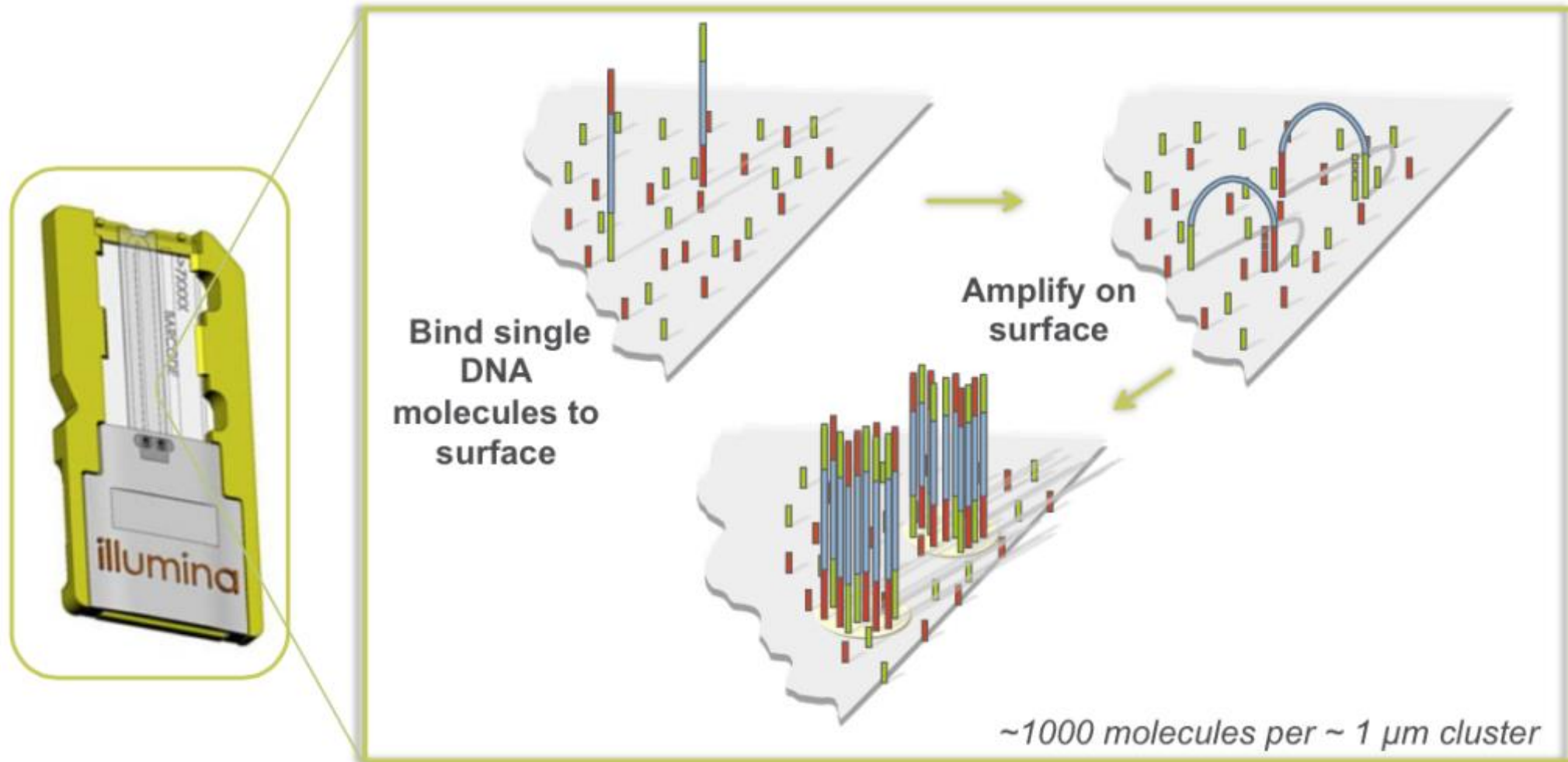
© Illumina

# Illumina Sequencing Workflow



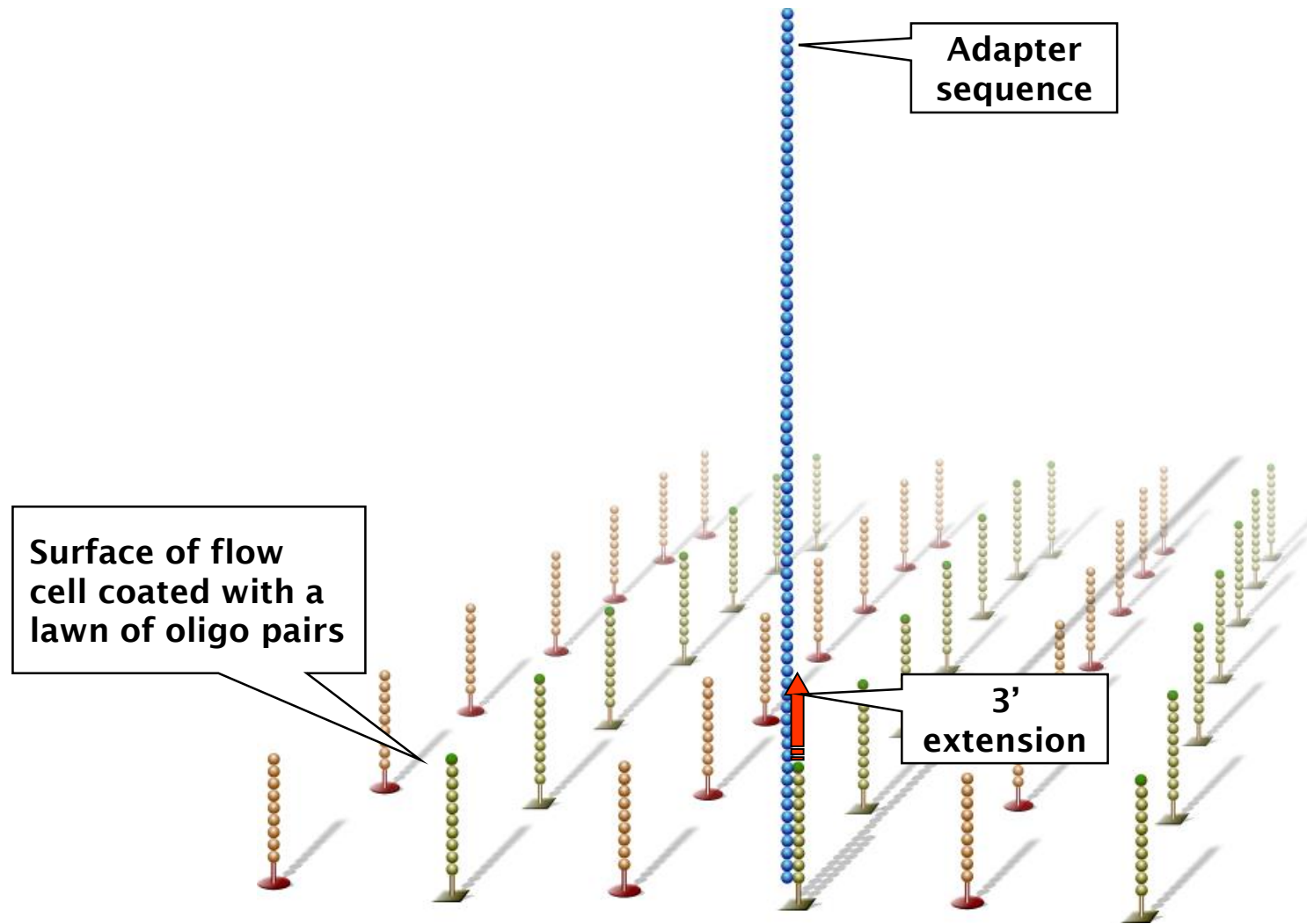


# Illumina MiniSeq - Technologie

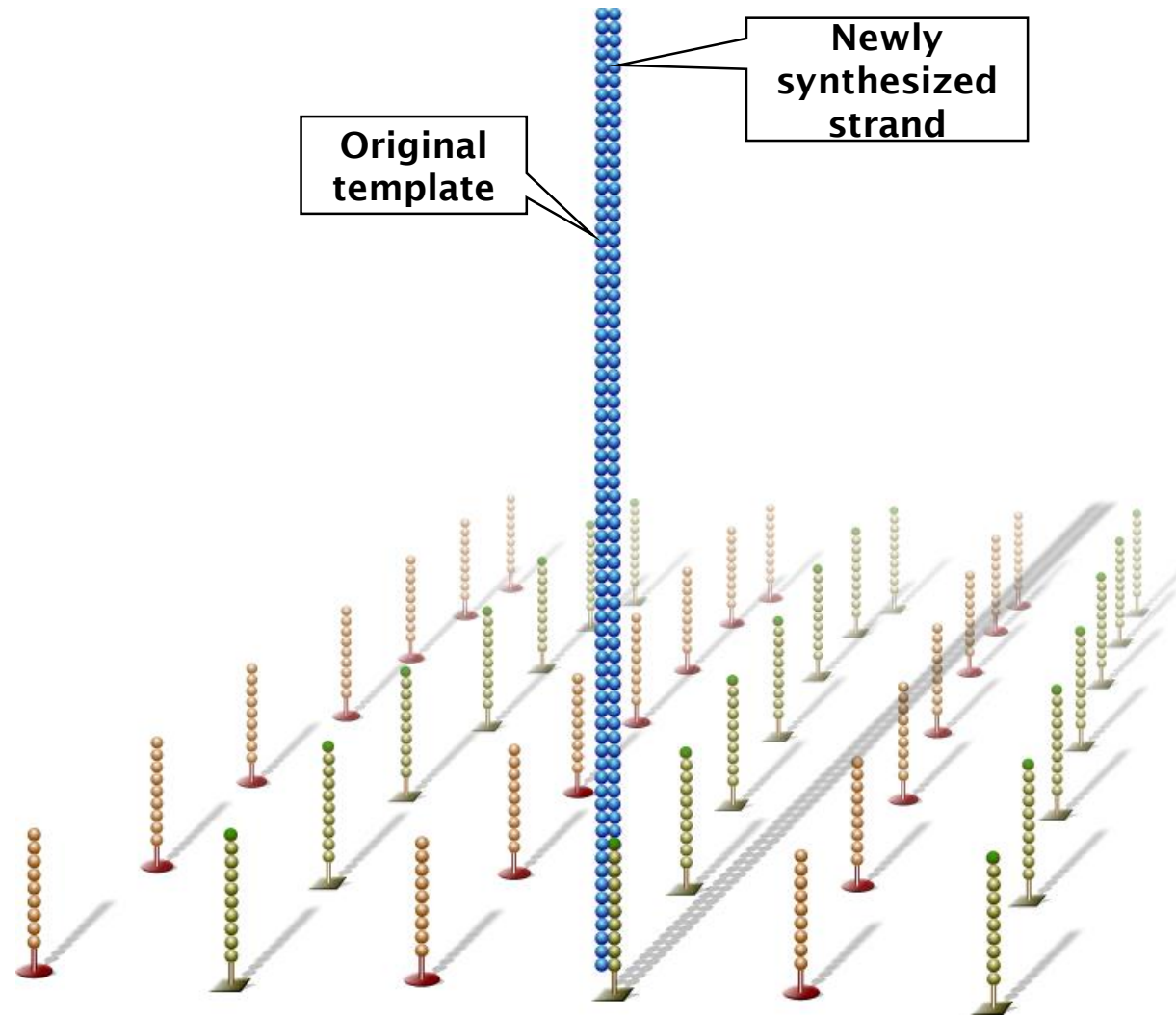


© Illumina

# Cluster Formation



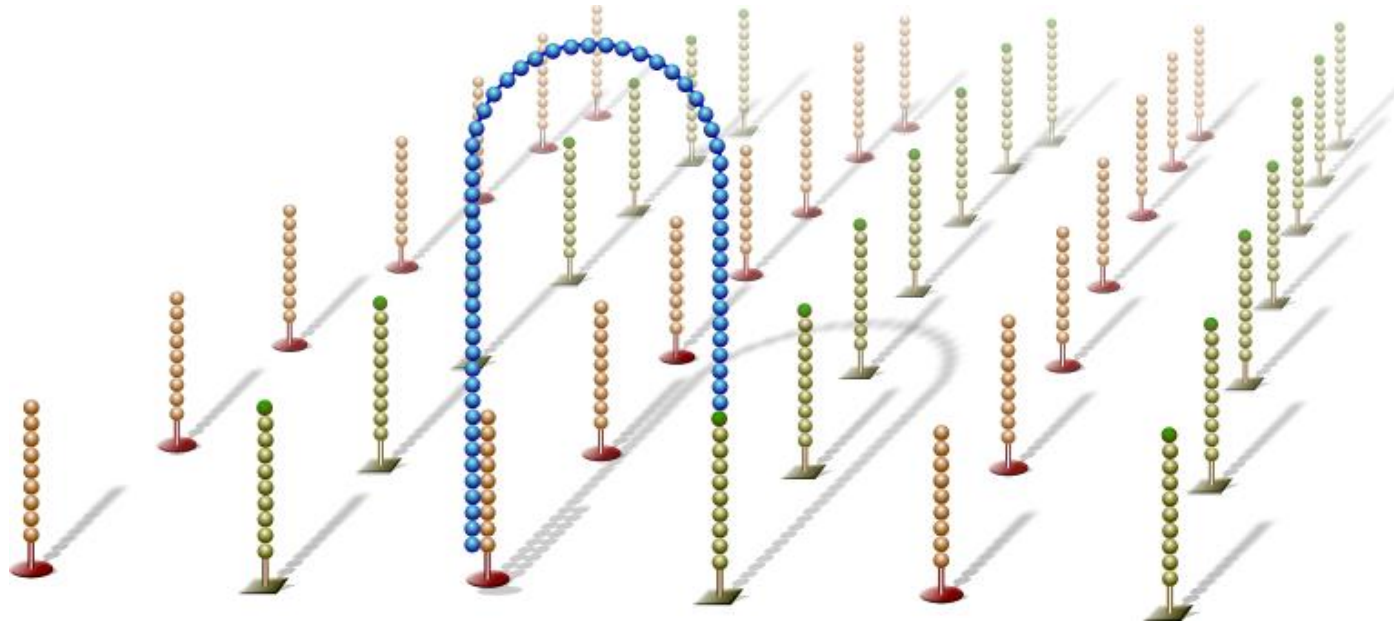
# Cluster Formation



© Illumina

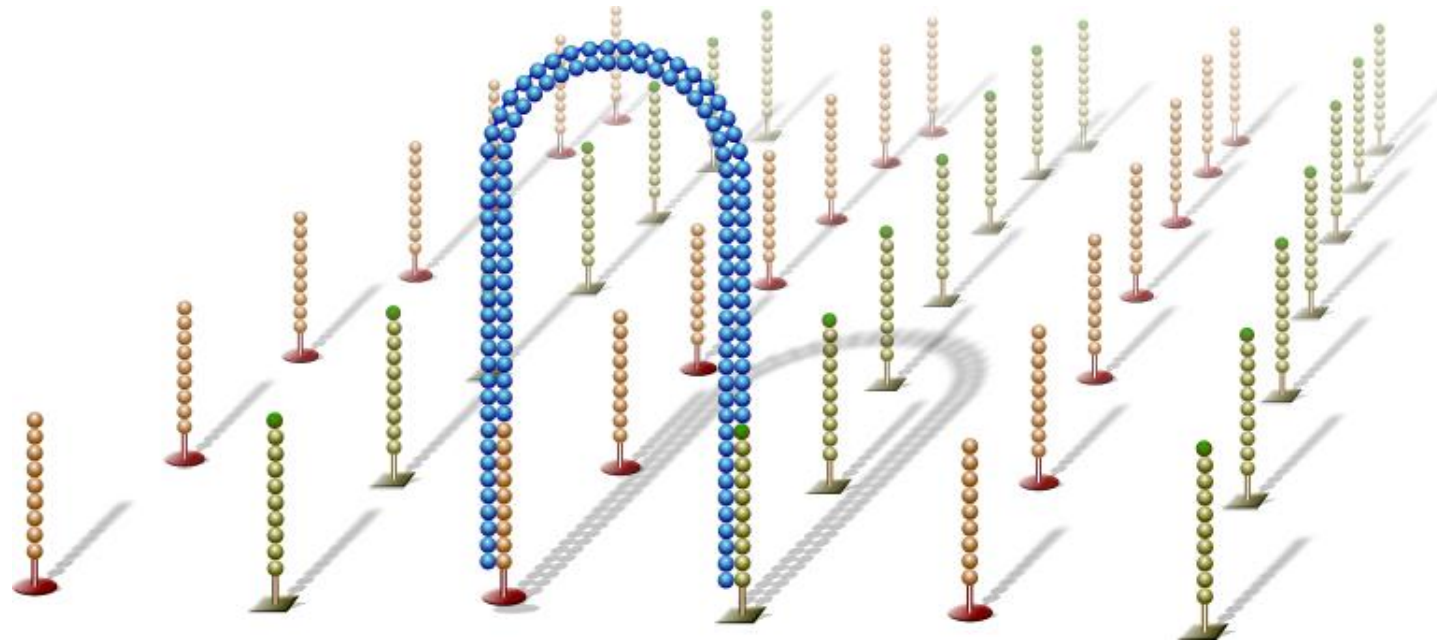
# Cluster Formation

## Bridge Amplification



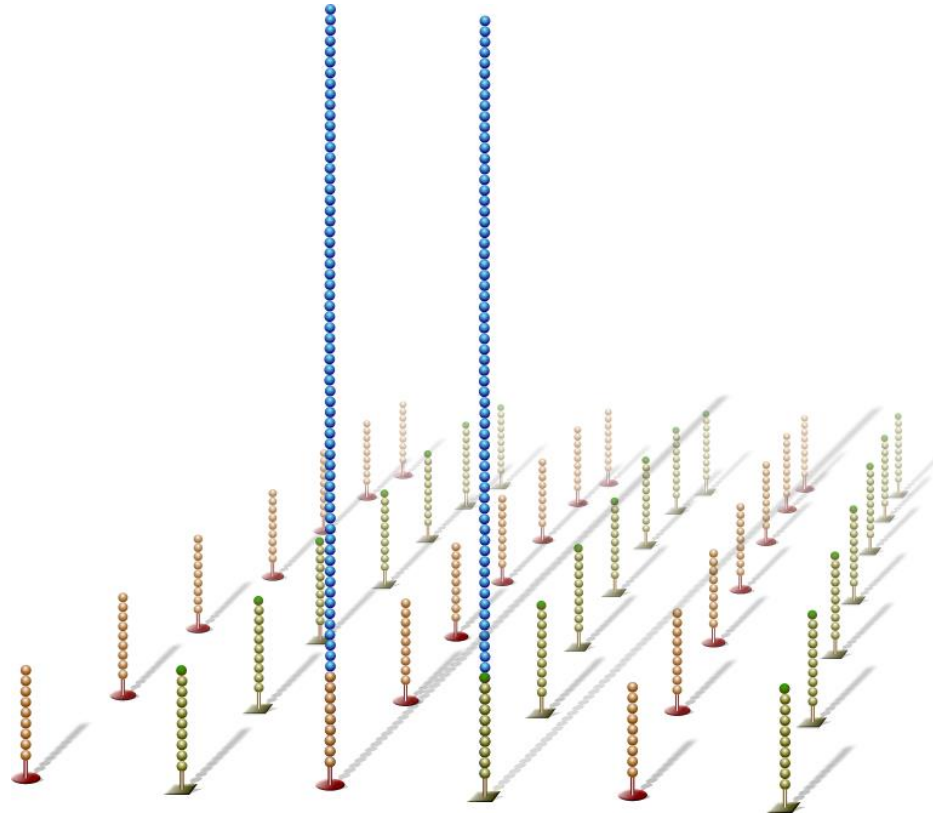
# Cluster Formation

## Bridge Amplification



# Cluster Formation

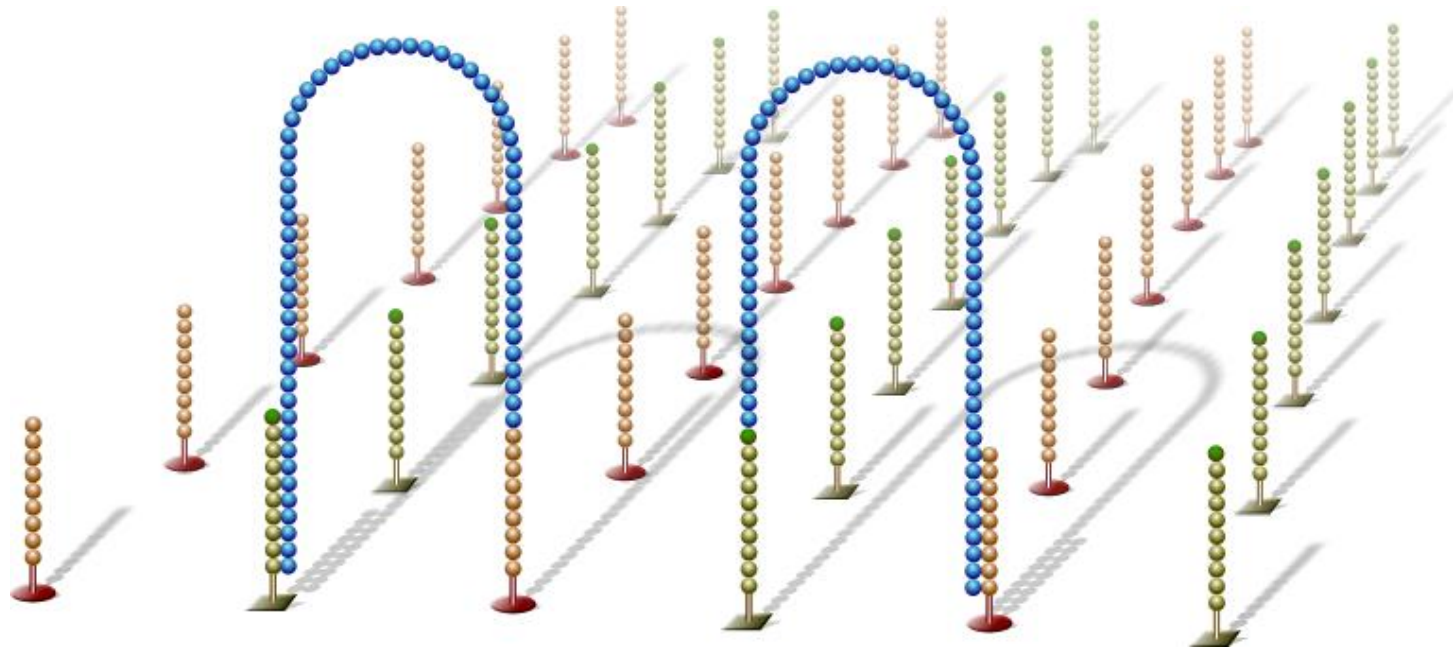
## Linearisierung



© Illumina

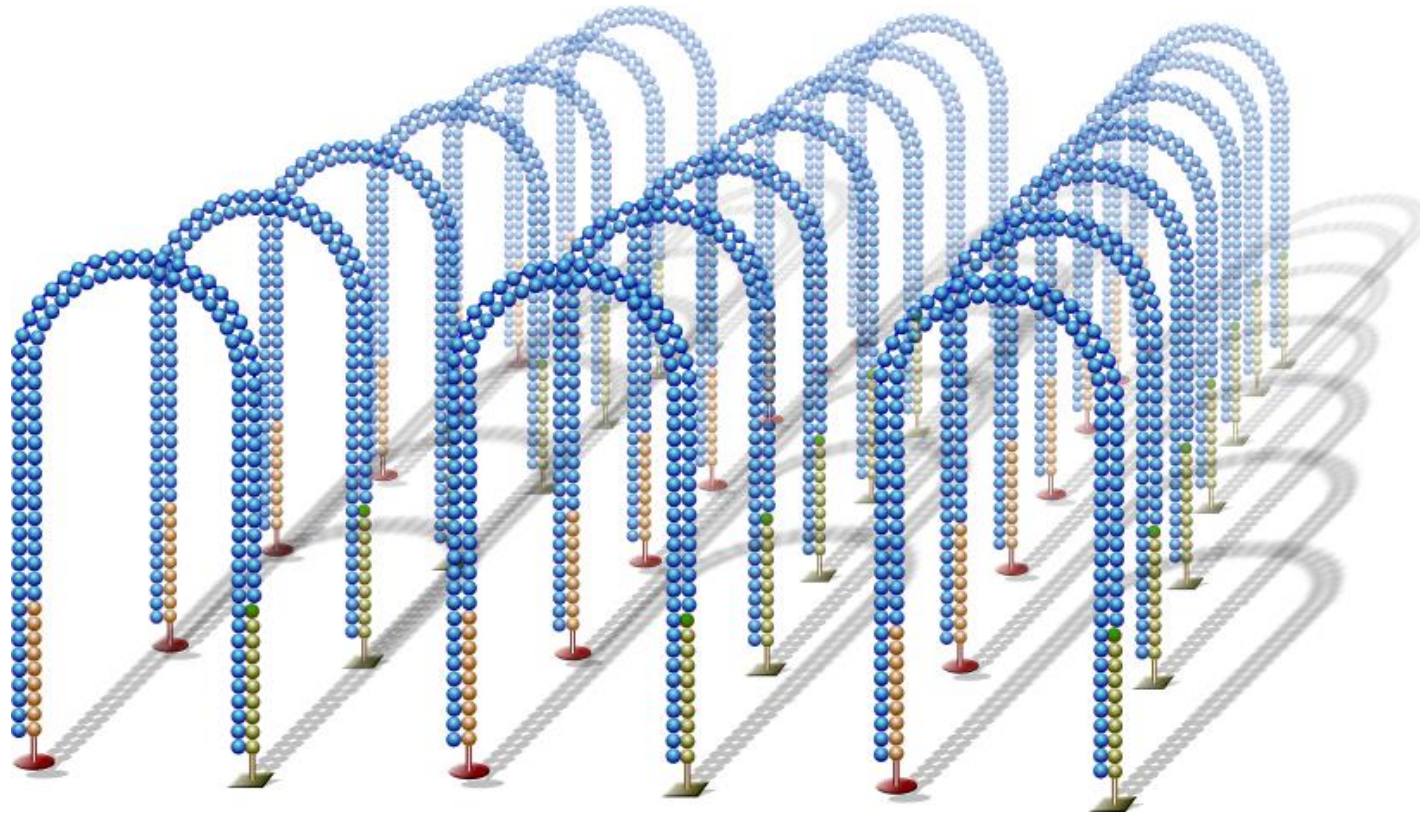
# Cluster Formation

## Bridge Amplification



# Cluster Formation

## Bridge Amplification



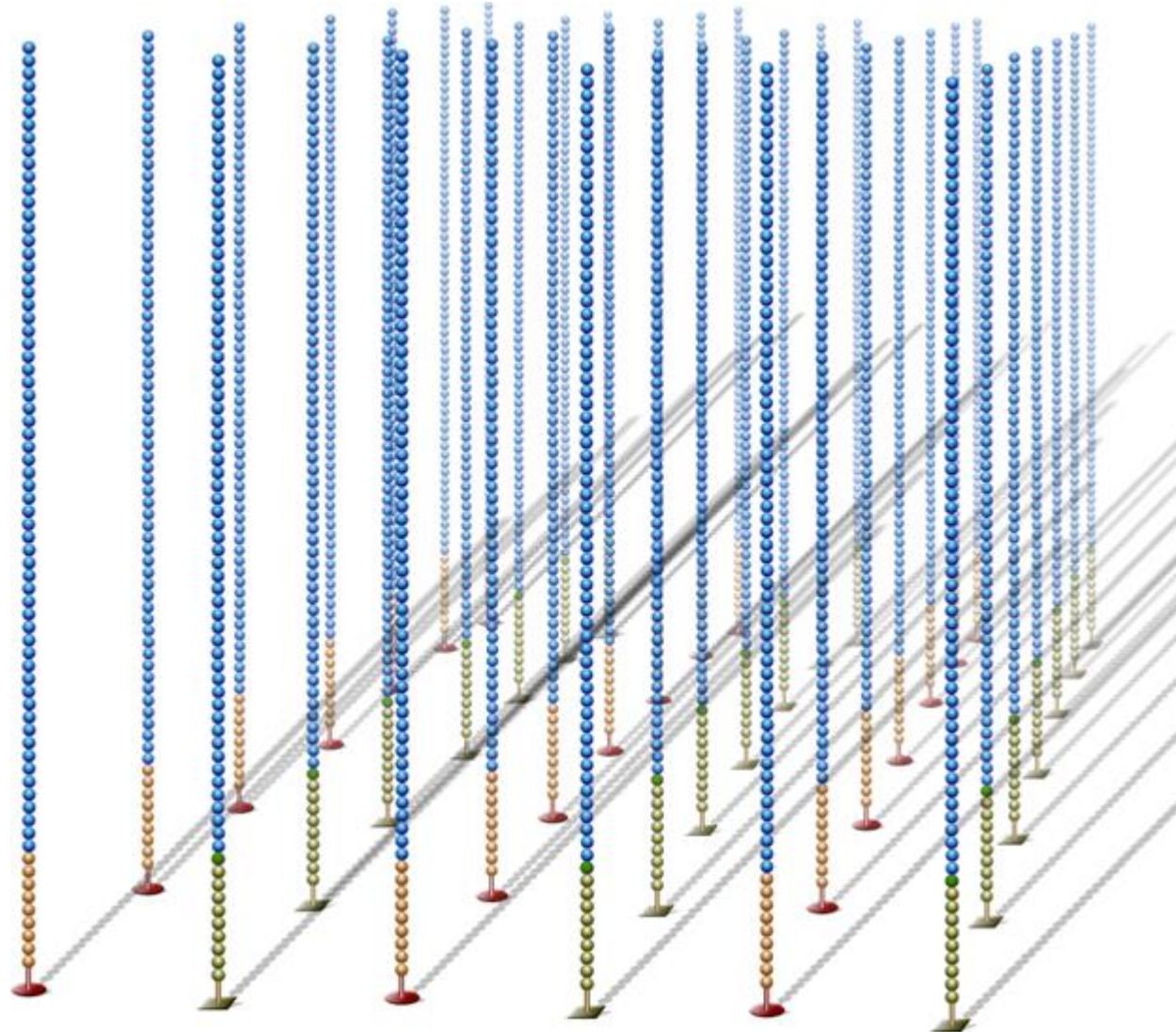
© Illumina



# Cluster Formation

Linearisierung

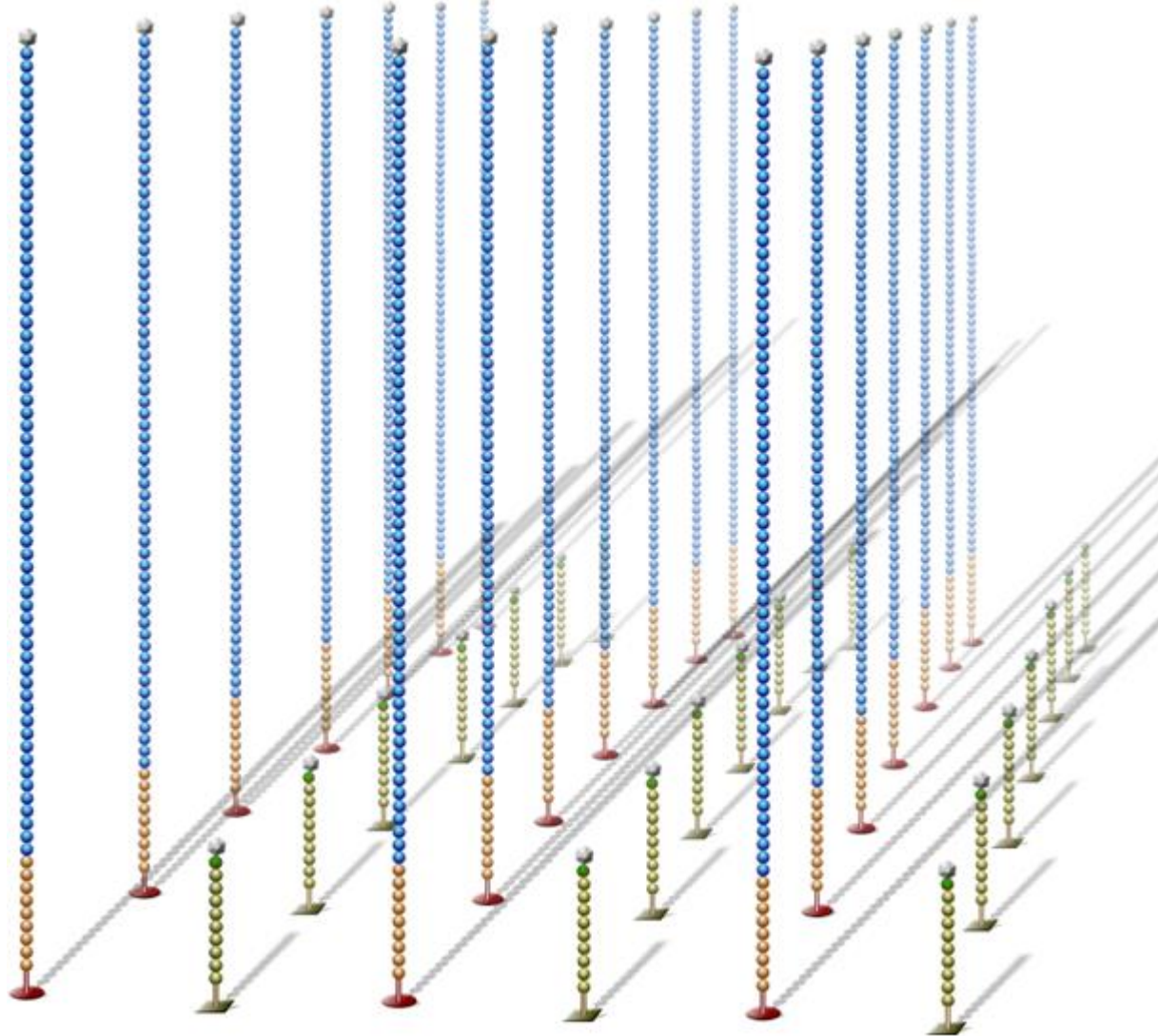
Verwerfen der  
R-Stränge



© Illumina

# Cluster Formation

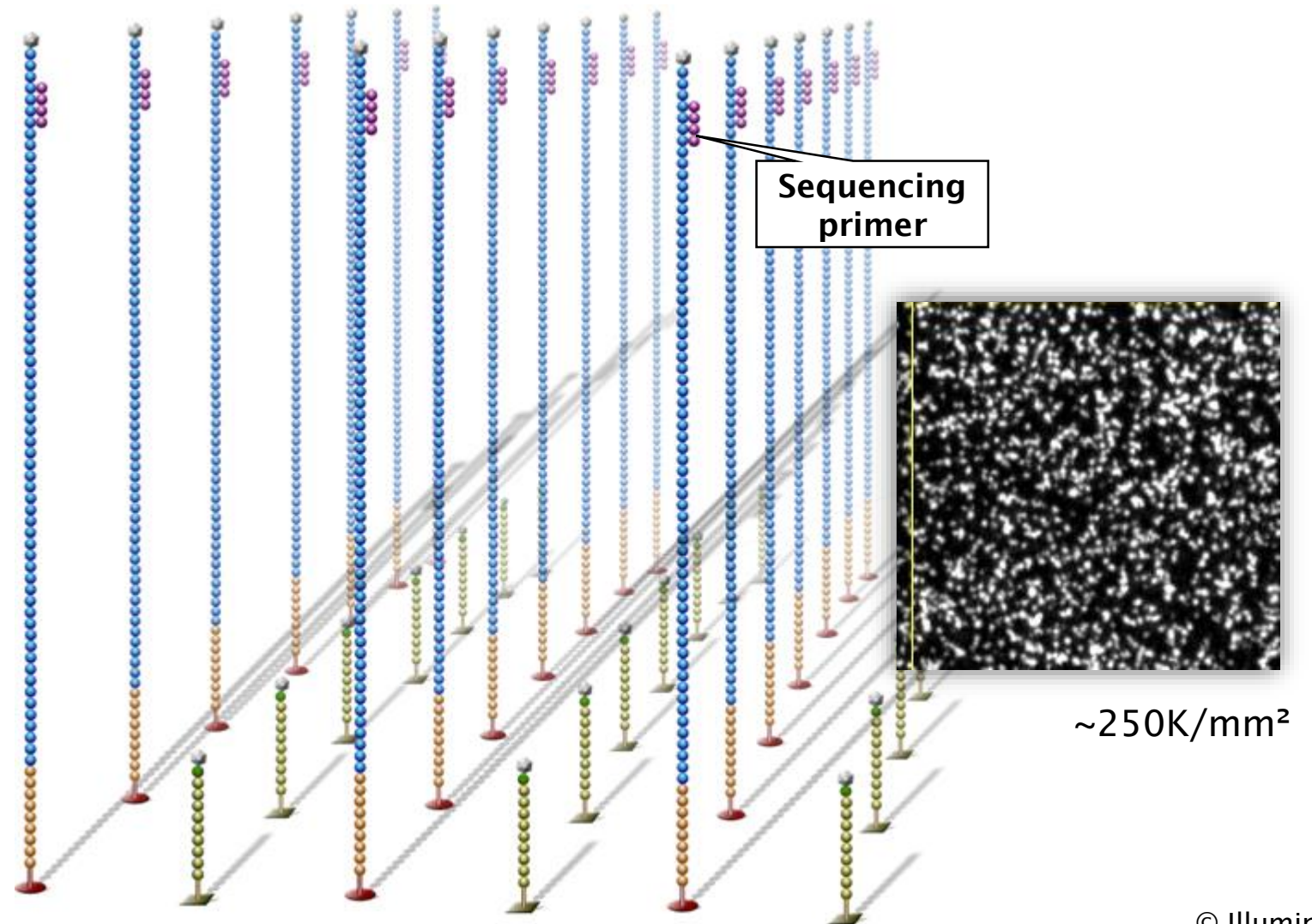
freie 3' Enden  
werden geblockt



© Illumina

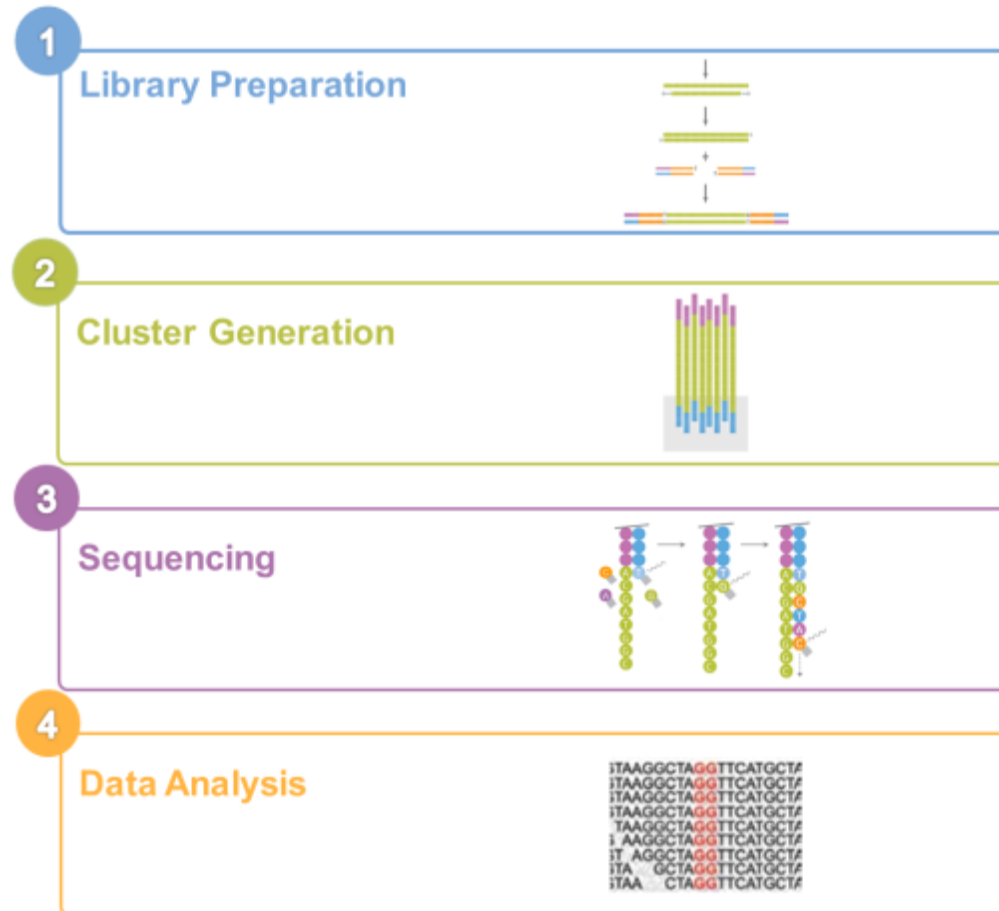
# Cluster Formation

## Primer Hybridisierung

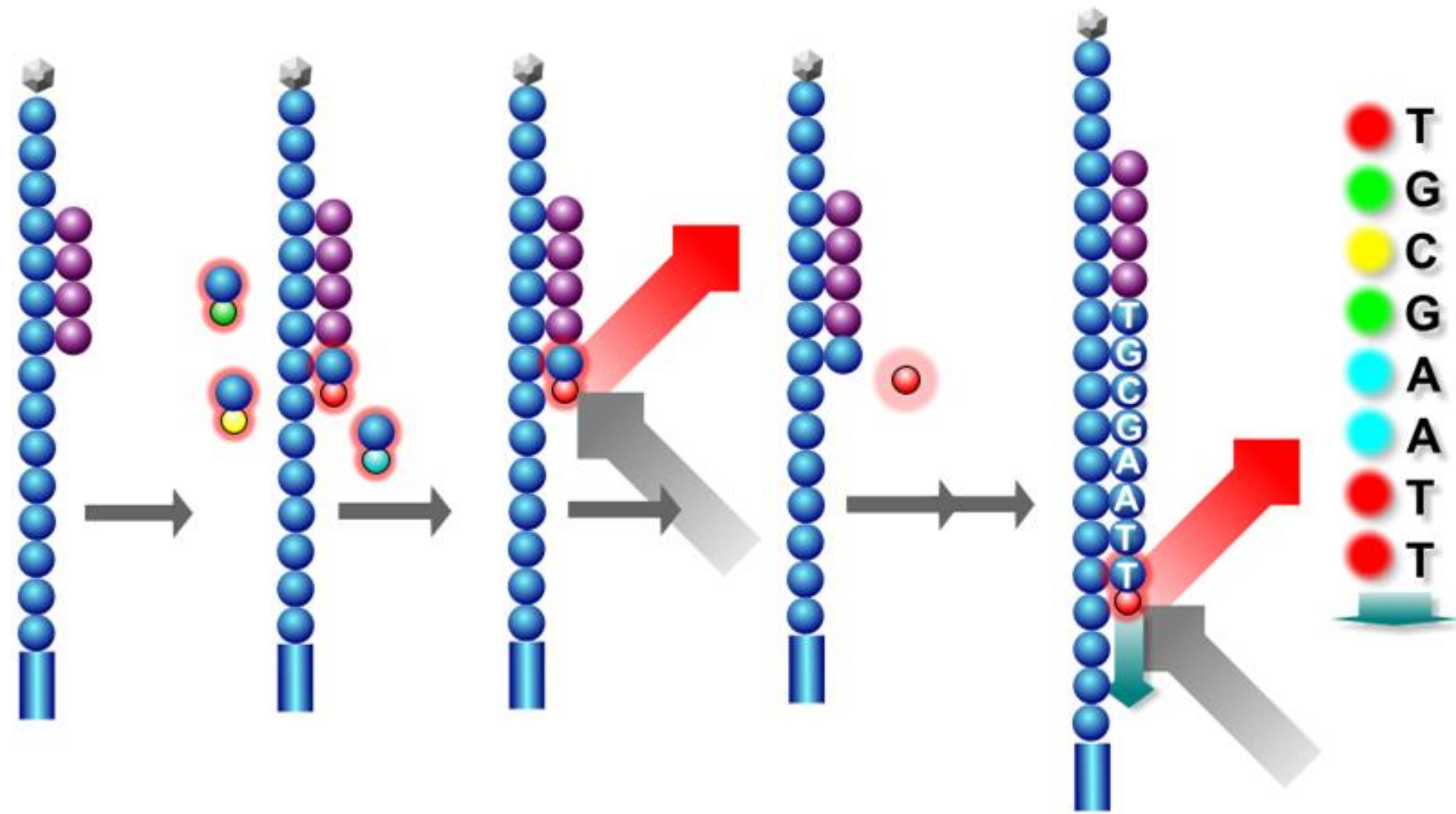


© Illumina

# Illumina Sequencing Workflow

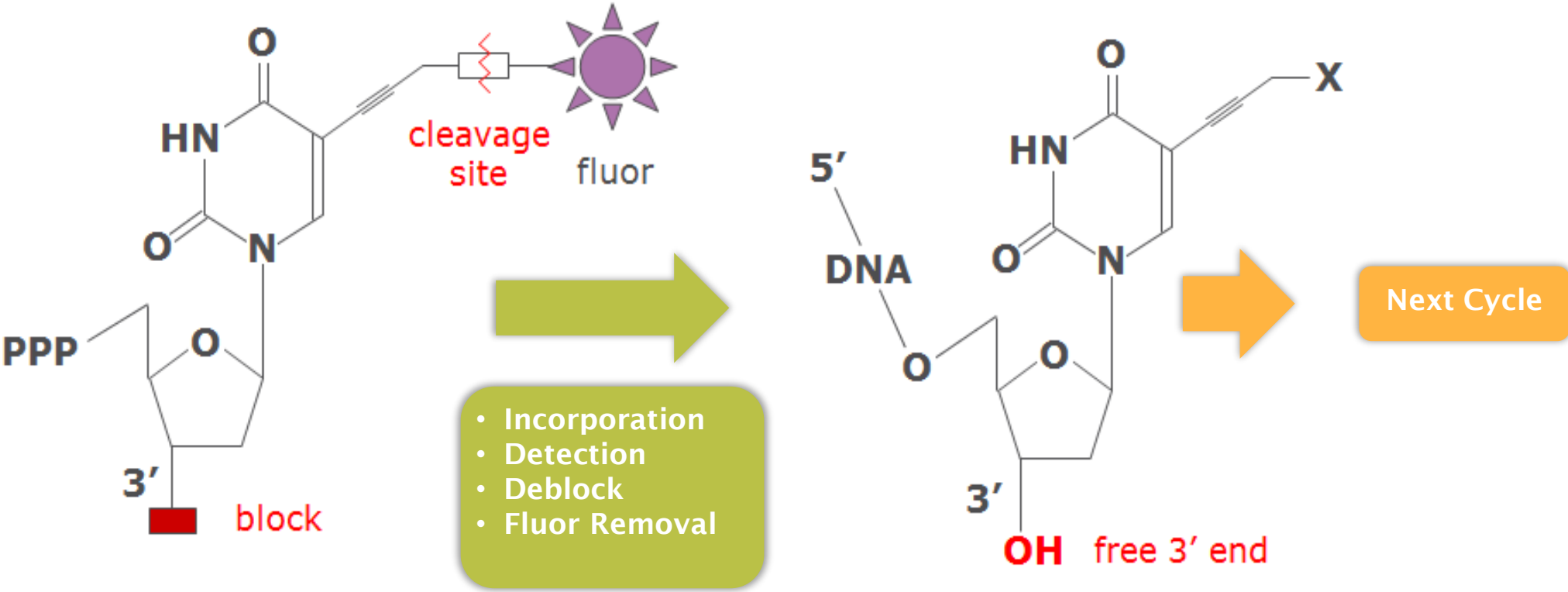


# Sequencing by Synthesis



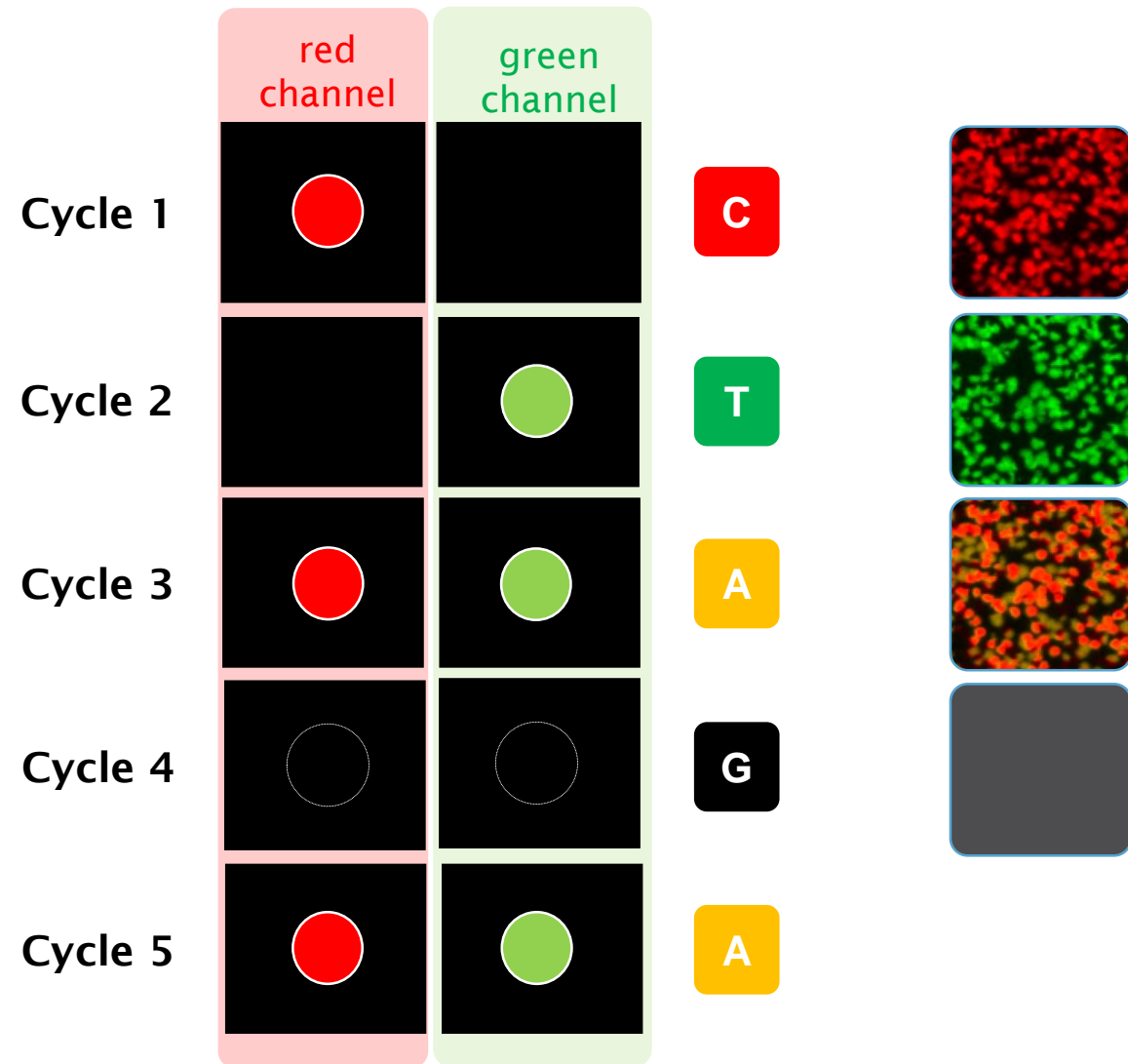
© Illumina

# Sequencing by Synthesis



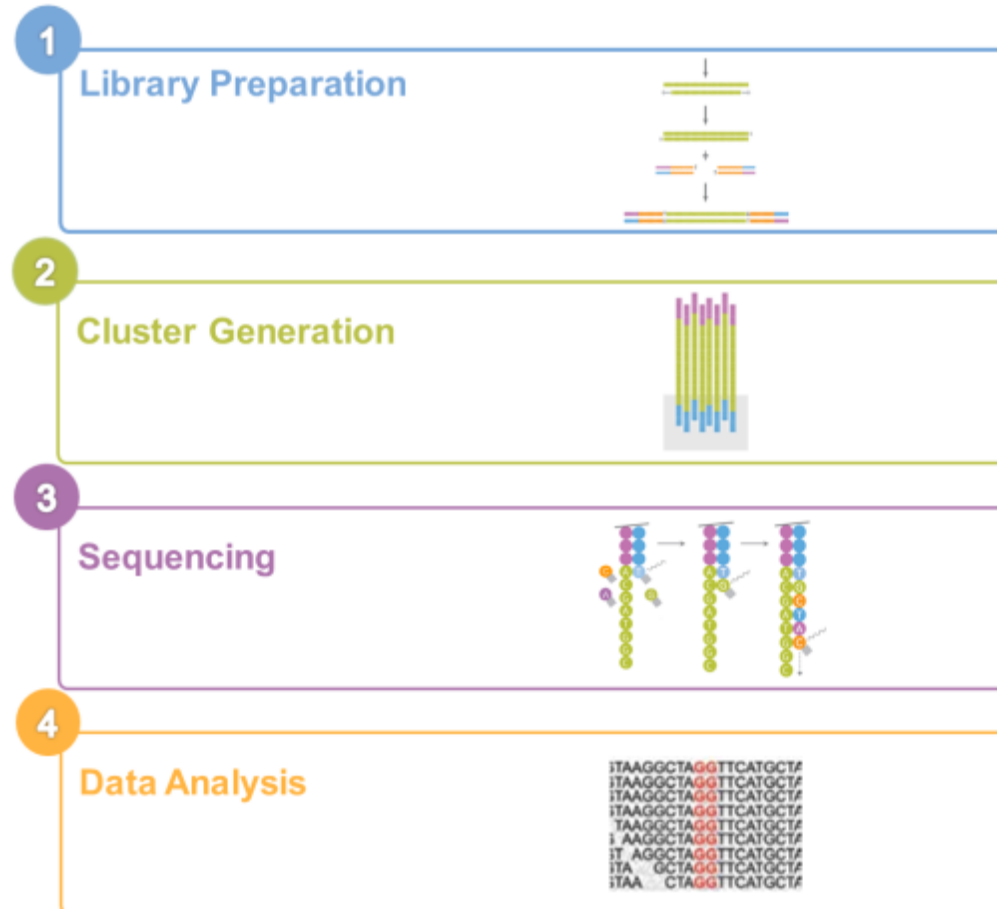
# Sequencing by Synthesis

## MiniSeq – 2 Kanal SBS



© Illumina

# Illumina Sequencing Workflow



```
ITAAGGCTAGGTTTCATGCT#  
ITAAGGCTAGGTTTCATGCT#  
ITAAGGCTAGGTTTCATGCT#  
ITAAGGCTAGGTTTCATGCT#  
TAAGGCTAGGTTTCATGCT#  
1 AAGGCTAGGTTTCATGCT#  
IT AGGCTAGGTTTCATGCT#  
ITA GCTAGGTTTCATGCT#  
ITAA CTAGGTTTCATGCT#
```

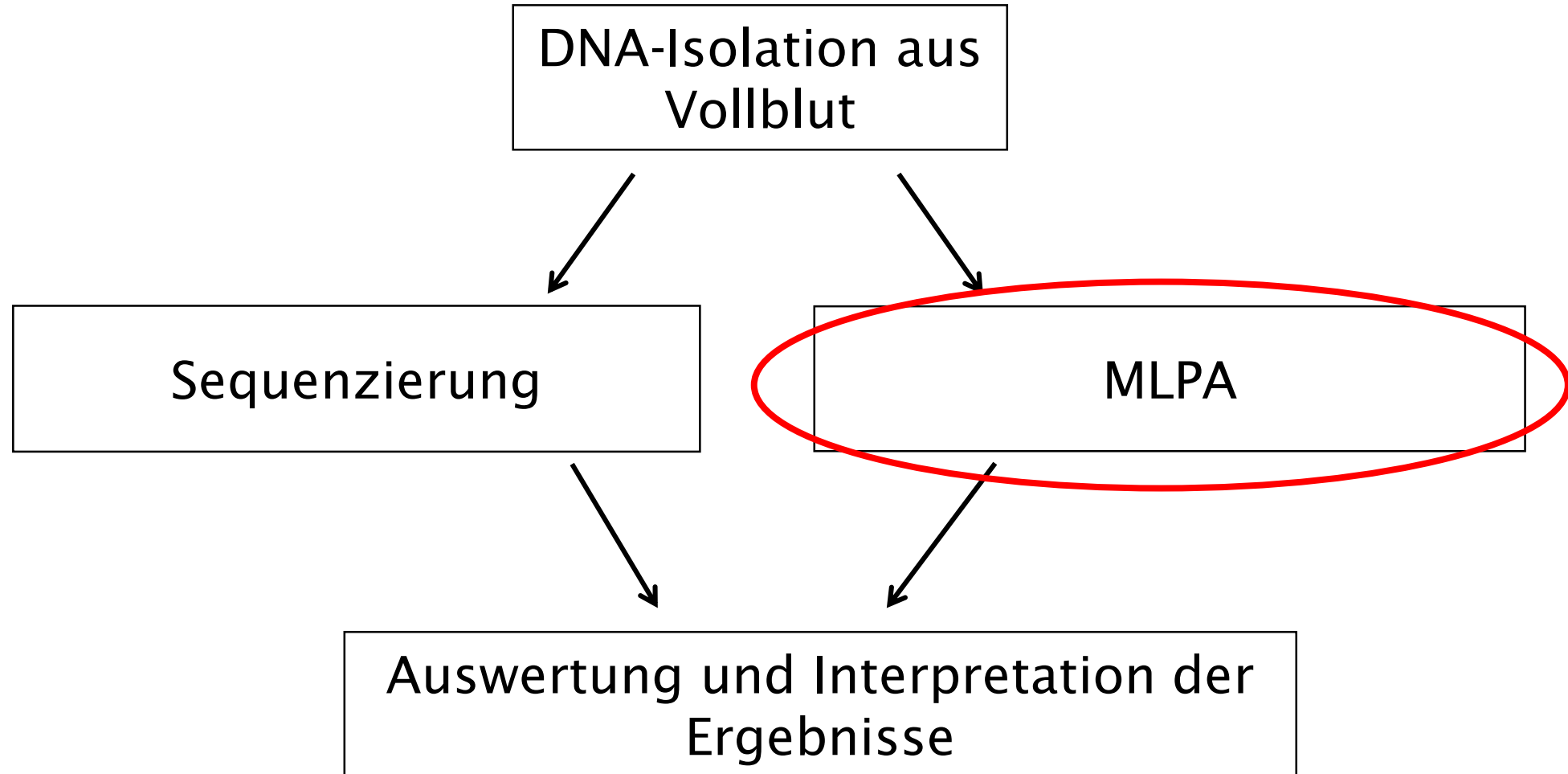


# Data Analysis – Sophia DDM

The screenshot displays the Sophia DDM TruSight\_Cancer v2 analysis interface. The top navigation bar includes 'WORKSPACE', 'VDB', and 'ANALYSIS #303-1 #20208-0130'. The main header shows 'TruSight\_Cancer v2 germline' and 'Patient's Disease ID'. The left sidebar contains 'SOPSEA Filters' with categories like 'Related Variants', 'Highly Pathogenic', 'Potentially Pathogenic', 'Unknown Significance', 'Likely Benign', 'Low Confidence Variants', and 'Flagged Variants'. The main panel shows a 'Variant List' with columns: Actionability, T..., Gene, Coding consequence, cDNA, Depth, VFS, ref, alt, Exon, and Exon ID. The table lists various variants across genes such as CHEK2, PALB2, ATM, BRCA1, BRCA2, BRRP1, CDH1, PMS2, PTEH, RAD51D, and STK11.

Actionability	T...	Gene	Coding consequence	cDNA	Depth	VFS	ref	alt	Exon	Exon ID
H/A	SNP	CHEK2	missense	c.470T>C	536	40.1	A	G	4	4
H/A	SNP	PALB2	missense	c.2794G>A	353	46.5	C	T	8	8
H/A	SNP	ATM	missense	c.5948A>G	794	100.0	A	G	40	40
H/A	BIDEL	ATM	intronic	c.3803-135dupA	498	34.1	TAA	TAAA	24	24
H/A	SNP	BRCA1	synonymous	c.4398T>C	351	49.0	A	G	18	18
H/A	SNP	BRCA1	3'UTR	c.*1287C>T	159	55.4	G	A	23	24
H/A	SNP	BRCA1	synonymous	c.2311T>C	321	46.1	A	G	10	11
H/A	SNP	BRCA1	synonymous	c.2087C>T	823	49.0	G	A	10	11
H/A	SNP	BRCA1	3'UTR	c.*1332G>A	174	44.3	C	T	23	24
H/A	SNP	BRCA1	missense	c.3113A>G	578	50.8	T	C	10	11
H/A	SNP	BRCA1	3'UTR	c.*421G>T	289	44.3	C	A	23	24
H/A	SNP	BRCA1	3'UTR	c.*7074C>G	67	47.8	G	C	2	2
H/A	SNP	BRCA2	3'UTR	c.*80A>G	429	59.4	A	G	2	2
H/A	SNP	BRCA2	missense	c.1114A>C	729	47.9	A	C	10	10
H/A	SNP	BRCA2	synonymous	c.6513G>C	956	100.0	G	C	11	11
H/A	SNP	BRCA2	3'UTR	c.*7105A>C	443	52.1	A	C	27	27
H/A	SNP	BRCA2	synonymous	c.4563A>G	789	100.0	A	G	11	11
H/A	SNP	BRCA2	synonymous	c.3877T>C	744	40.0	T	C	11	11
H/A	SNP	BRRP1	synonymous	c.3411T>C	493	49.8	A	G	20	20
H/A	SNP	BRRP1	synonymous	c.2617A>G	441	46.0	T	C	19	19
H/A	SNP	CDH1	synonymous	c.2074T>C	323	59.1	T	C	13	13
H/A	SNP	CDH1	intronic	c.*48+6G>T	369	100.0	C	T	1	1
H/A	SNP	CDH1	intronic	c.*564-14827G>A	817	49.5	G	A	3	3
H/A	BIDEL	CDH1	intronic	c.2364+173dupA	344	96.3	CAA	CAAA	13	13
H/A	SNP	MSH6	intronic	c.*3438-16A>T	31	56.9	A	T	3	3
H/A	SNP	PMS2	intronic	c.*705+17A>G	393	47.1	T	C	6	6
H/A	SNP	PMS2	missense	c.*2570G>C	113	58.3	C	G	15	15
H/A	SNP	PMS2	synonymous	c.*783C>G	818	99.8	G	C	7	7
H/A	SNP	PTEH	3'UTR	c.*511G>A	71	46.5	G	A	1	1
H/A	BIDEL	PTEH	3'UTR	c.*396delT	86	80.7	CT	C	1	1
H/A	SNP	PTEH	3'UTR	c.*326G>C	152	88.7	G	C	1	1
H/A	SNP	RAD51D	synonymous	c.*214C>T	204	52.0	G	A	3	3
H/A	SNP	STK11	intronic	c.*920+7G>C	128	45.2	G	C	7	7
H/A	SNP	BRCA1	missense	c.*2612C>T	721	44.0	G	A	10	11
H/A	SNP	BRCA1	missense	c.*3548A>G	322	45.8	T	C	10	11
H/A	SNP	BRCA1	missense	c.*4877A>G	399	41.9	T	C	15	16

# Genetische Analyse



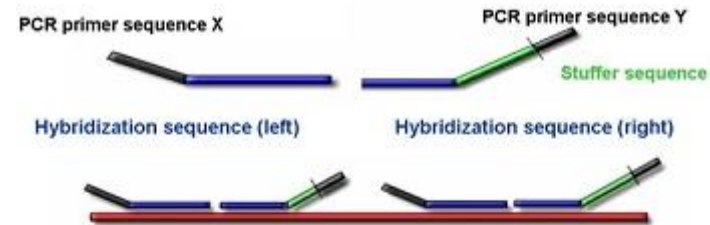
# MLPA

- 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)



# MLPA

## 1. Denaturation and Hybridization



## 2. Ligation



## 3. PCR with universal primers X and Y

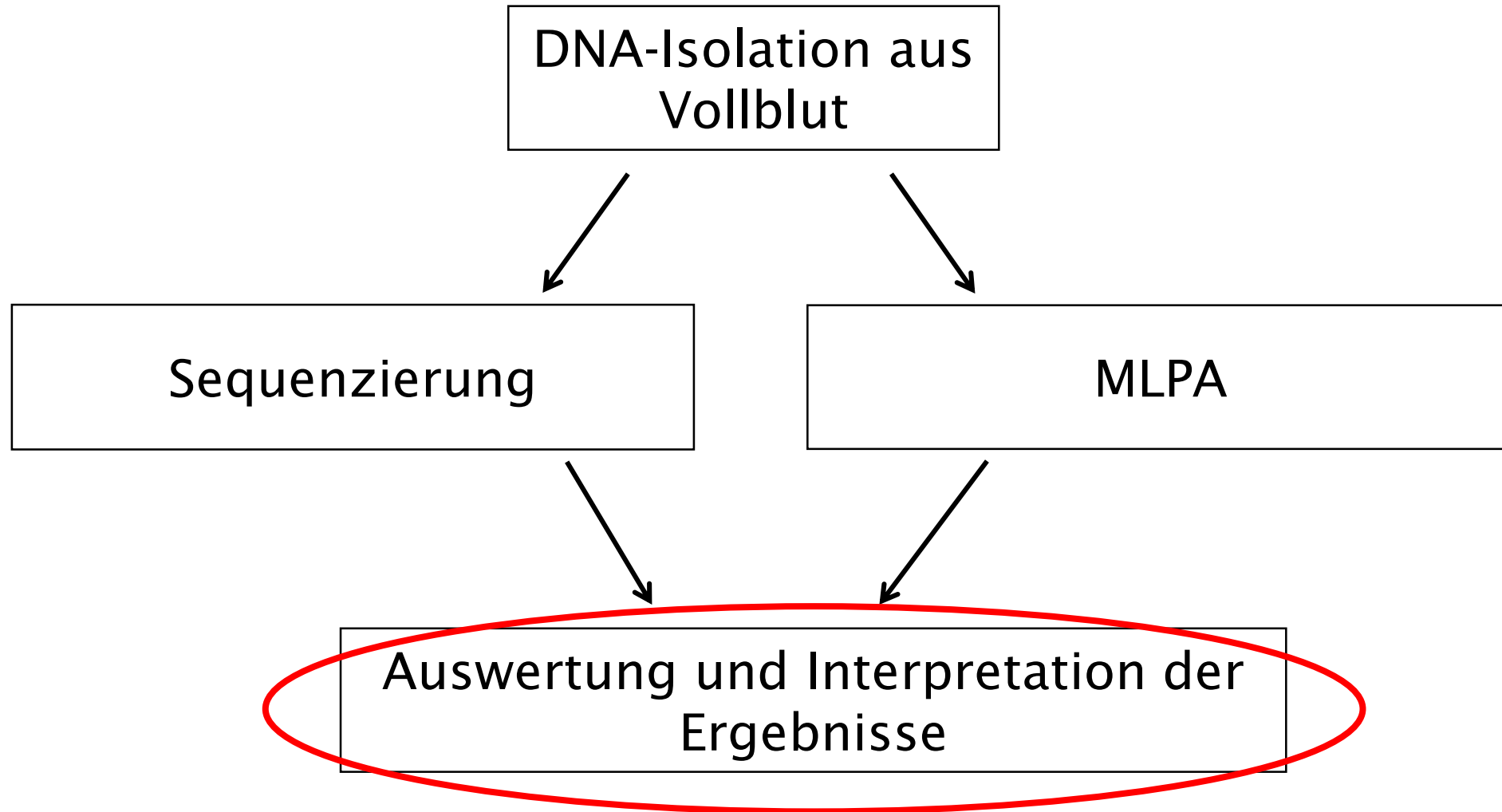
exponential amplification of ligated probes only



## 4. Fragment analysis



# 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