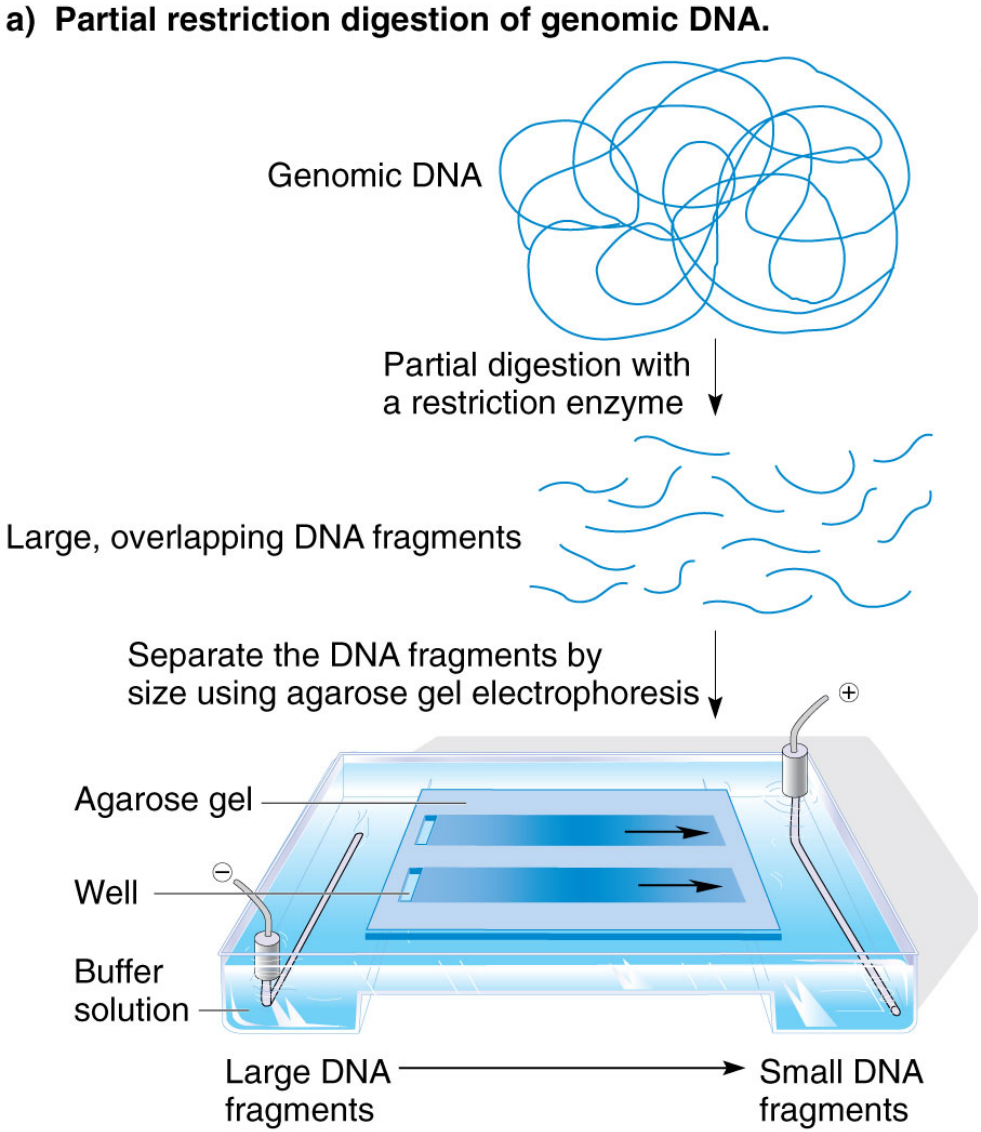
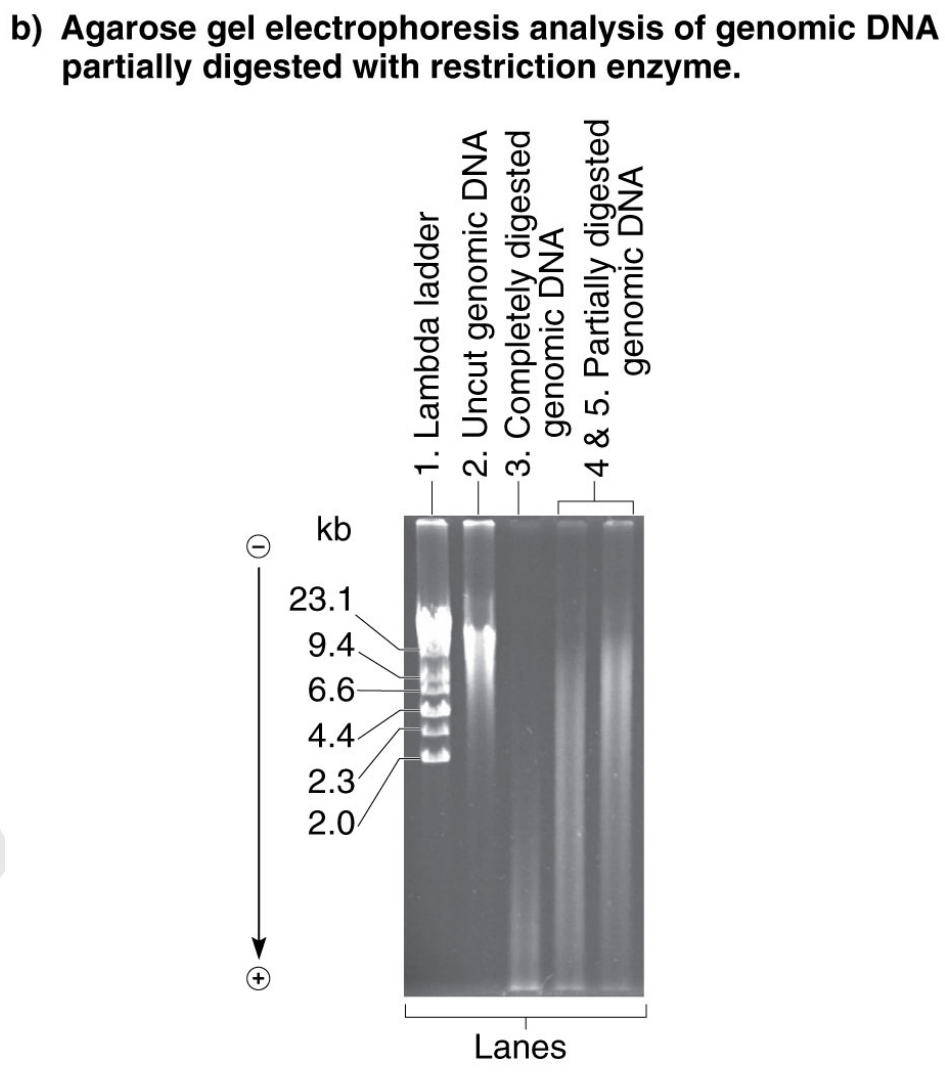
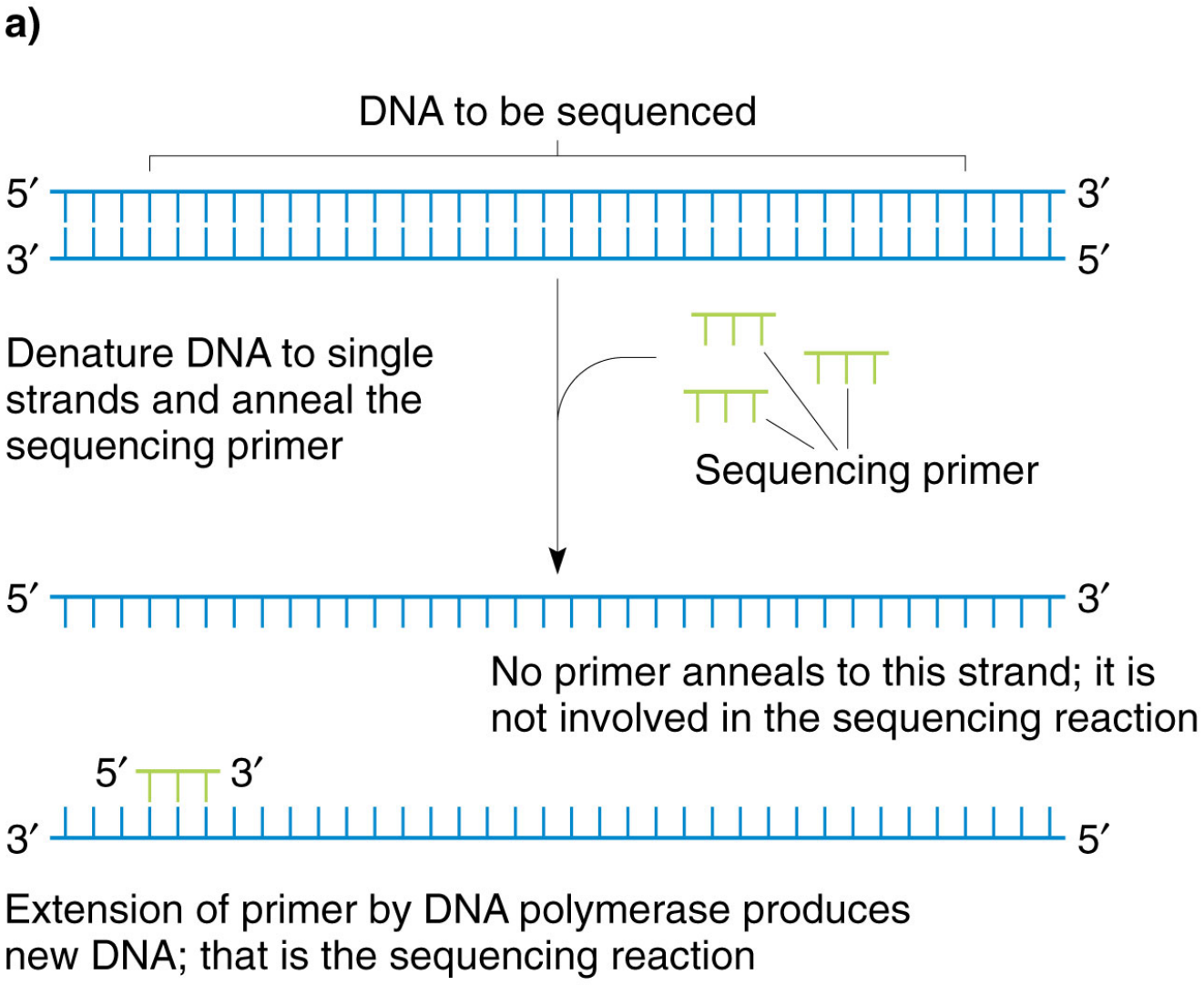
# Gelelektrophorese von DNA erlaubt deren Auftrennung nach Grösse im elektrischen Feld



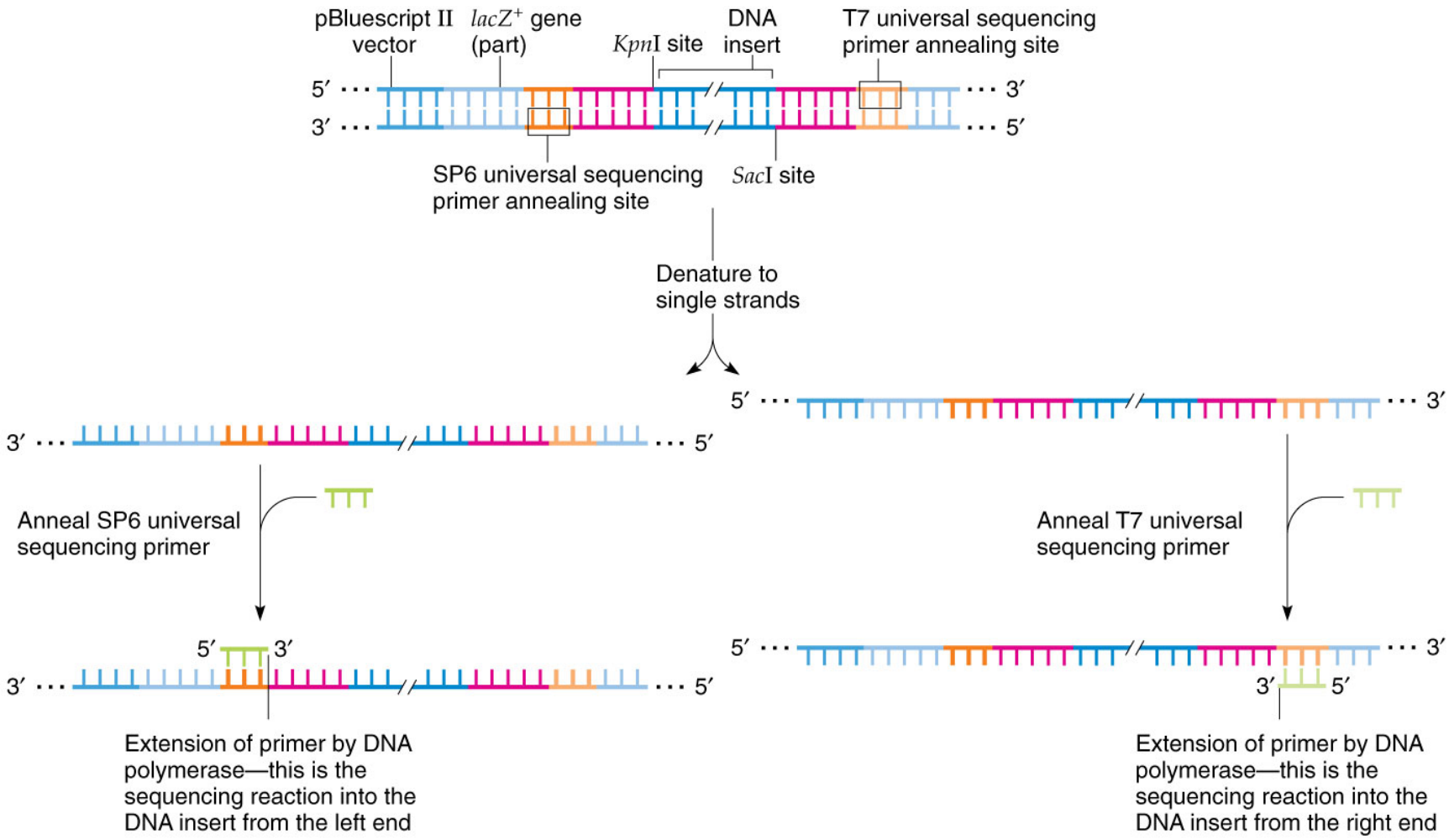


# DNA-Sequenzierung

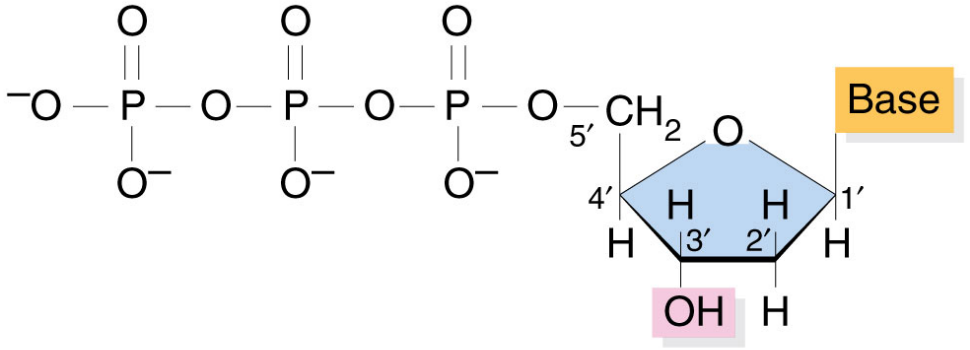


b)

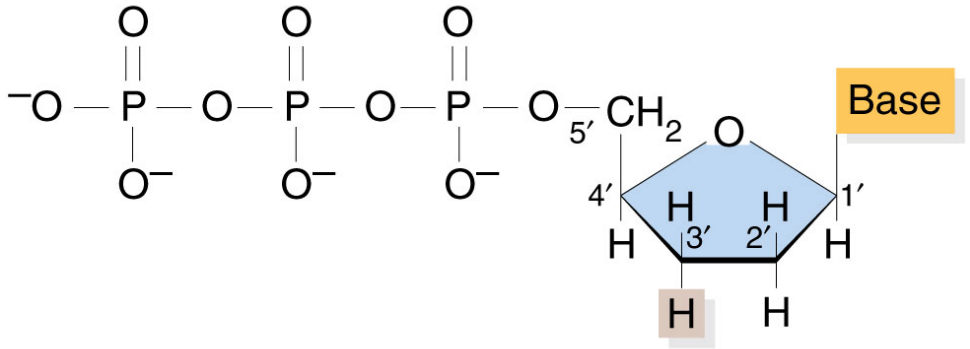


# Der Einbau eines didesoxy-Nukleotids führt zum Strangabbruch

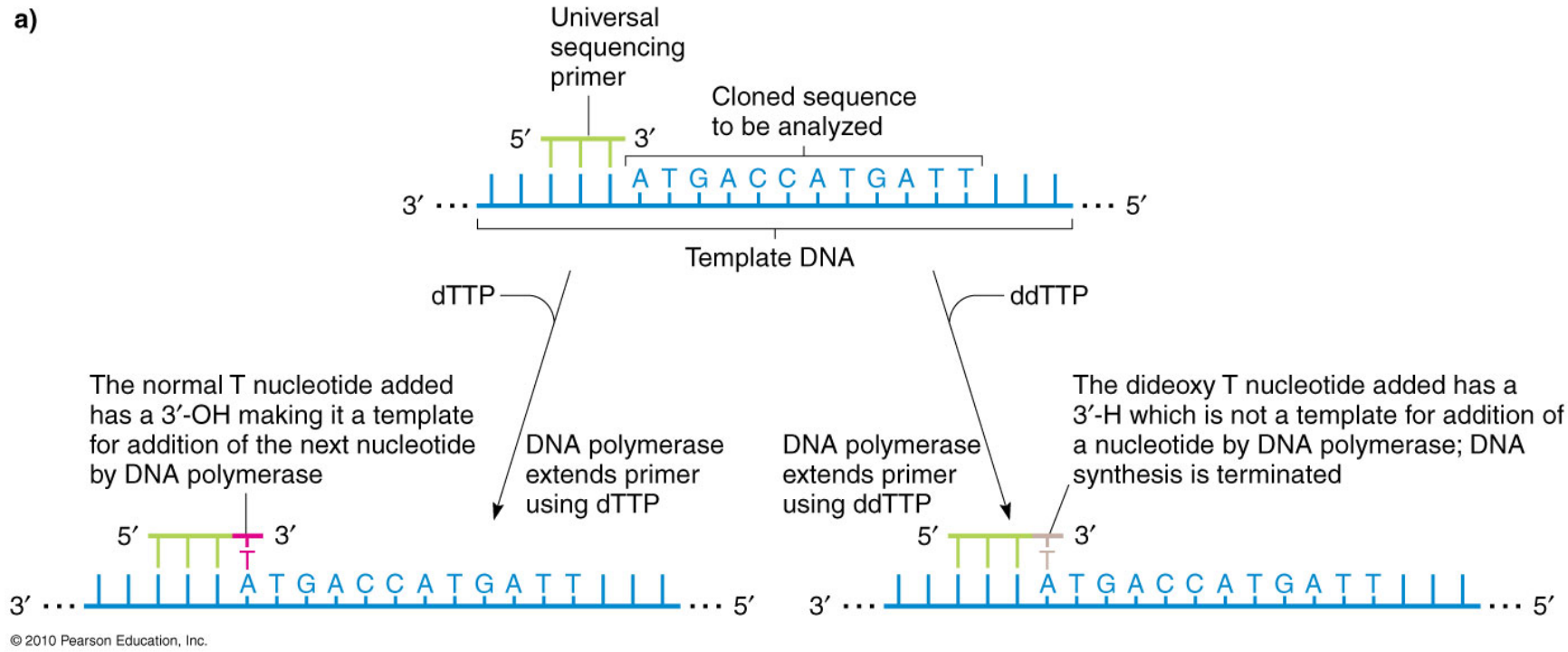
**a) Deoxynucleotide (dNTP) DNA precursor**



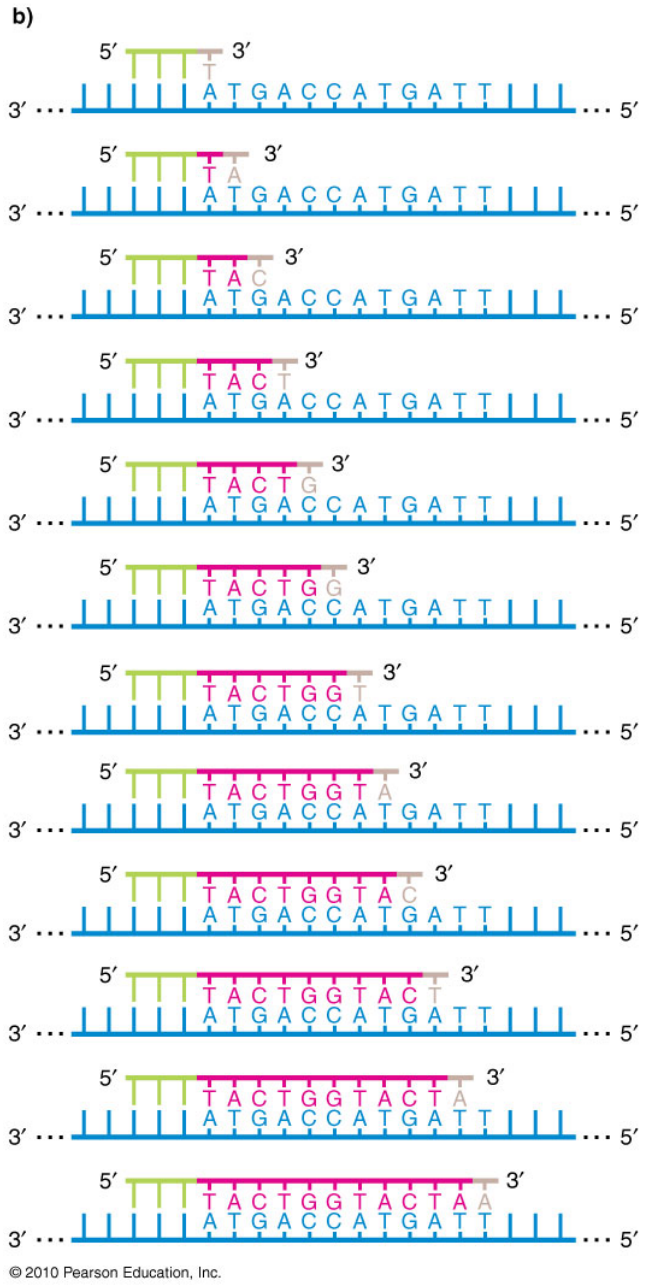

**b) Dideoxynucleotide (ddNTP) DNA precursor**



Durch eine Mischung von normalen und didesoxy Nukleotiden kommt es nicht an jeder Position zu einem Abbruch.



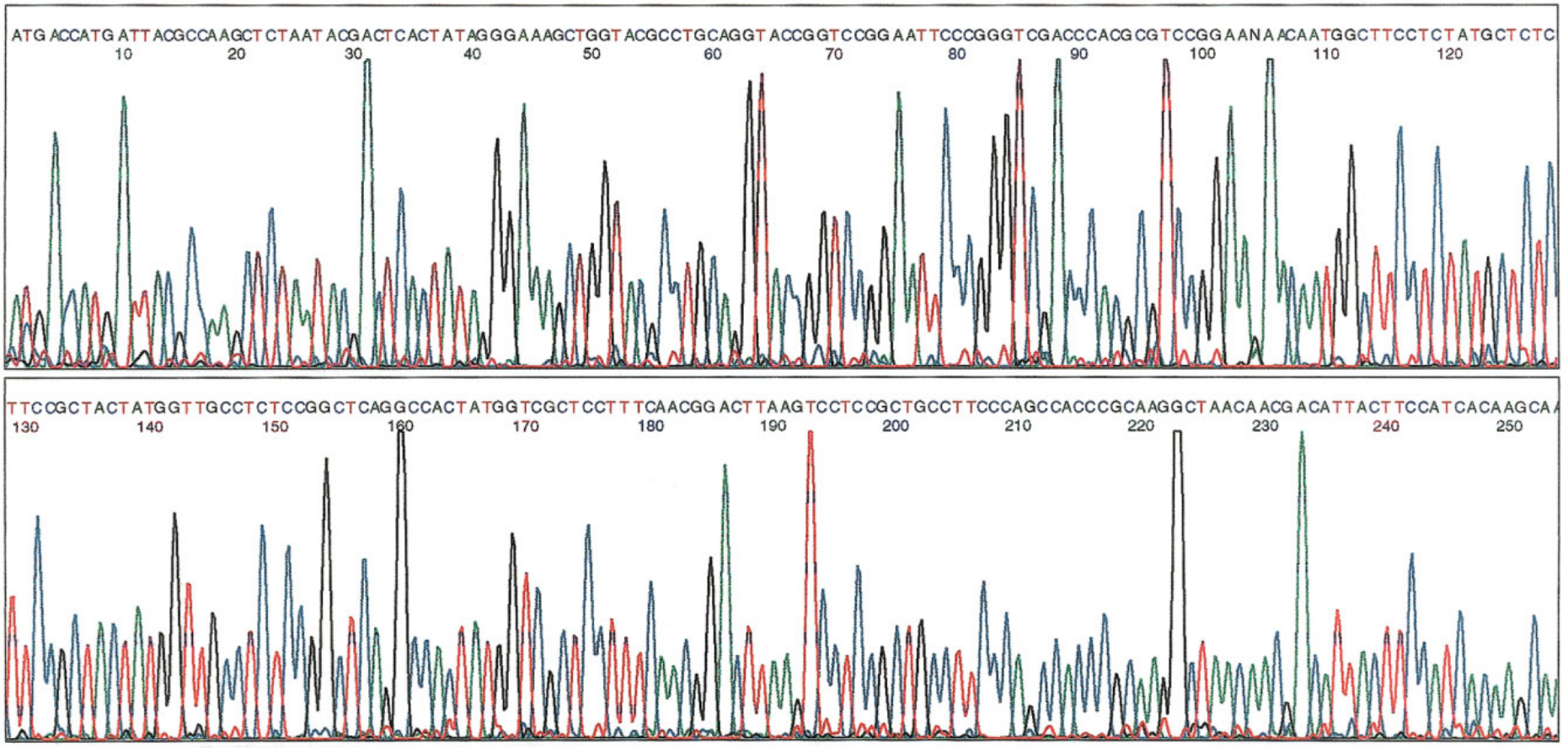
Didesoxy Nukelotide sind mit Fluoreszierenden Farbstoffen markiert. Daher sind alle Moleküle, welche mit einem ddG enden zB Grün, alle mit ddC terminierte Moleküle blau, alle ddT rot und alle ddA Gelb.



# Gelelektrophorese in Acrylamid Kapillaren und Abtasten der Signale mit einem Laser, erlaubt die Sequenzbestimmung.

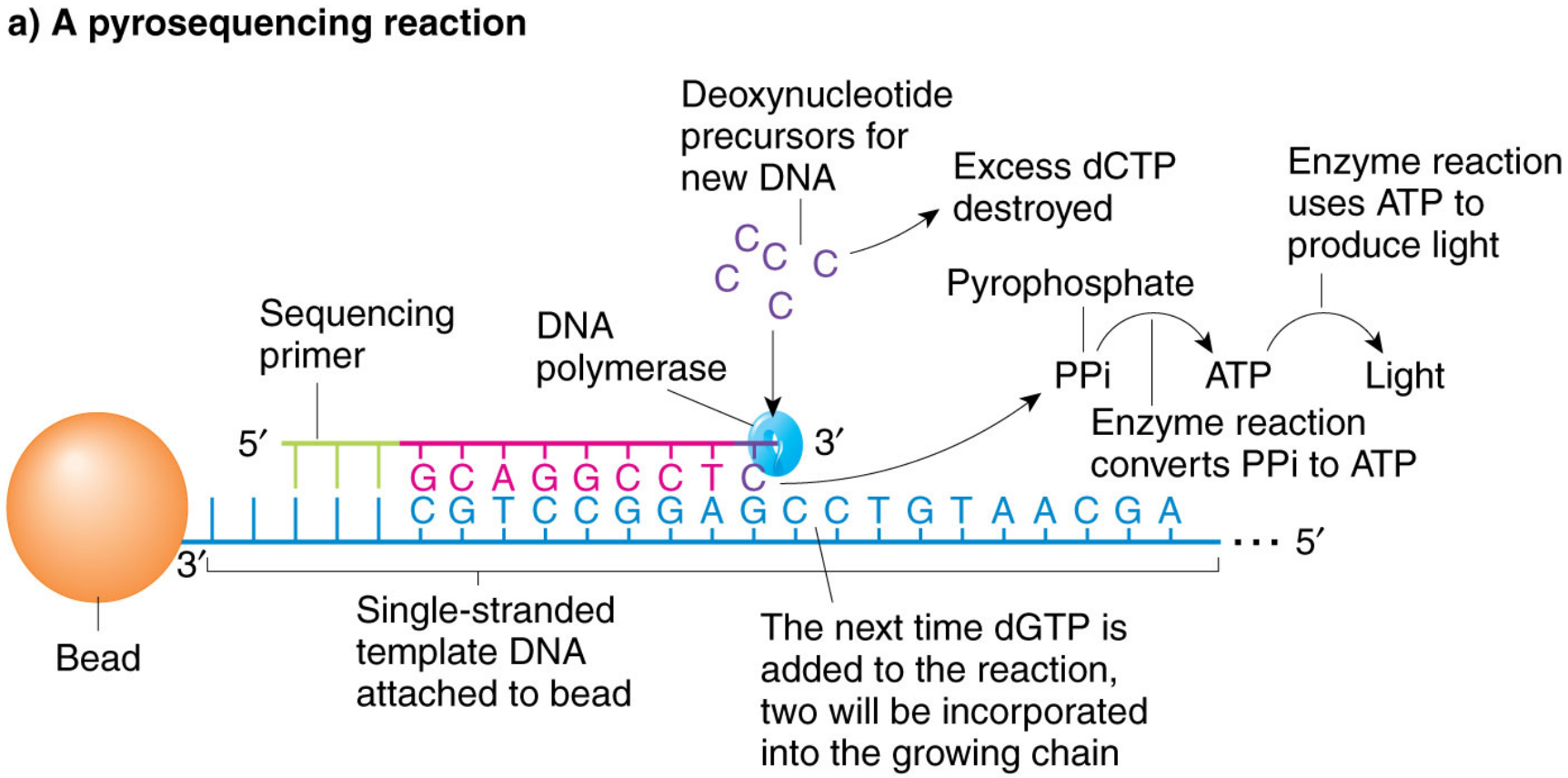
Sanger Sequenzierung, Kettenabbruchverfahren

c)

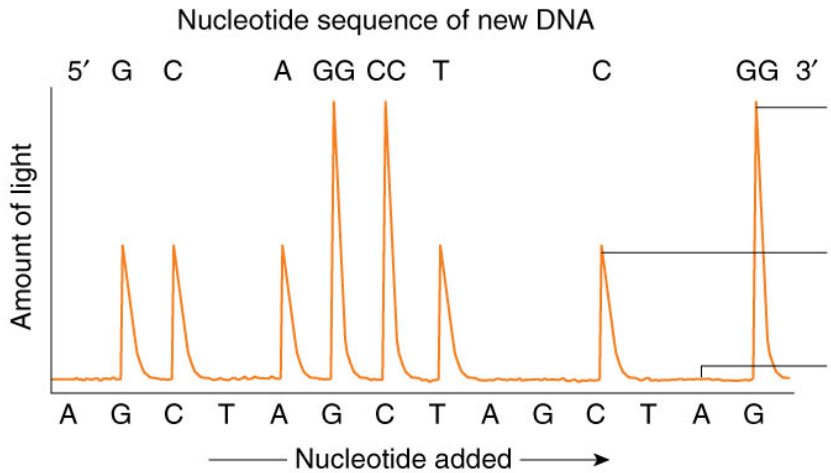


# Next Generation Sequencing: Pyrosequencing.

Nachteil: Kurze Sequenzen, teuer pro Lauf
Vorteil: Millionen an Sequenzen gleichzeitig, billig pro Base



**b) Pyrogram result of pyrosequencing**




Double-height peak indicates that two nucleotides were incorporated into the new DNA when the precursor was added. In this case a G was incorporated meaning that there were two adjacent C nucleotides on the template.

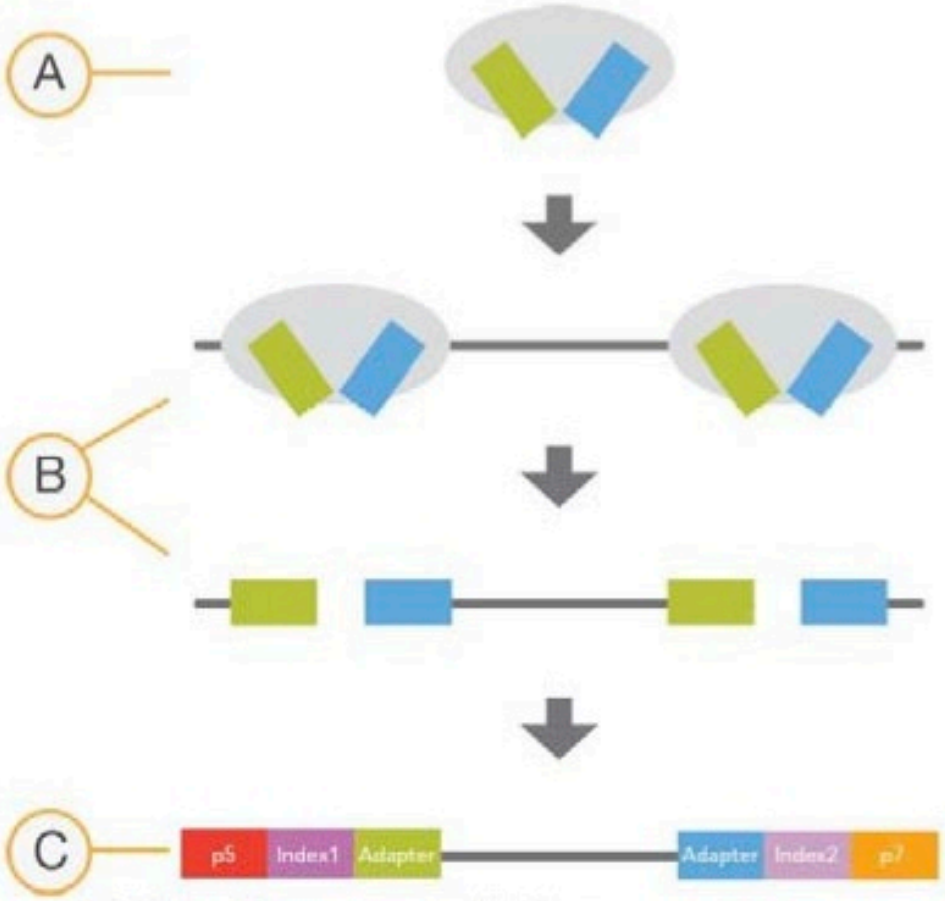
Single-height peak indicates that one nucleotide was incorporated into the new DNA when the precursor was added. In this case a T was incorporated meaning that the template had an A.

The absence of peaks when nucleotides are added means that they could not be incorporated into new DNA, meaning that the template did not have complementary bases.

# Illumina Sequencing

# Library Preparation

*Illumina Nextera DNA Sample Preparation Kit*




A Nextera Transposome with Adaptors
B Tagmentation to Fragment and Add Adaptors
C Limited Cycle PCR to Add Sequencing Primer Sequences and Indicies

**Separate fragmentation not required**

**Tag with enzyme mix**

**PCR**
**Polishes fragment ends and incorporates optional indices**



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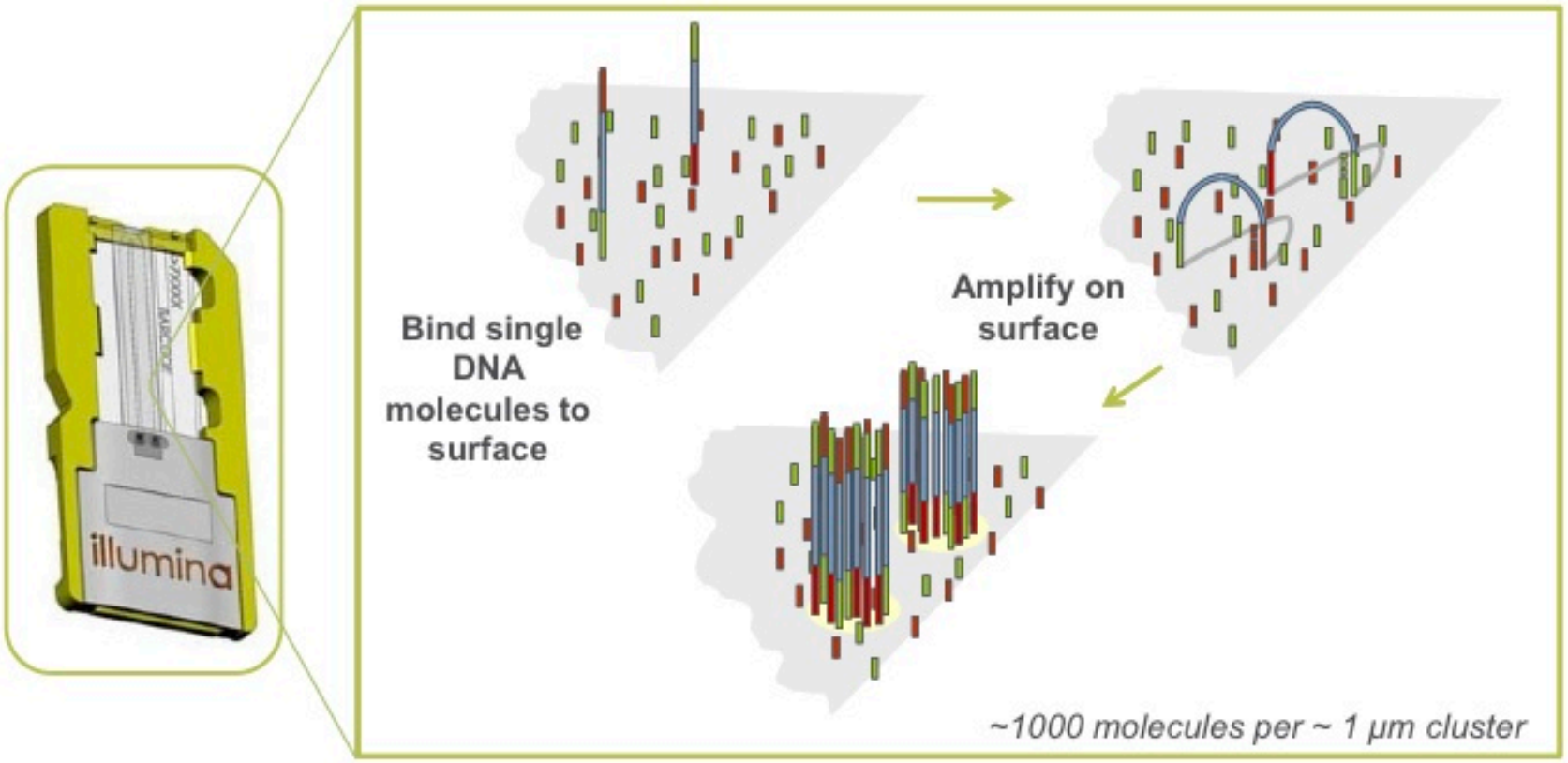
# Cluster Generation



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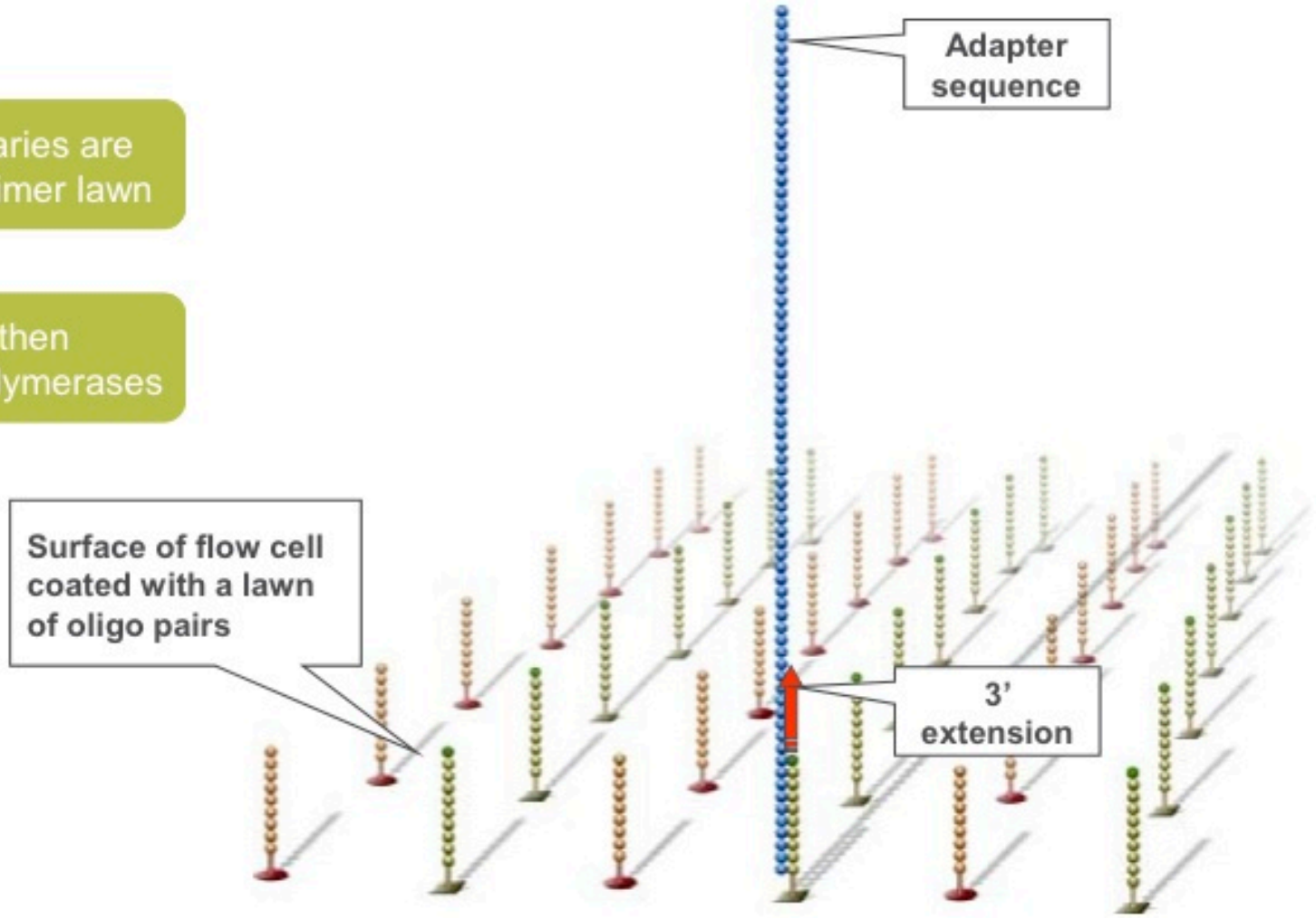
# Hybridize Fragment & Extend

Single DNA libraries are hybridized to primer lawn

Bound libraries then extended by polymerases



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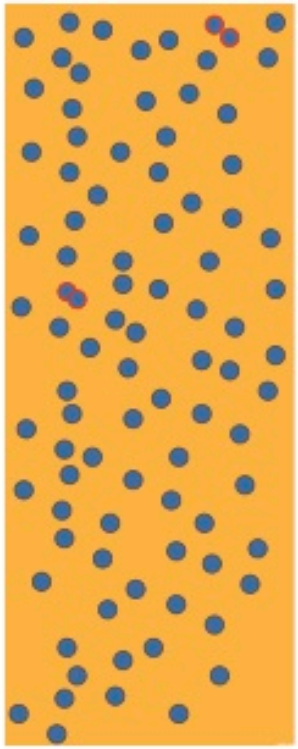
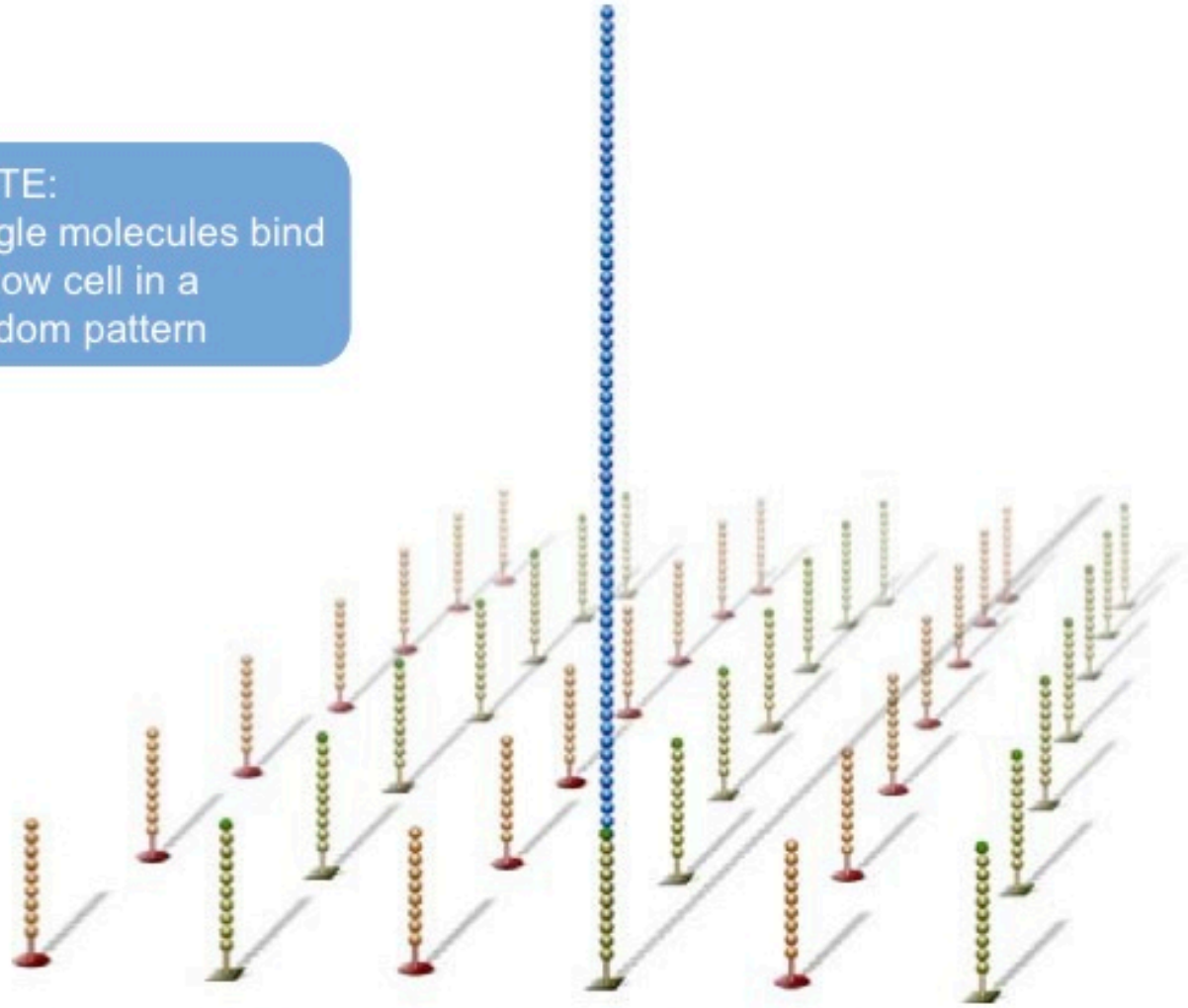
# Hybridize Fragment & Extend

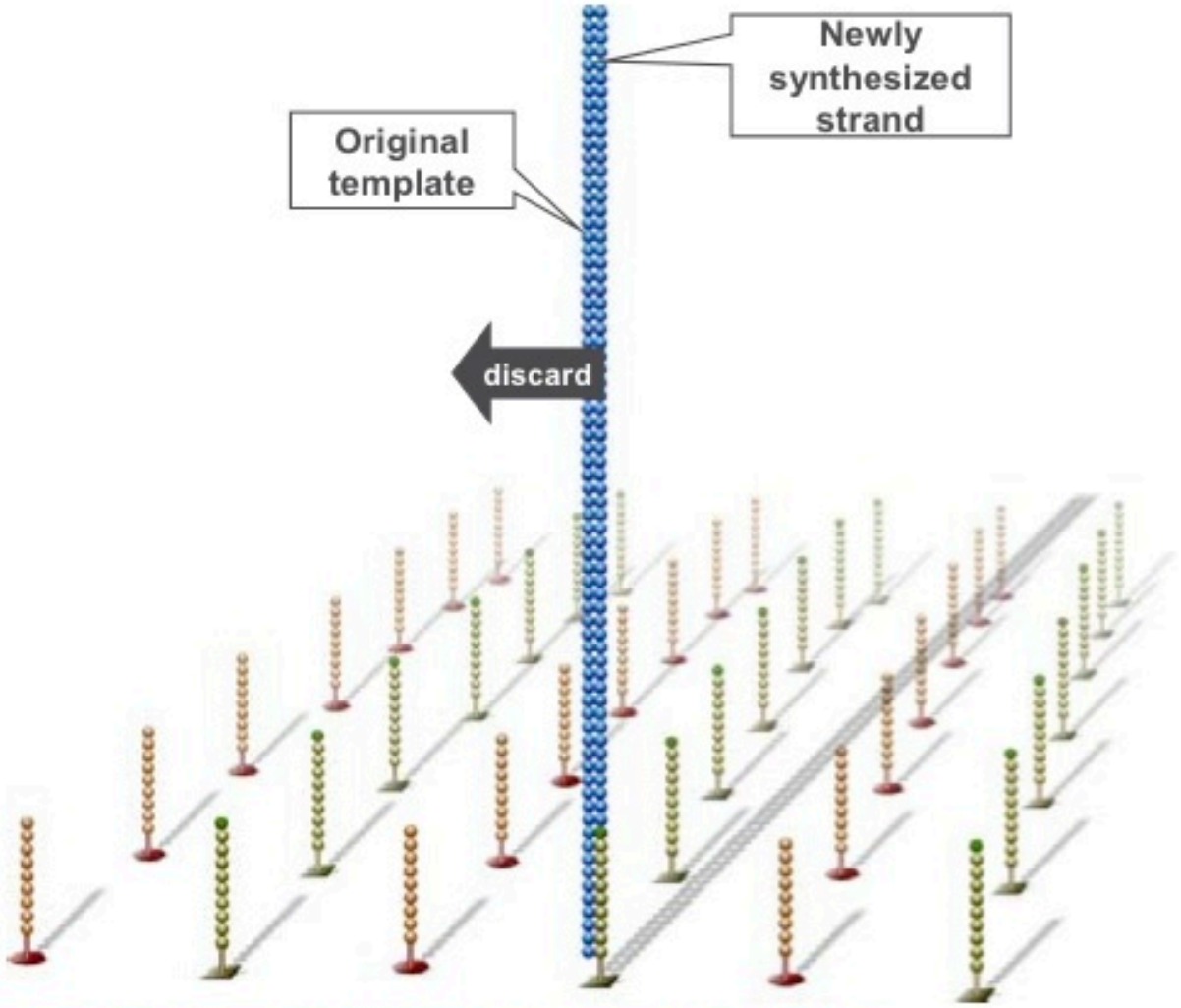


NOTE:
Single molecules bind to flow cell in a random pattern



# Denature Double-Stranded DNA

- Double-stranded molecule is denatured
- Original template washed away
- Newly synthesized strand is covalently attached to flow cell surface



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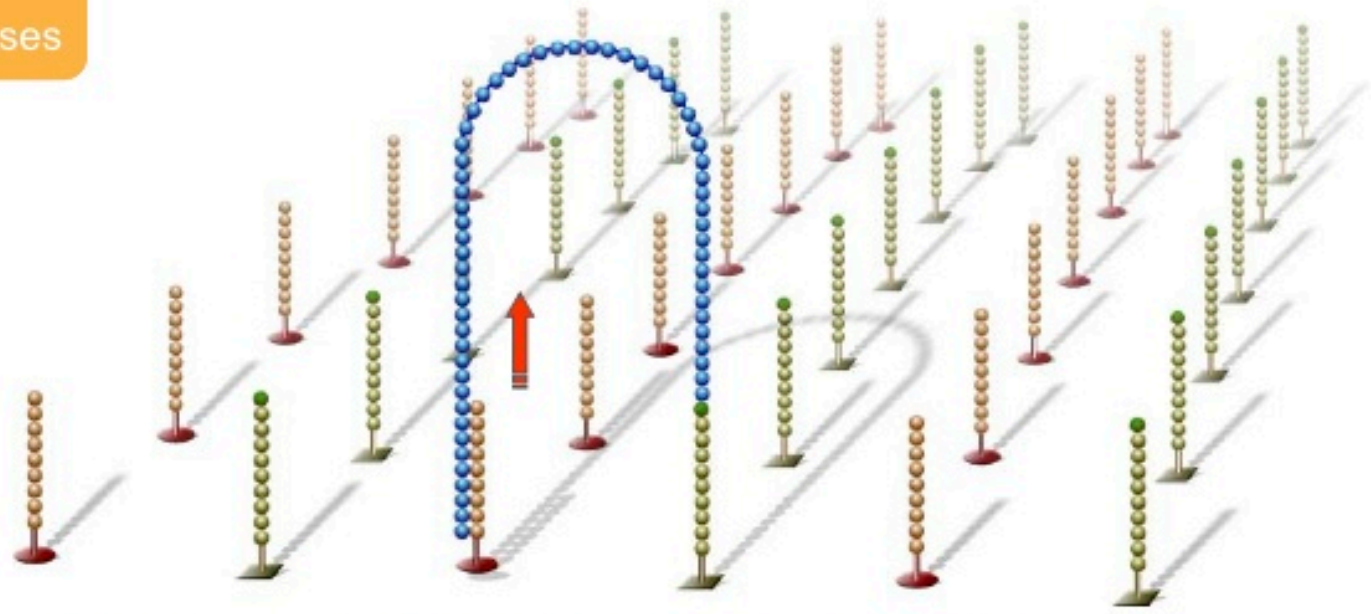
# Bridge Amplification

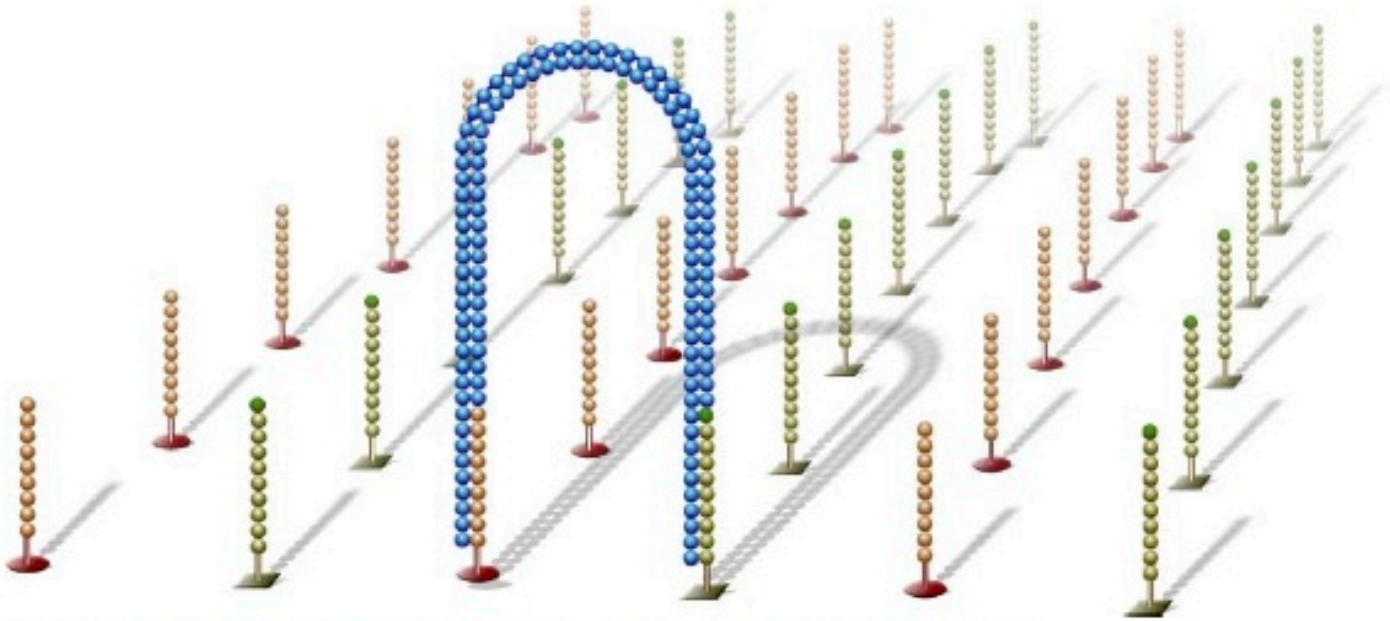
Single-stranded molecule flips over and forms a bridge by hybridizing to adjacent, complementary primer

Hybridized primer is extended by polymerases



# Bridge Amplification

Double-stranded bridge is formed





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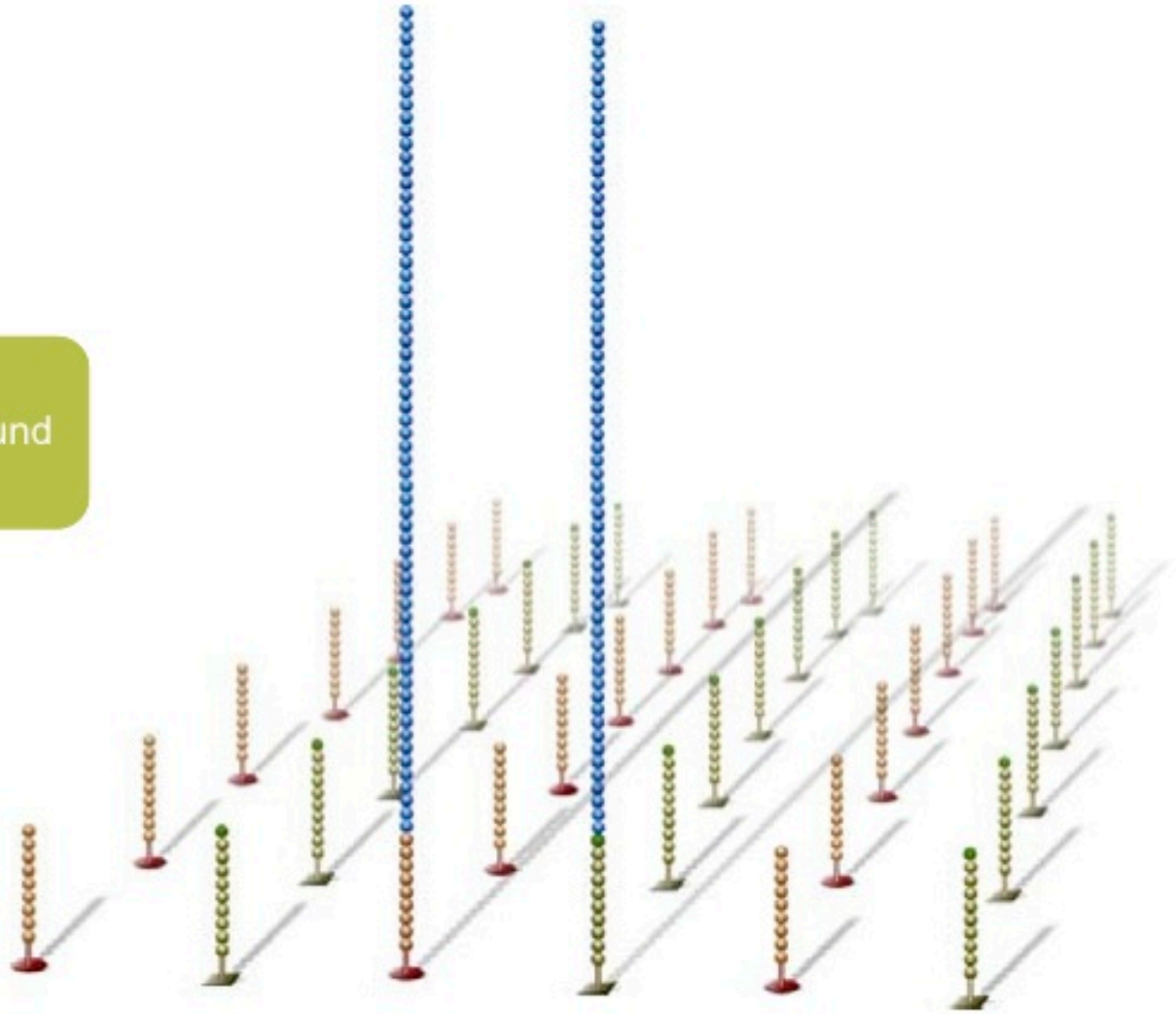
# Denature Double-Stranded Bridge

Double-stranded bridge is denatured

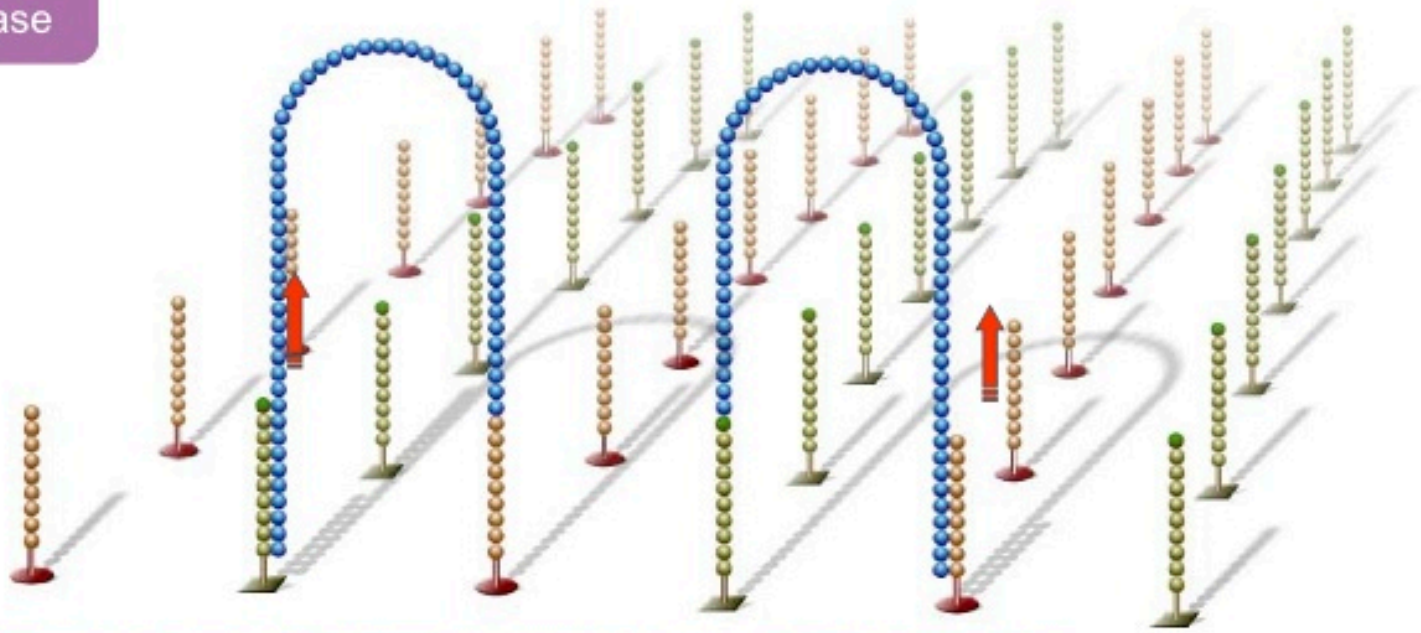
Result:
Two copies of covalently bound single-stranded templates



# Bridge Amplification

Single-stranded molecules flip over to hybridize to adjacent primers

Hybridized primer is extended by polymerase





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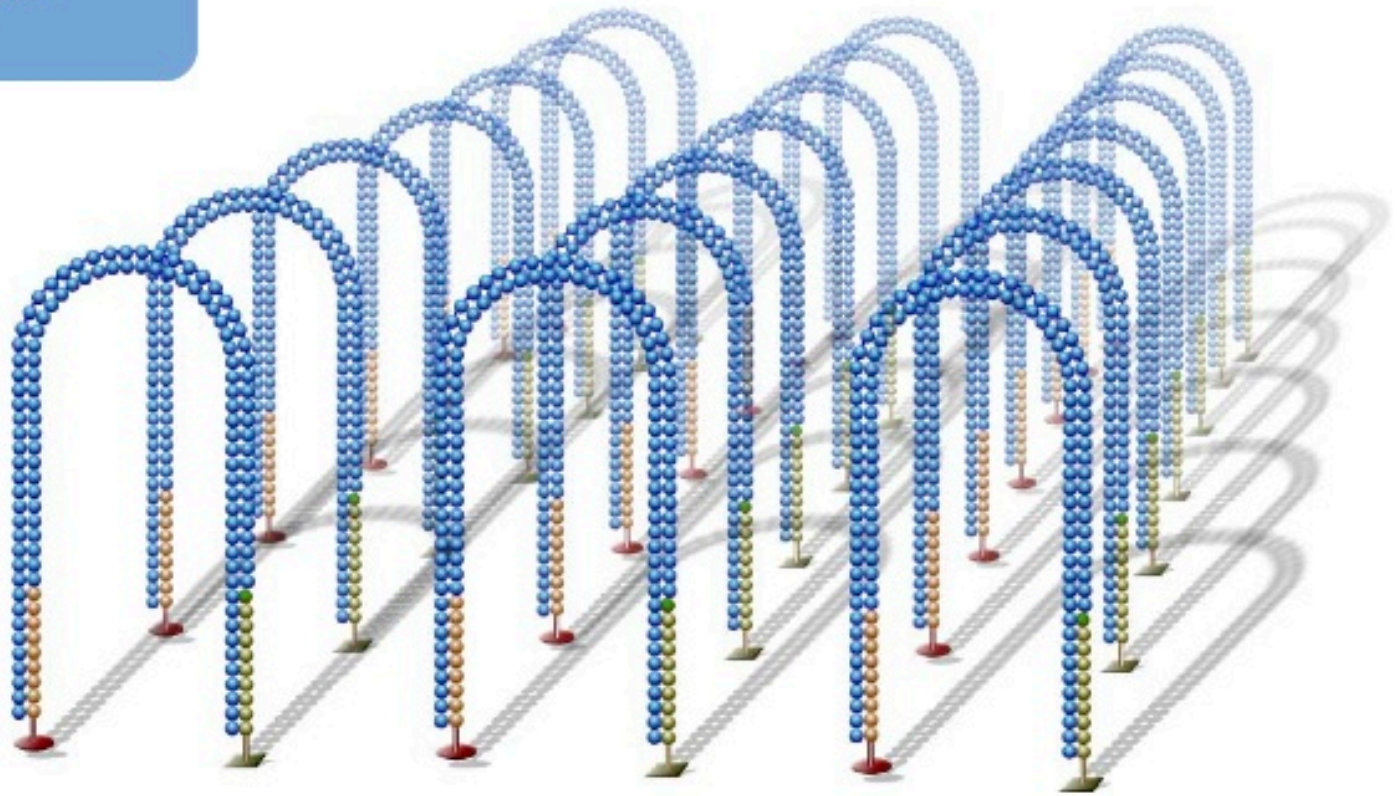
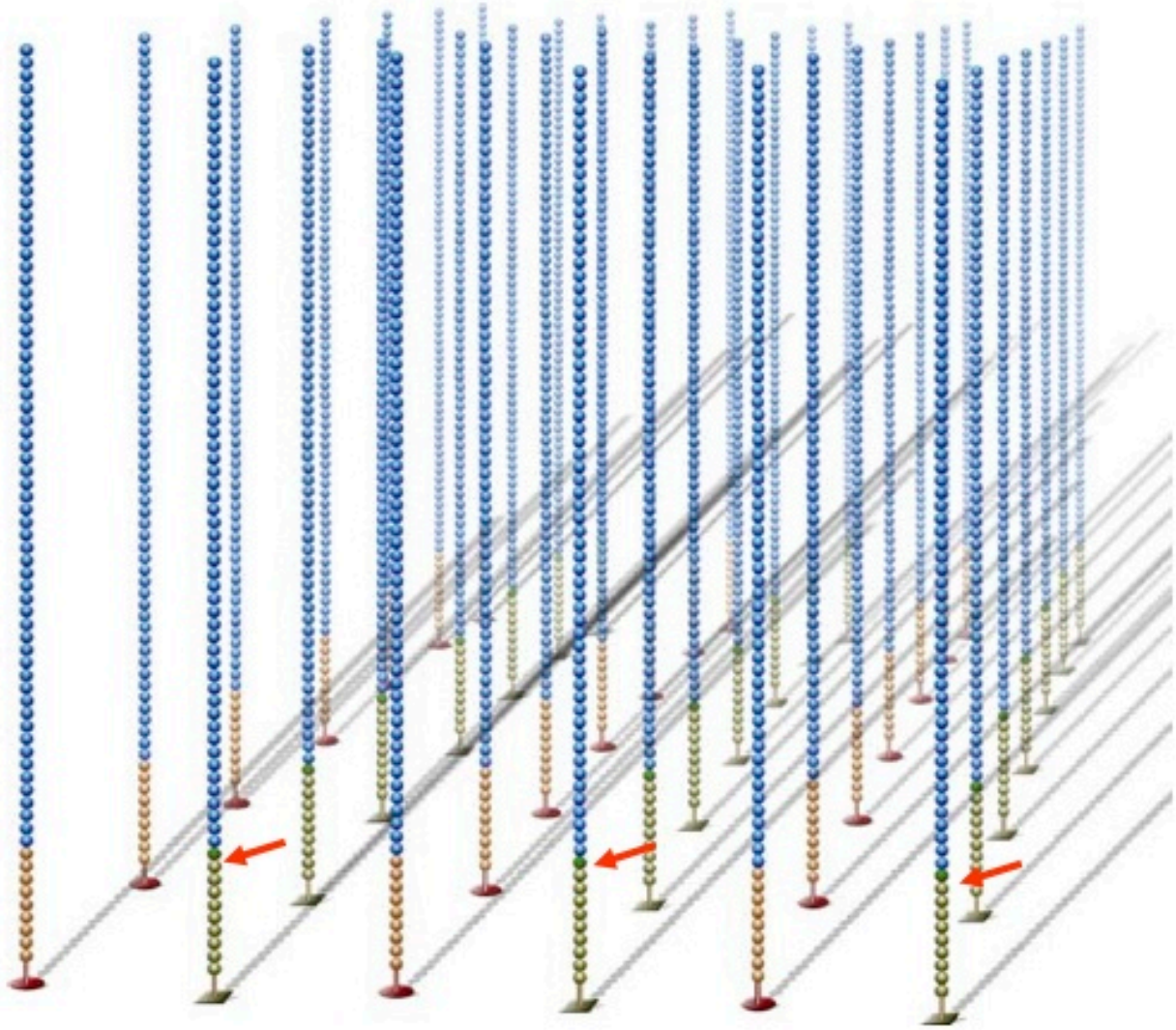
# Bridge Amplification

Bridge amplification cycle repeated until multiple bridges are formed



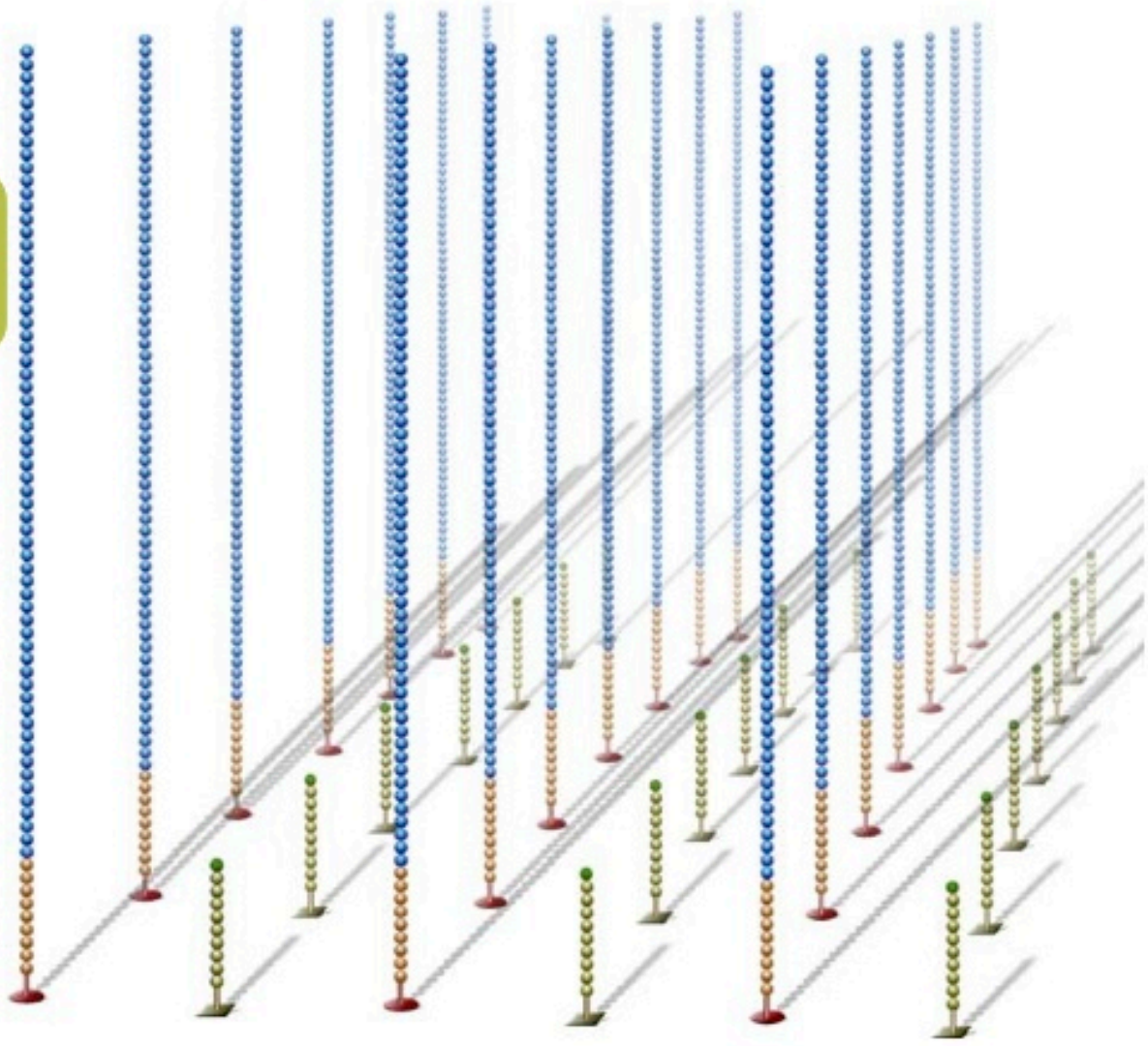
# Linearization

dsDNA bridges are denatured



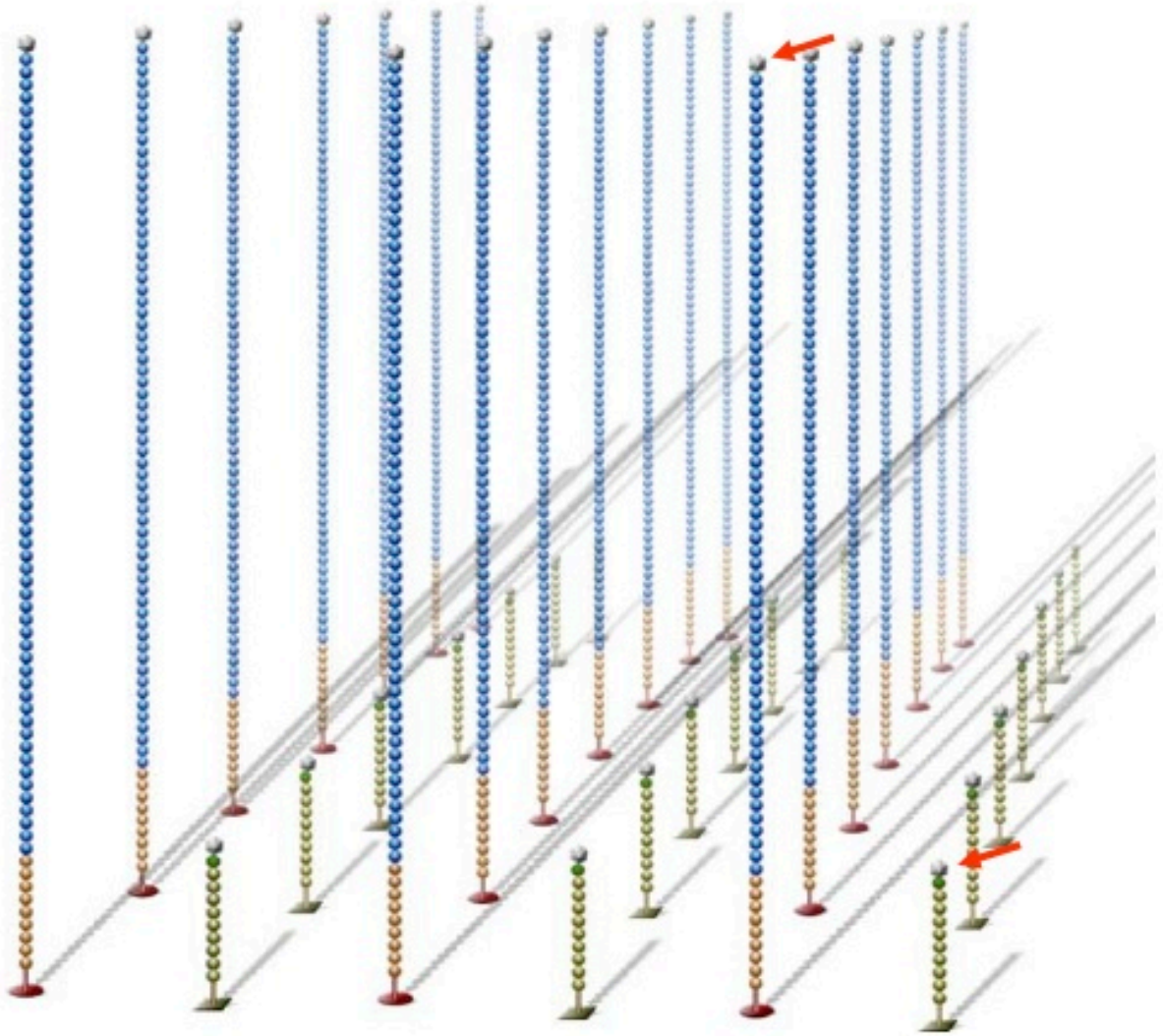
# Reverse Strand Cleavage

Reverse strands cleaved and washed away, leaving a cluster with forward strands only



# Blocking

Free 3' ends are blocked to prevent unwanted DNA priming





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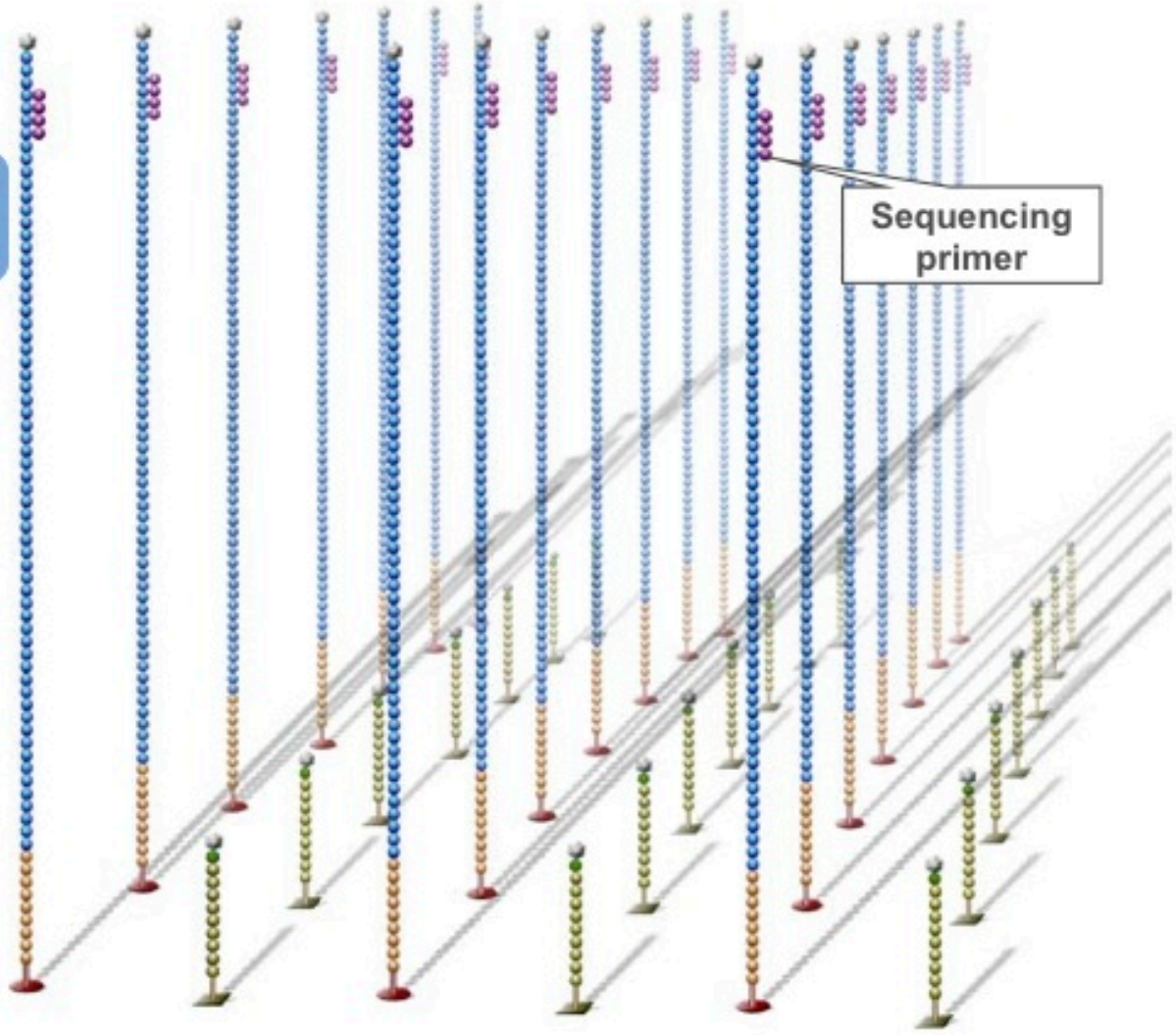
# Read 1 Primer Hybridization

Sequencing primer is hybridized to adapter sequence



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# MiSeq Sequencing Workflow

1 Library Preparation

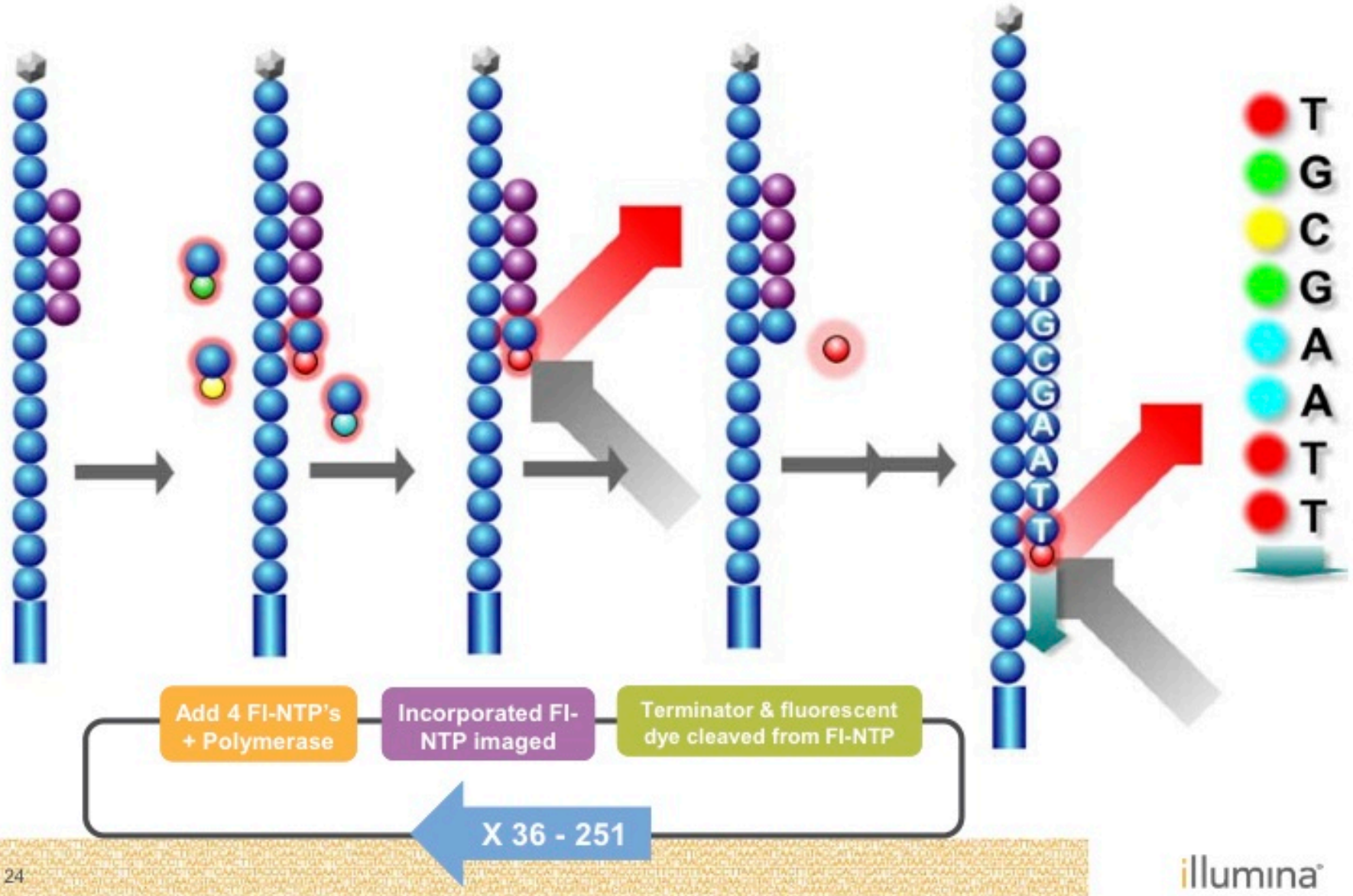


2 Cluster Generation

3 Sequencing

4 Data Analysis

# Sequencing by Synthesis



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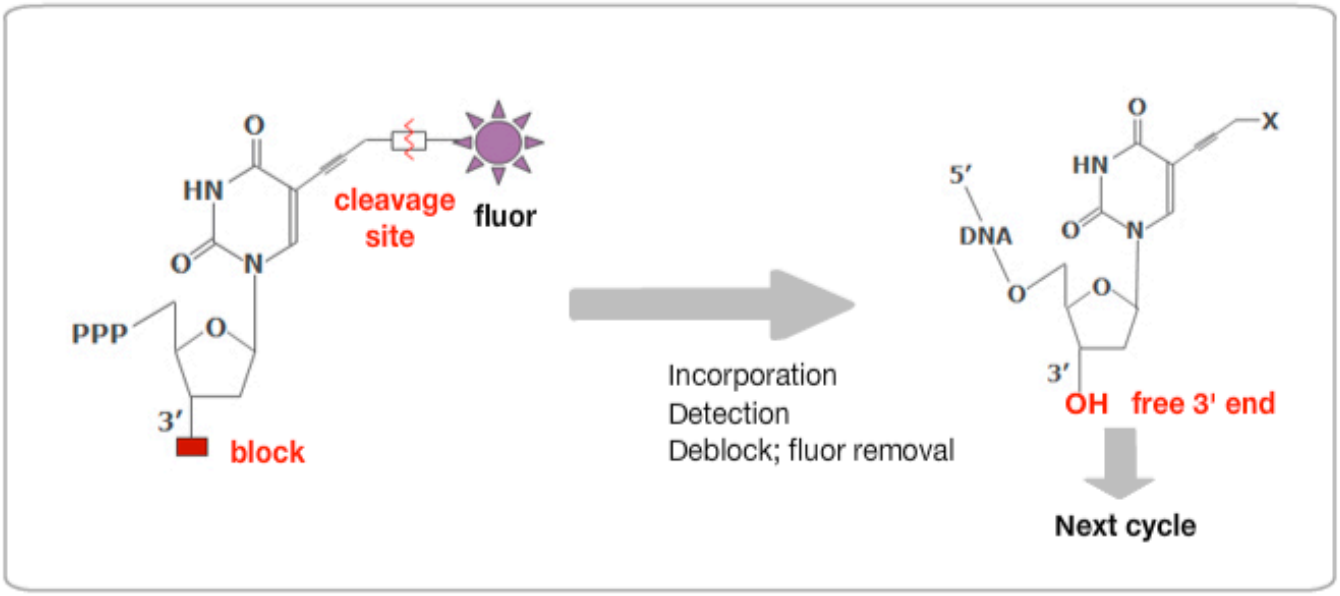
# MiSeq Sequencing Chemistry

Menu Sequencing

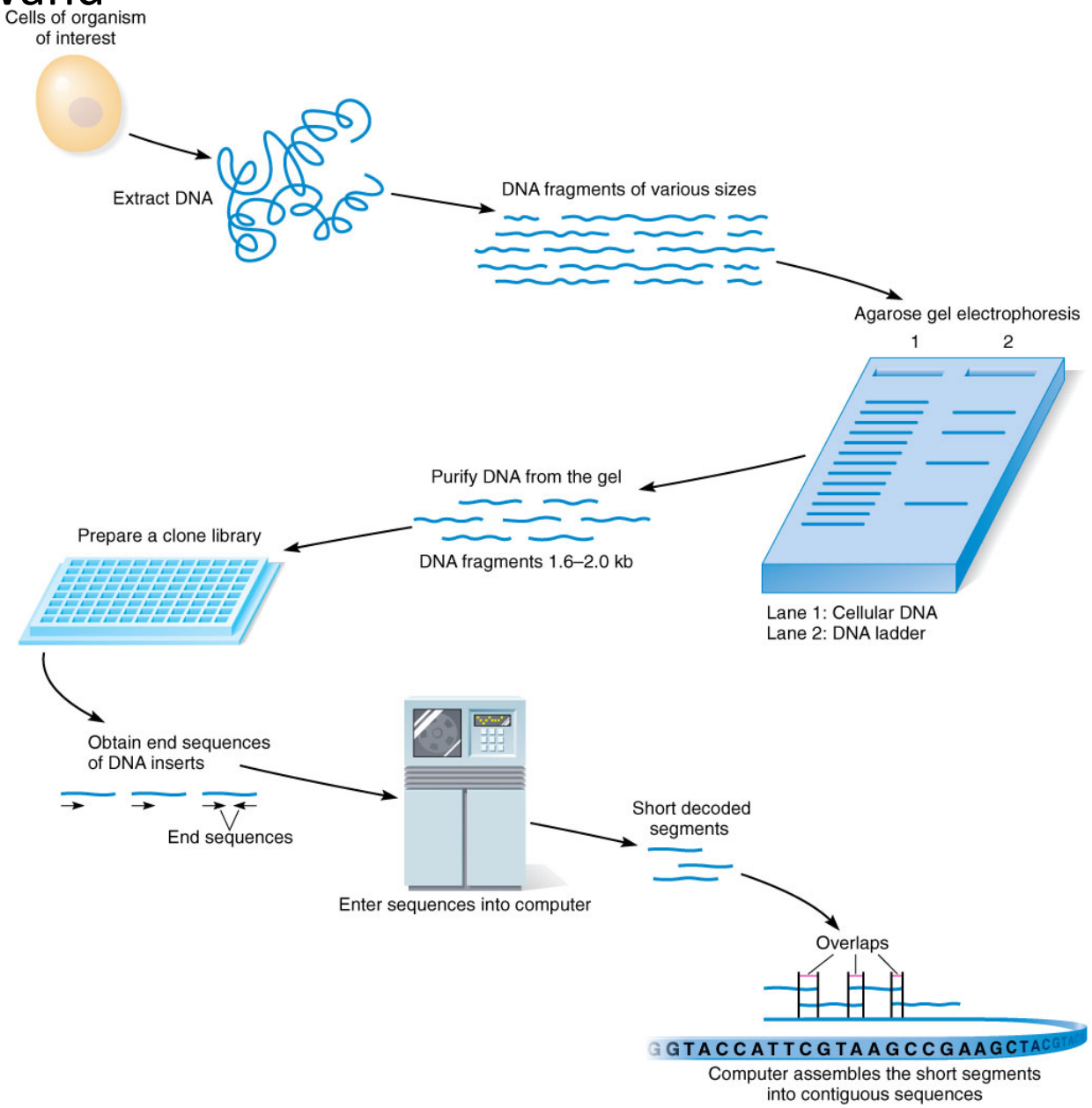
## Sequencing

The MiSeq sequences the DNA clusters using Illumina's Sequencing By Sythesis (SBS) Chemistry which relies on Reversible Terminator Chemistry (RTC).

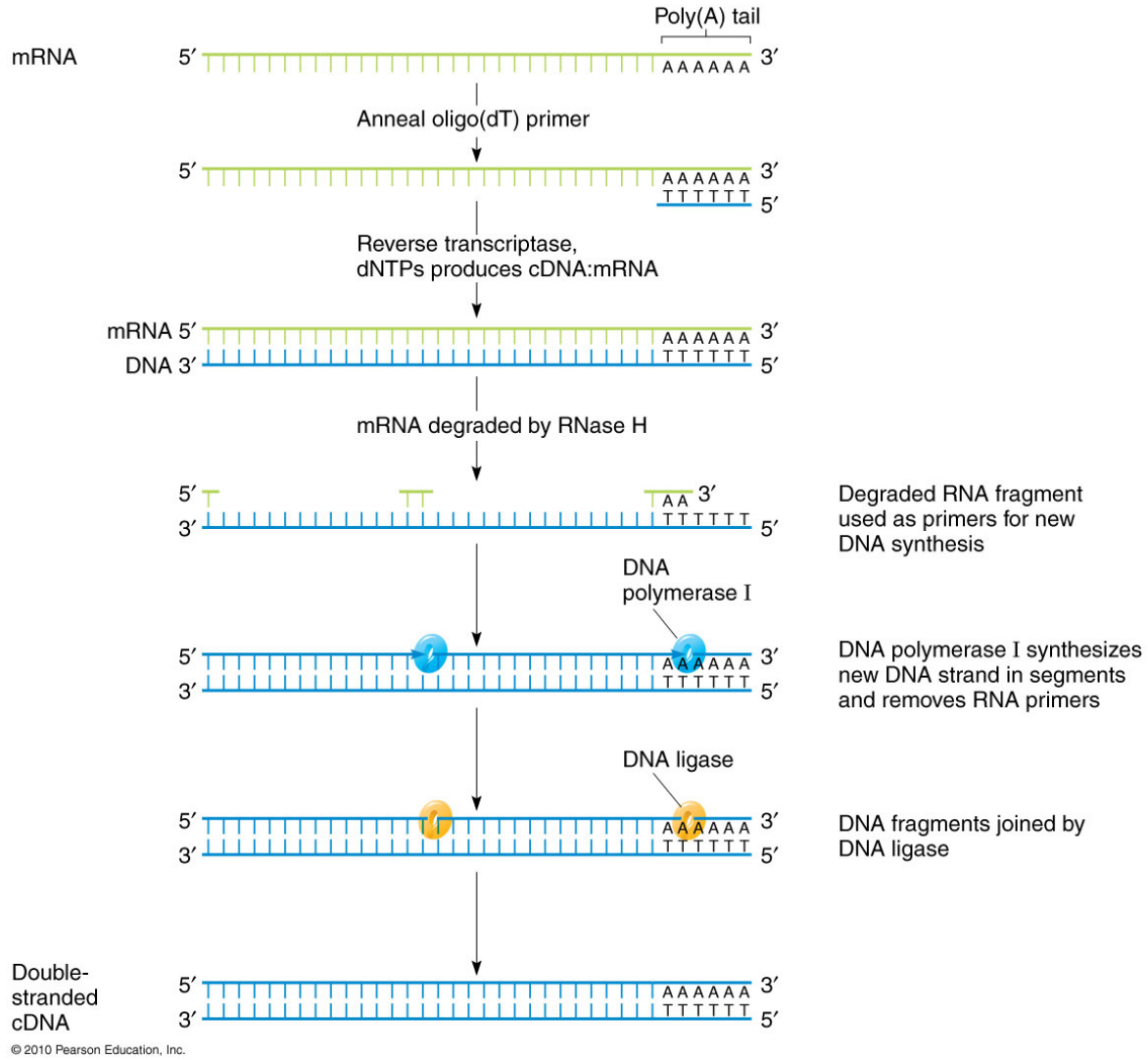
- All 4 labeled nucleotides in 1 reaction
- Higher accuracy



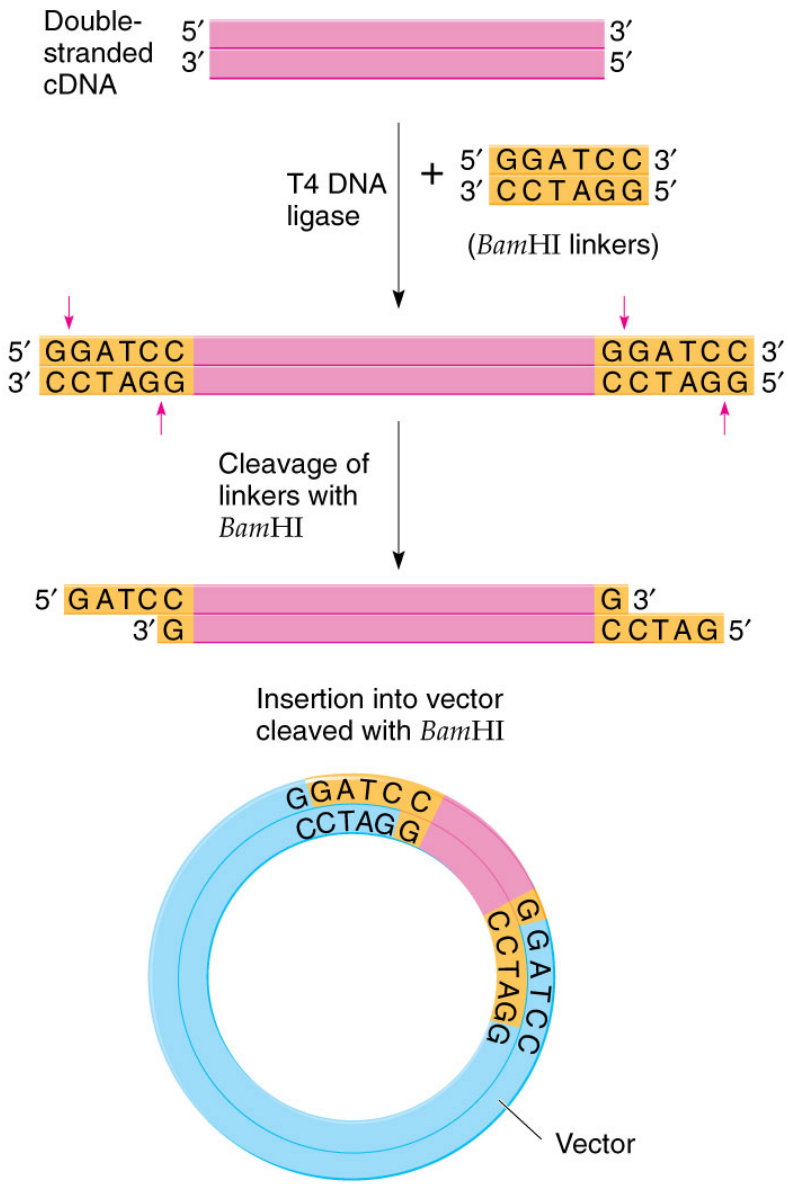
# Assemblierung von genomischen Sequenzen erfordert grossen Rechneraufwand



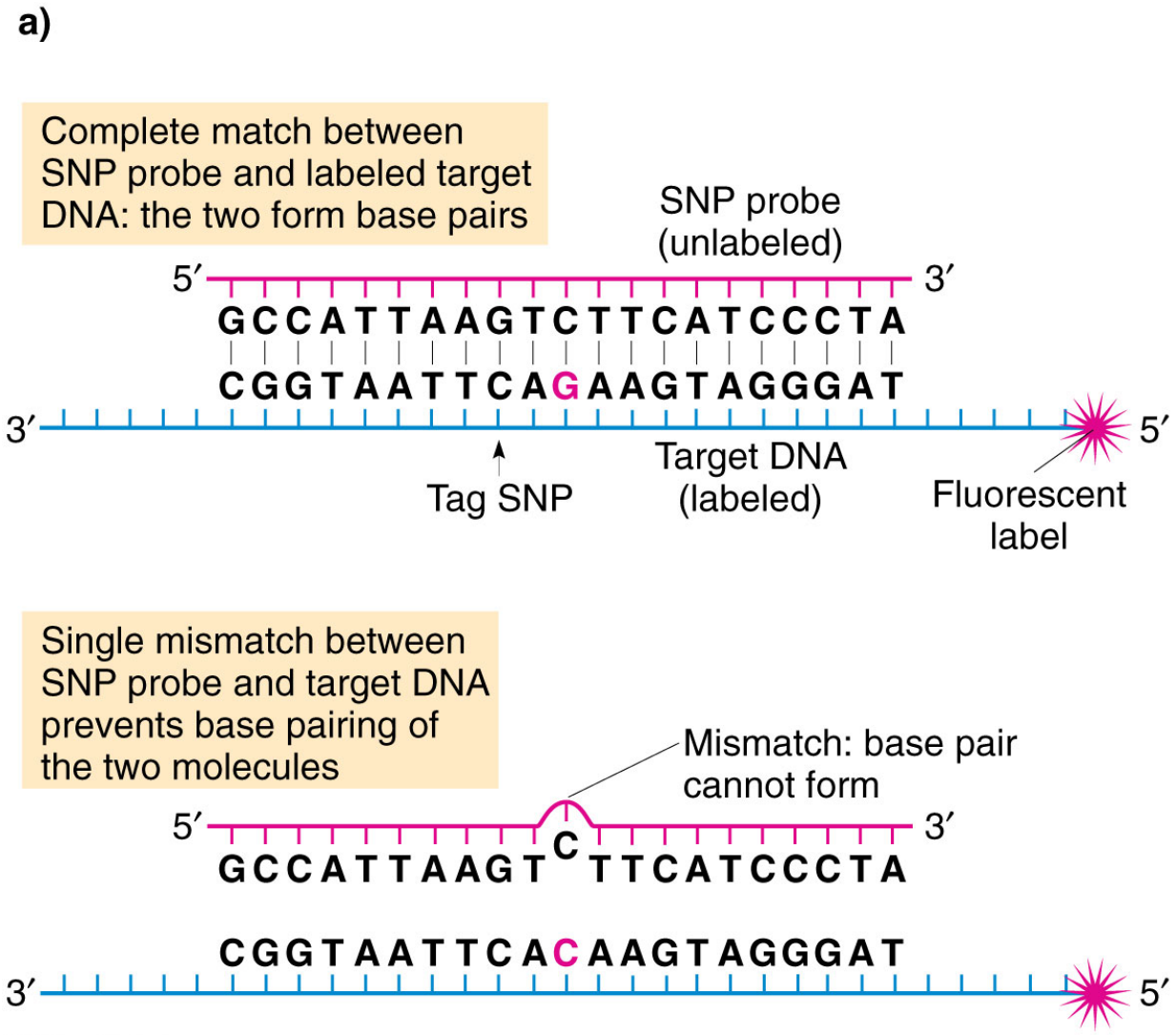
cDNA Banken: mRNA wird mittels Hybridisierung an oligo dT Säulen gereinigt. Von einem oligo dT primer wird mittels REVERSER TRANSKRIPTASE eine cDNA hergestellt. DNA-Polymerase stellt den zweiten DNA Strang her.



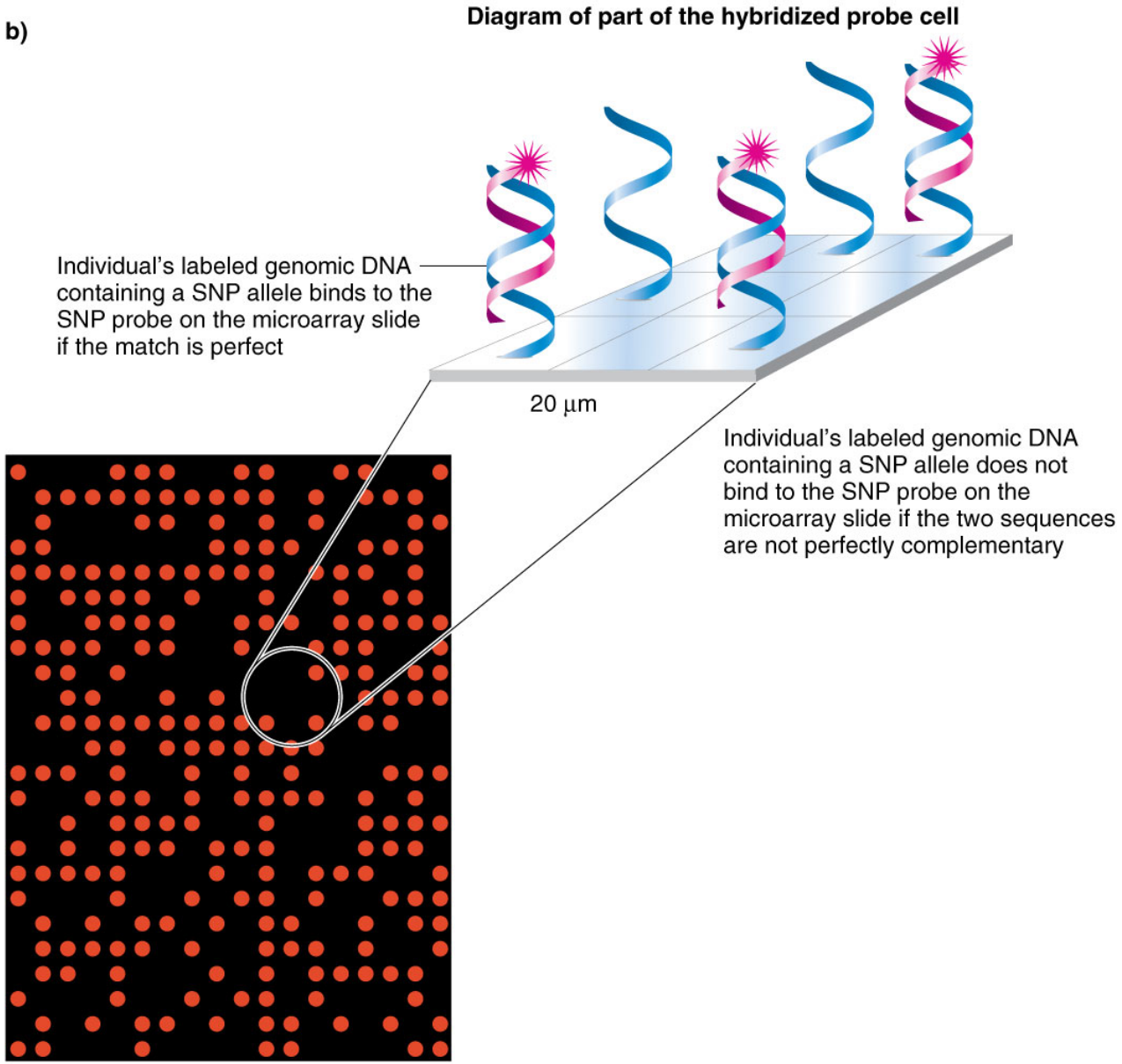
# Die cDNA Fragmente werden mittels LINKER in einen Vektor kloniert



Variationen in Genomen können mit Single nucleotide polymorphisms (SNPs) identifiziert werden. Korrelationen von SNPs und bestimmten Merkmalen können für die Bestimmung komplexer Erbgänge verwendet werden.



SNP-maps können mittels Microarrays identifiziert werden.
Auf einem Objektträger sind Oligonukleotide, welche das gesamte Genom abdecken aufgebracht. An diese Sequenzen können markierte Sequenzen hybridisiert werden.



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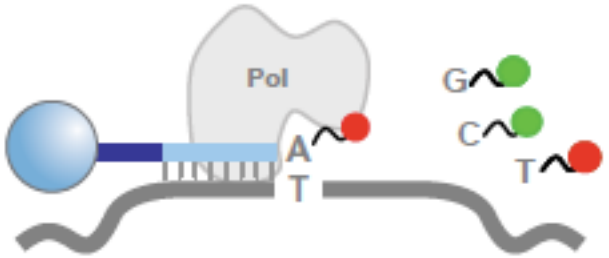
## Two-Step Allele Detection


**Step 1. Selectivity**
Hybridization of unlabeled DNA fragment to 50-mer probe on array




**Step 2. Specificity**
Enzymatic single base extension with labeled nucleotide

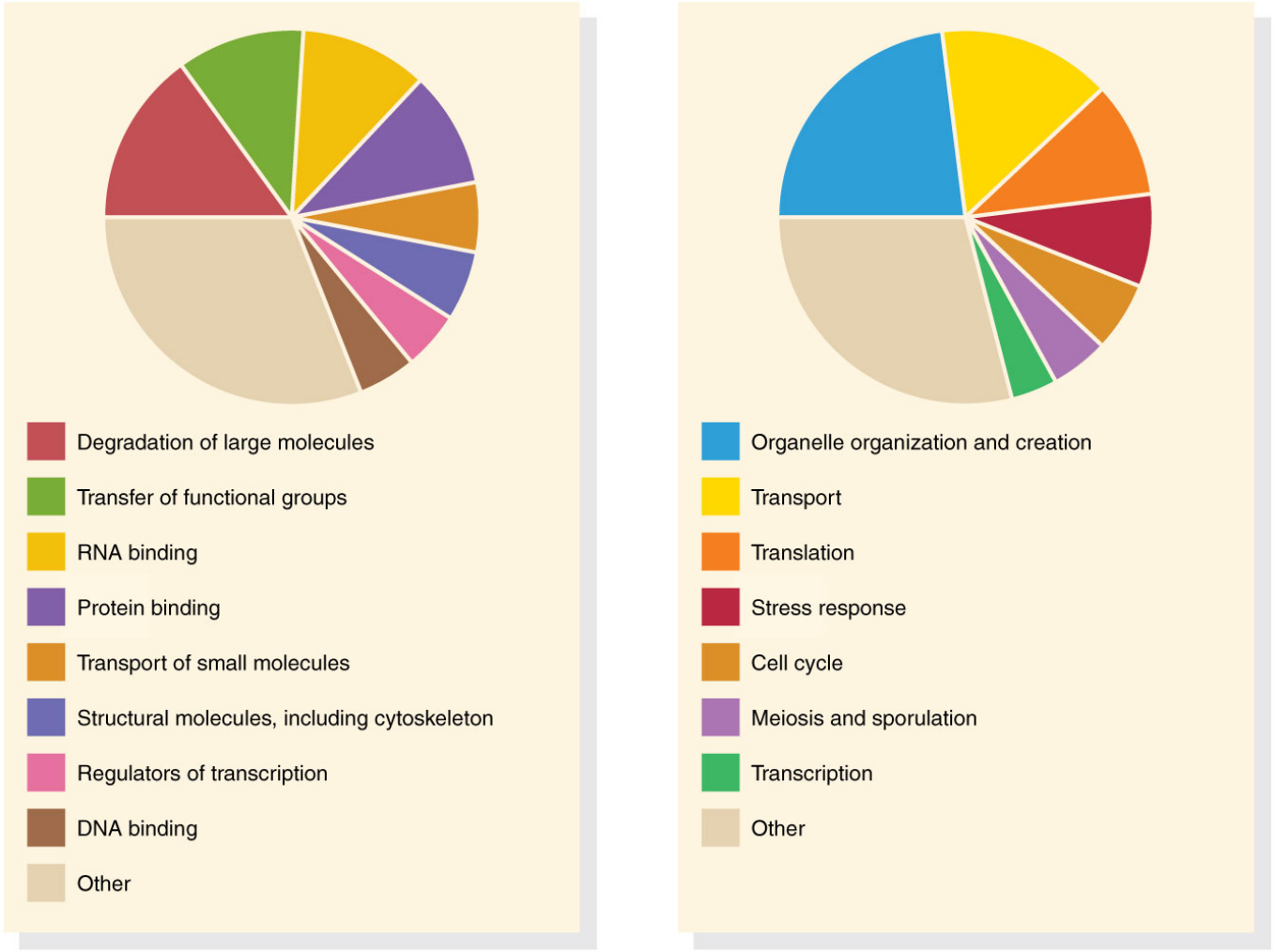
SNP Variationen in einer isolierten Population (zB Island) können gut zur Identifikation von genetischen Risikofaktoren und Prädispositionen herangezogen werden. Die Isländische Firma DeCode Genetics hat Stammbäume aller Isländer und deren Krankengeschichte zur Identifikation derartiger Risikofaktoren verwendet und genetische Tests hierfür vermarktet.

Genanalyse im 21. Jahrhundert:
Vergleichende Genomik.
Komplett sequenzierte Genome erlauben die Identifikation homologer Gene mittels Bioinformatik.

```
Query 2072  RPRPY--PPNVGQEALSQTTISWAPFQDT 2098
            +  P       GQEALSQTTISW PFQ++
Sbjct 1982  KSEPLIGRKKTGQEALSQTTISWTPFQES 2010
```

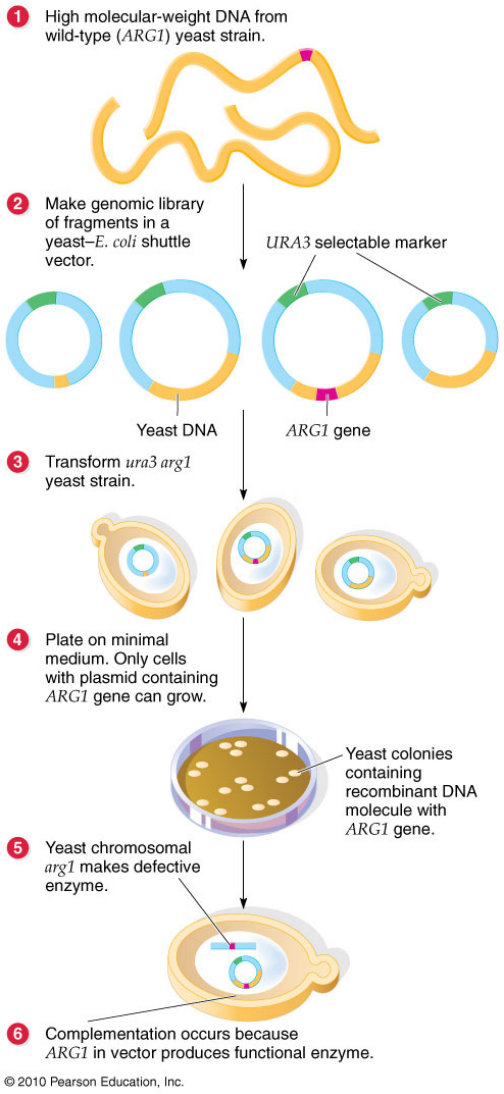
Bioinformatische Vergleiche erlauben die funktionelle Zuordnung vieler Gene.

Hier: Mehr als 60 % aller S. cerevisiae Gene kann eine Funktion aufgrund bioinformatischer Ähnlichkeiten zugeordnet werden.



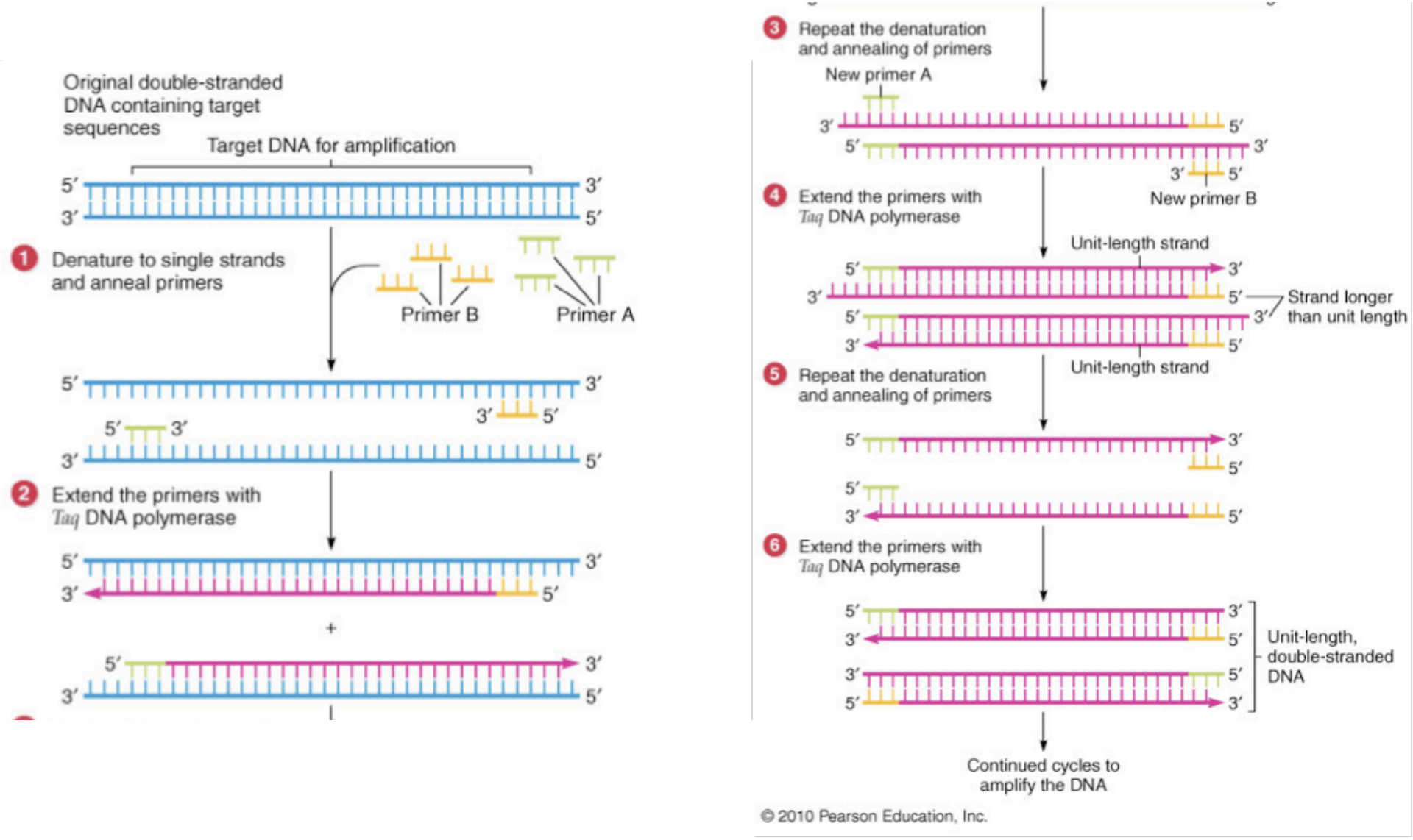
# Isolation von homologen Genen mittels Komplementation

Hier: Hefe DNA wird in einen Expressionsvektor kloniert.
Alle Plasmide werden in S. cervisiae Zellen transformiert welche KEIN arginin synthetisieren können.
Die Rekombinanten Zellen werden auf Wachstum auf Medium OHNE Arginin getestet. Nur Zellen, welche das fehlende Gen am Plasmid enthalten können wieder wachsen und Kolonien bilden.



Reverse Genetik: Vom Gen zum Phän.
Erlaubt die Analyse von Gen-Funktionen aufgrund spezifischer Inhibition eines bestimmten Genes

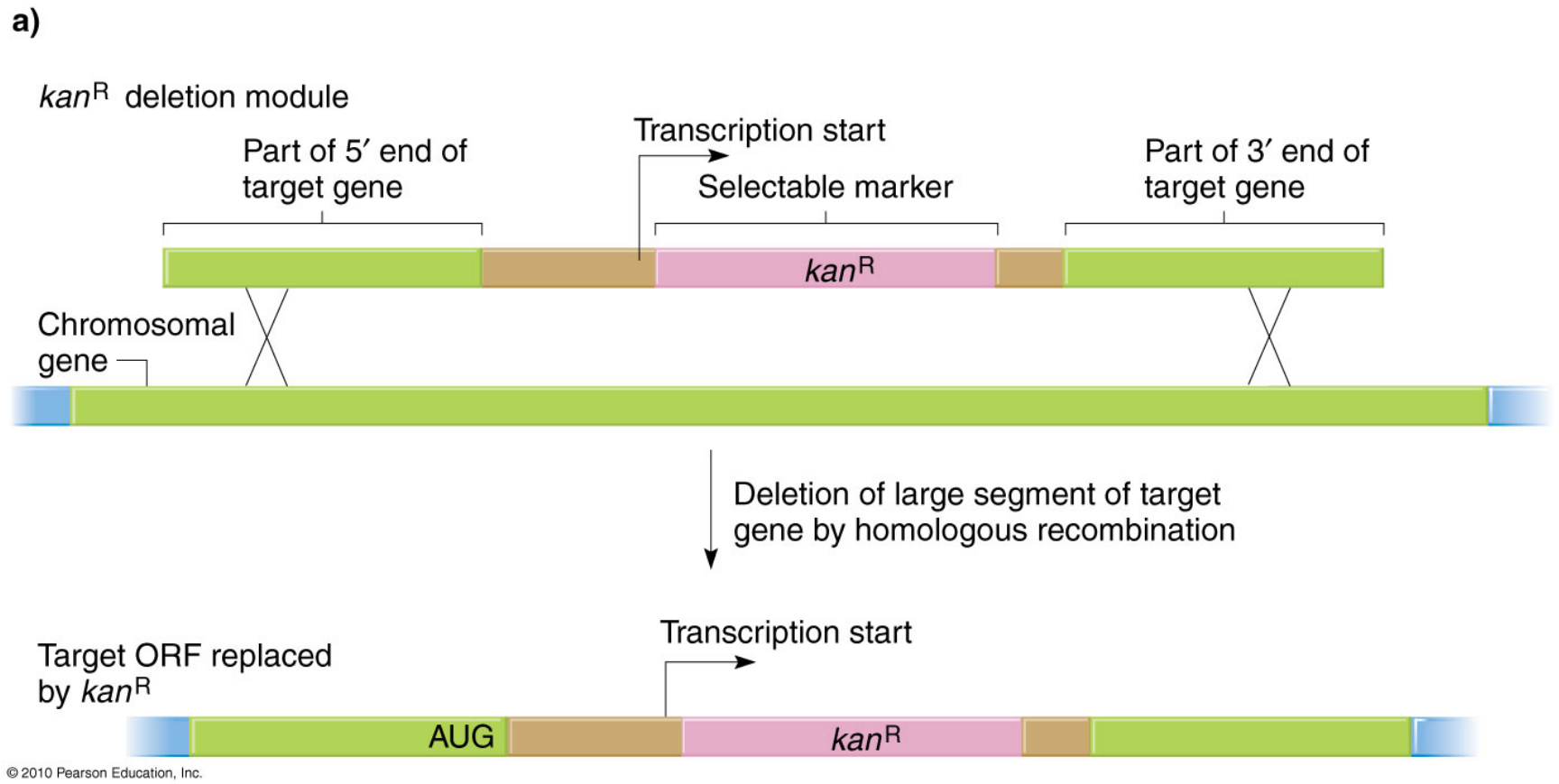
# Isolation eines bestimmten Gens mittels Polymerase Chain Reaction (PCR)



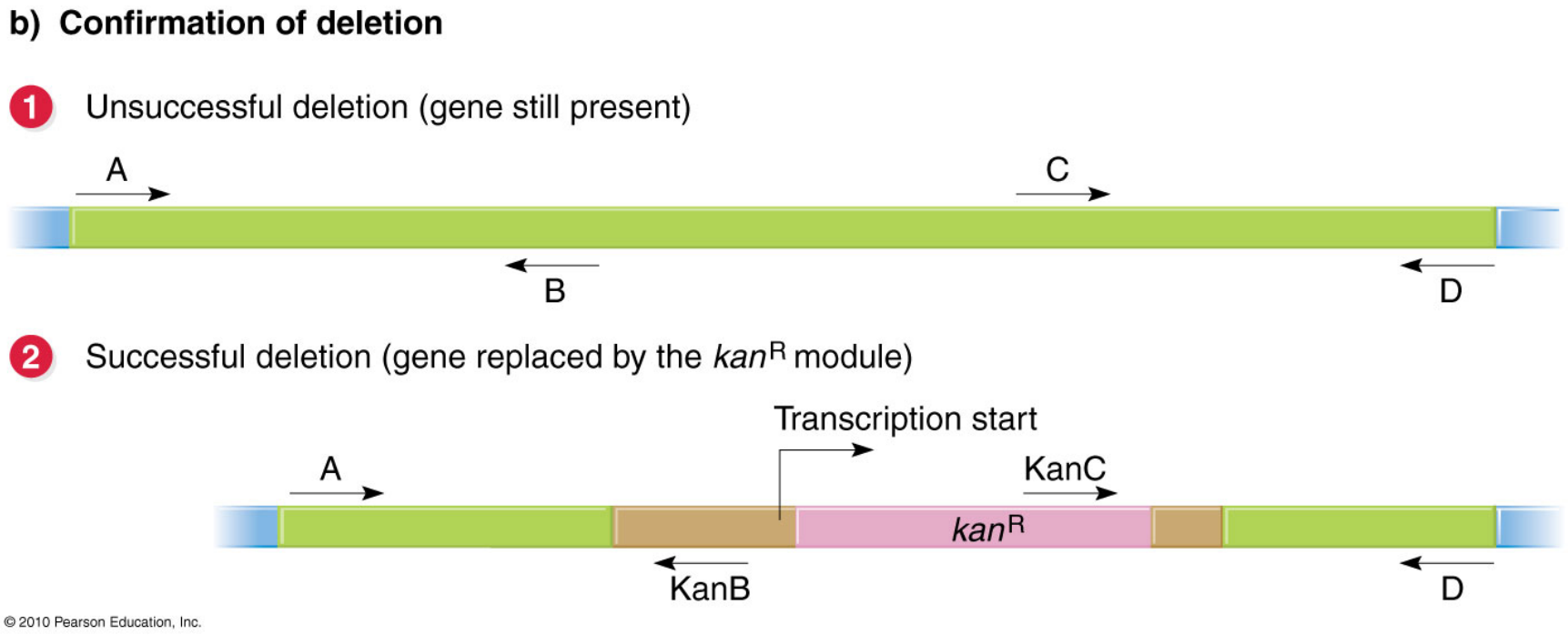
Voraussetzung für die erfolgreiche Anwendung der PCR war die Isolierung von thermostabilen DNA-Polymerasen. Diese wurden in Thermophilen Prokaryoten gefunden. *Thermus aquaticus, Pyrococcus woisei, Pyrococcus furiosus.*

Diese Organismen leben zum Grossteil in heissen Quellen

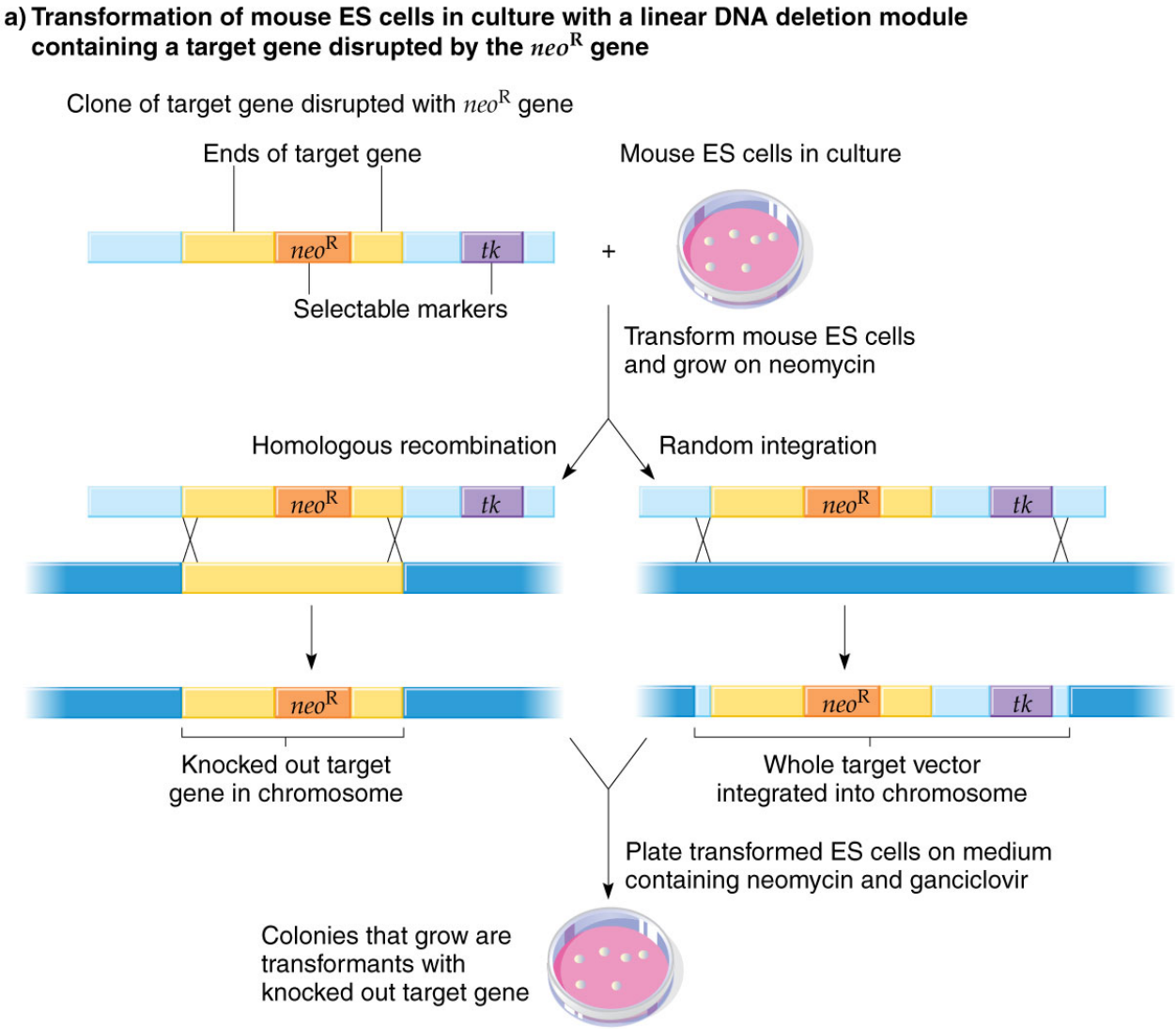
Das Gen, welches mittels PCR amplifiziert wurde, wird in einen Vektor kloniert und kann mit einem Resistenzgen zerstört werden.
Das resultierende Konstrukt kann mittels homologer Rekombination das endogene Gen zerstören.



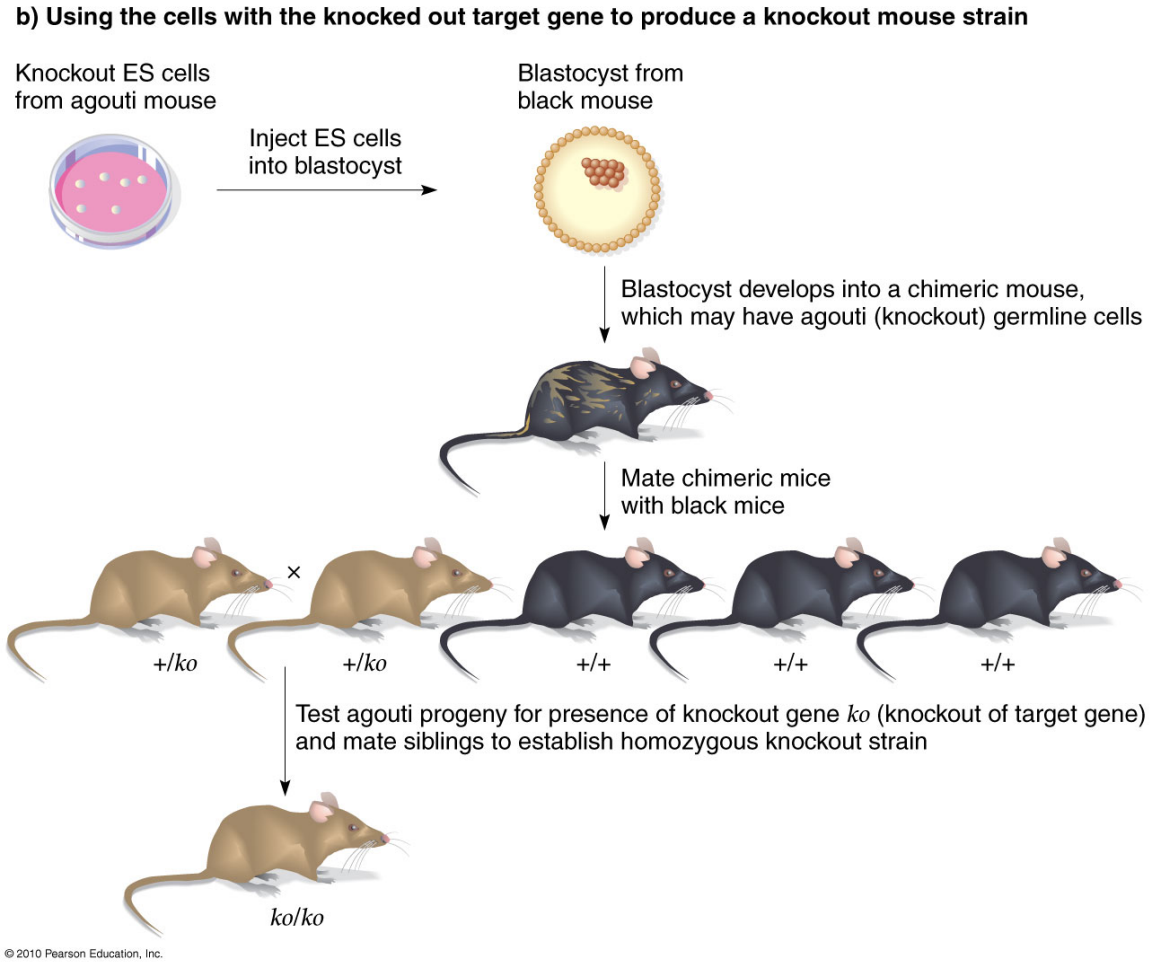
# Die erfolgreiche Insertion des Konstrukts wird wieder mittels PCR überprüft



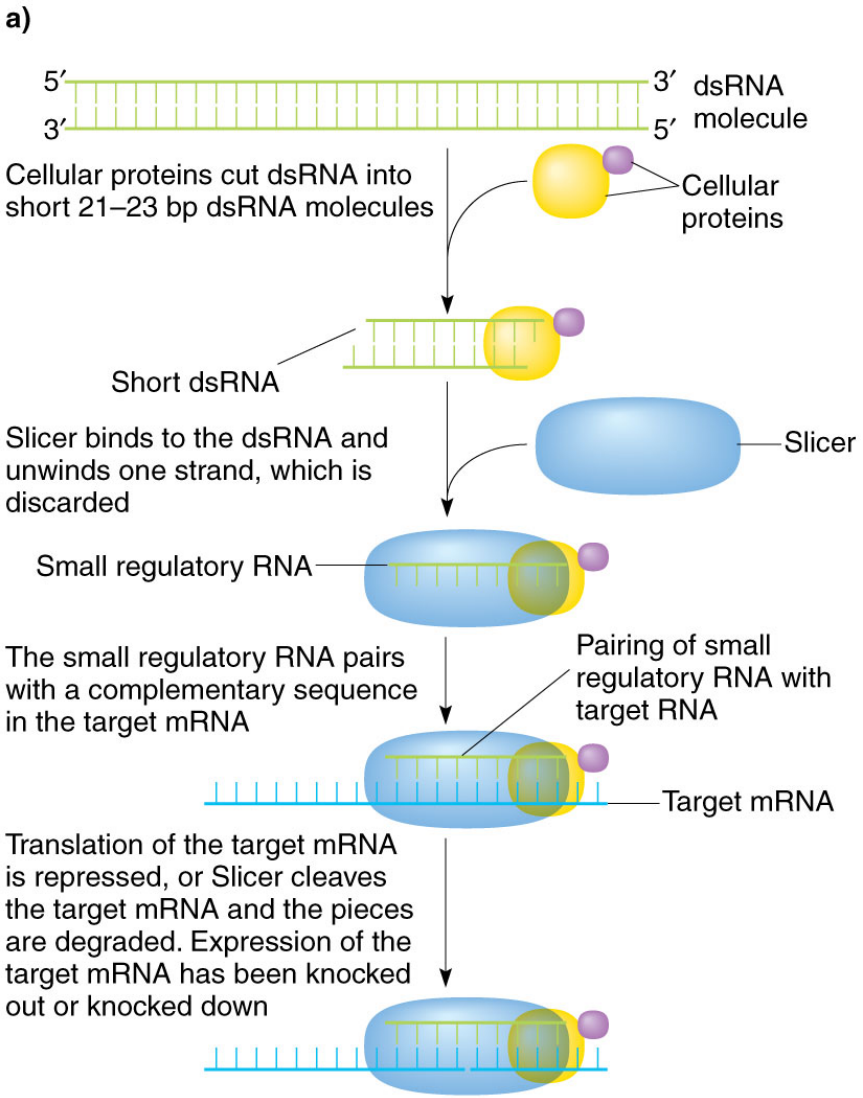
Um rekombinante Mäuse zu generieren, wird das Zielgen in Embryonalen Stammzellen (ES) Zellen durch homologe Rekombination zerstört.



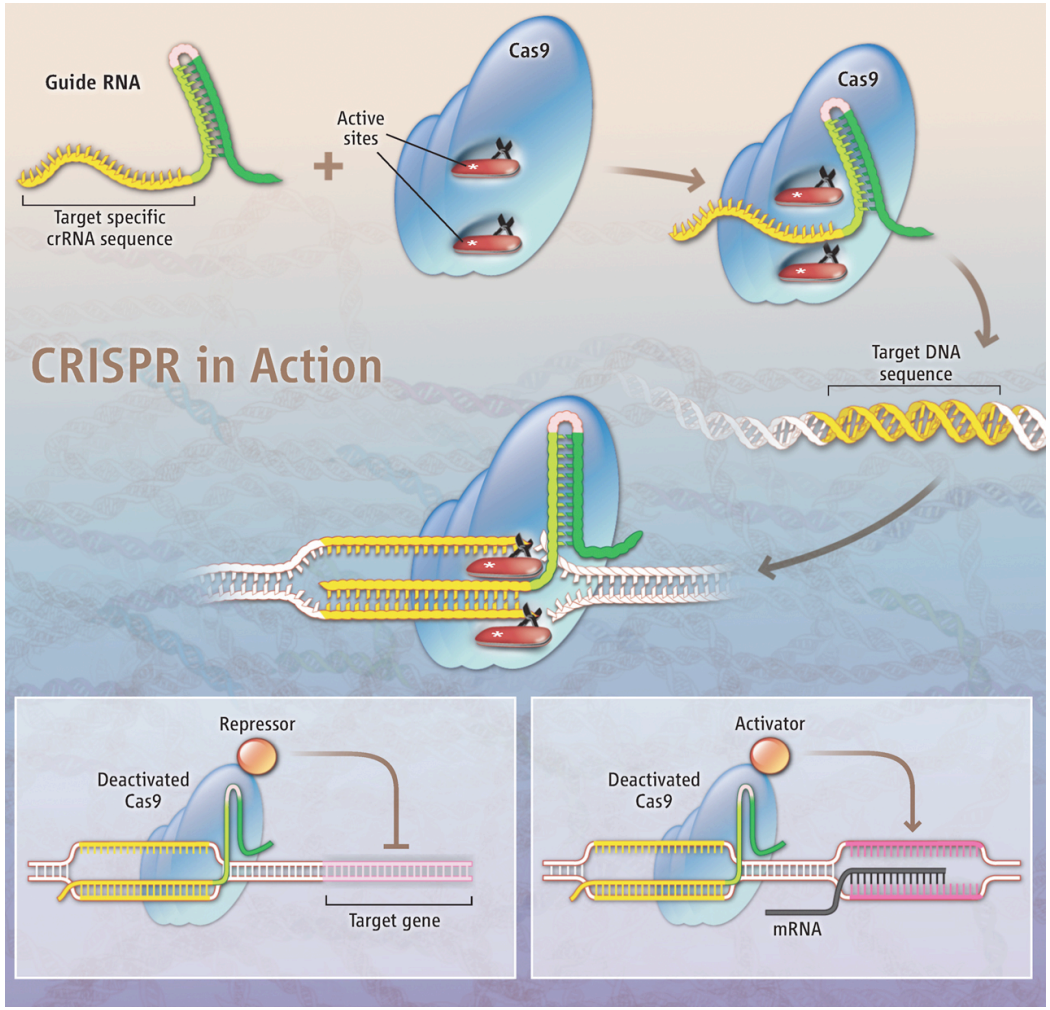
Rekombinante ES Zellen werden in Blastozysten injiziert, wo diese zum sich entwickelnden Embryo beitragen. Die Blastozysten werden in scheinschwanger Mäuse injiziert. Ist die Injektion erfolgreich entsteht eine chimäre Maus. Haben die transgenen Zellen zur Keimbahn beigetragen, kann eine heterozygote Maus entstehen, die anschliessend homozygotisiert wird.



# Reverse Genetik kann auch mittels RNA-interferenz (RNAi) durchgeführt werden



# Gene Manipulation mittels CRISPR/ Cas9



# Viel Erfolg!