

Methoden zum Studium der Genregulation

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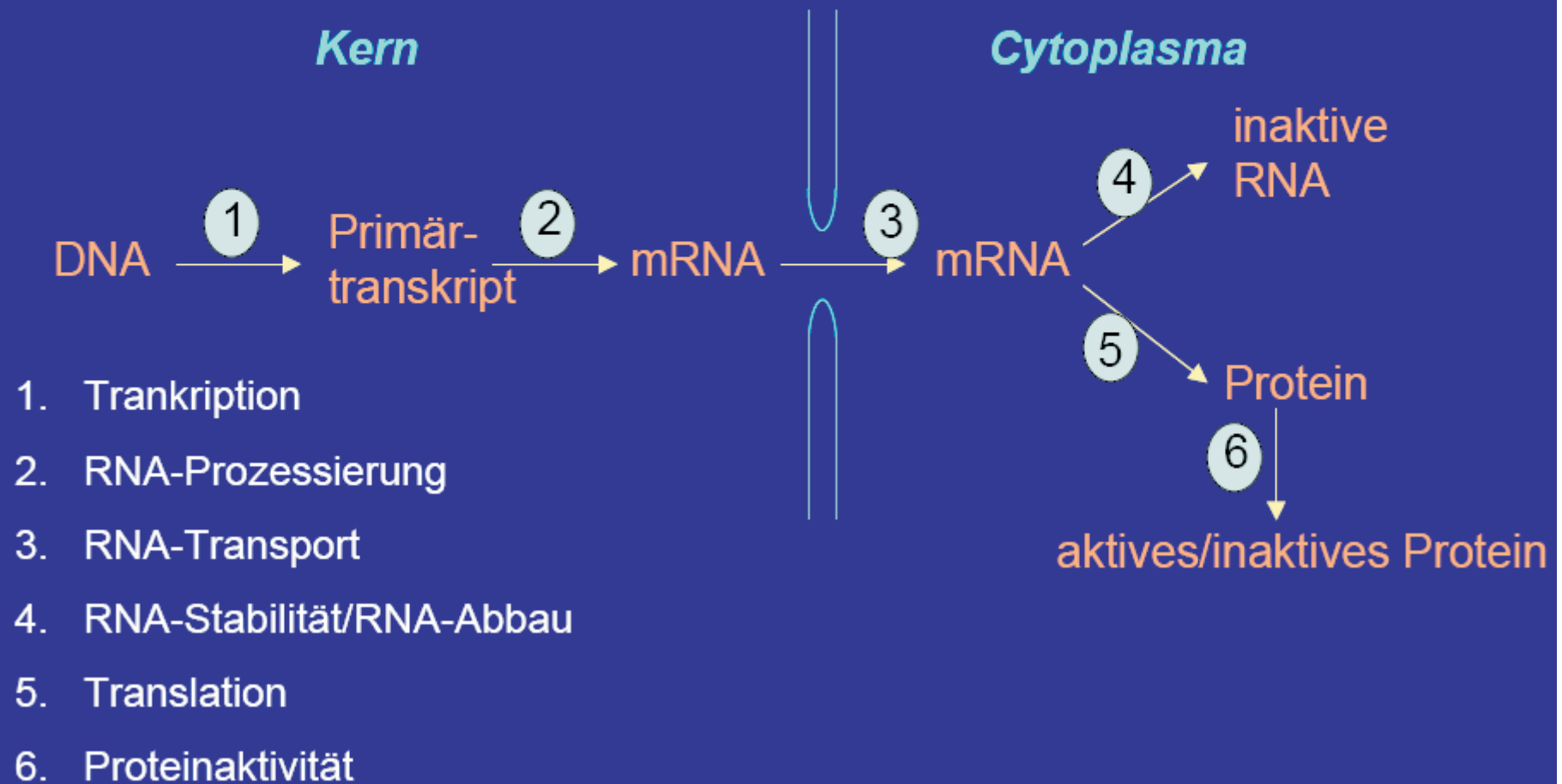
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Ebenen zur Regulation der Genexpression



Methoden zum Studium der Genregulation

Isolierung von RNA

- Gesamt-RNA
- m-RNA

Isolierung von Gesamt-RNA



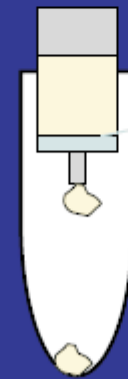
Lyse der Zellen (Guanidiniumisothiocyanat,
 β -ME, N-Lauroylsarkosin)



Phenol/Chloroform Extraktionen



Fällung der RNA

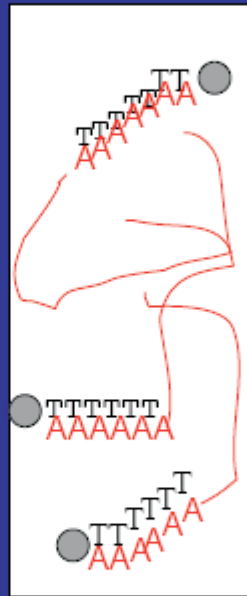


Silicagel Membran

mRNA Isolierung

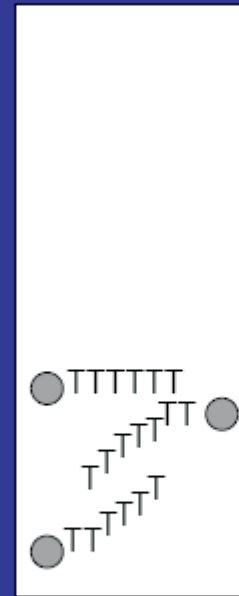
Gesamt-RNA

PolyA⁺-RNA
bindet

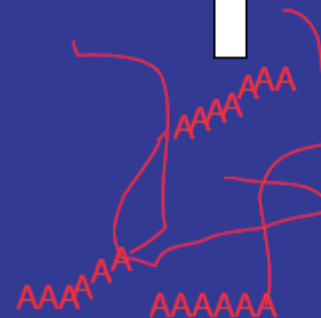


tRNA, rRNA
Durchlauf

Elution der mRNA



eluierte
PolyA⁺-RNA

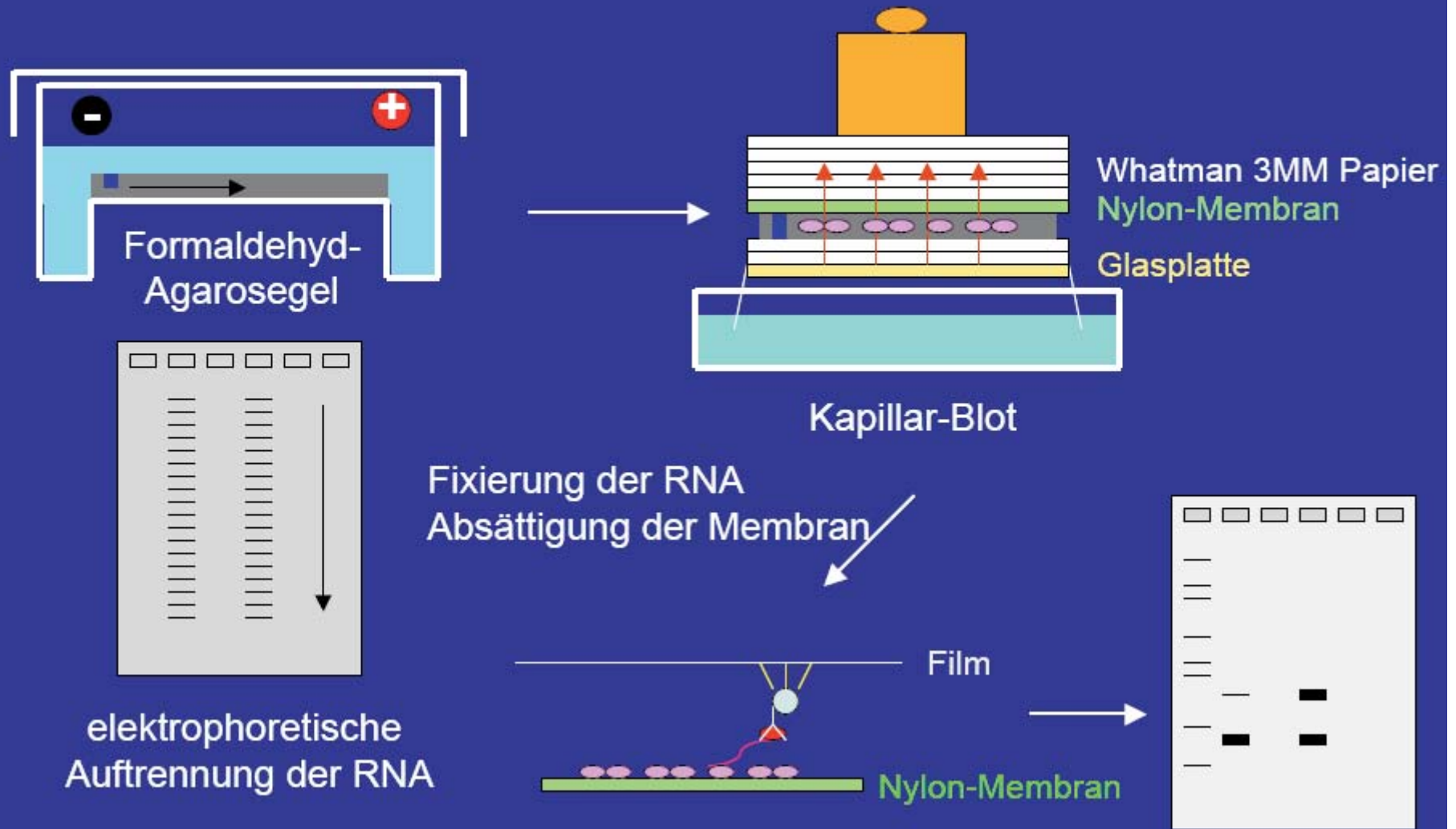


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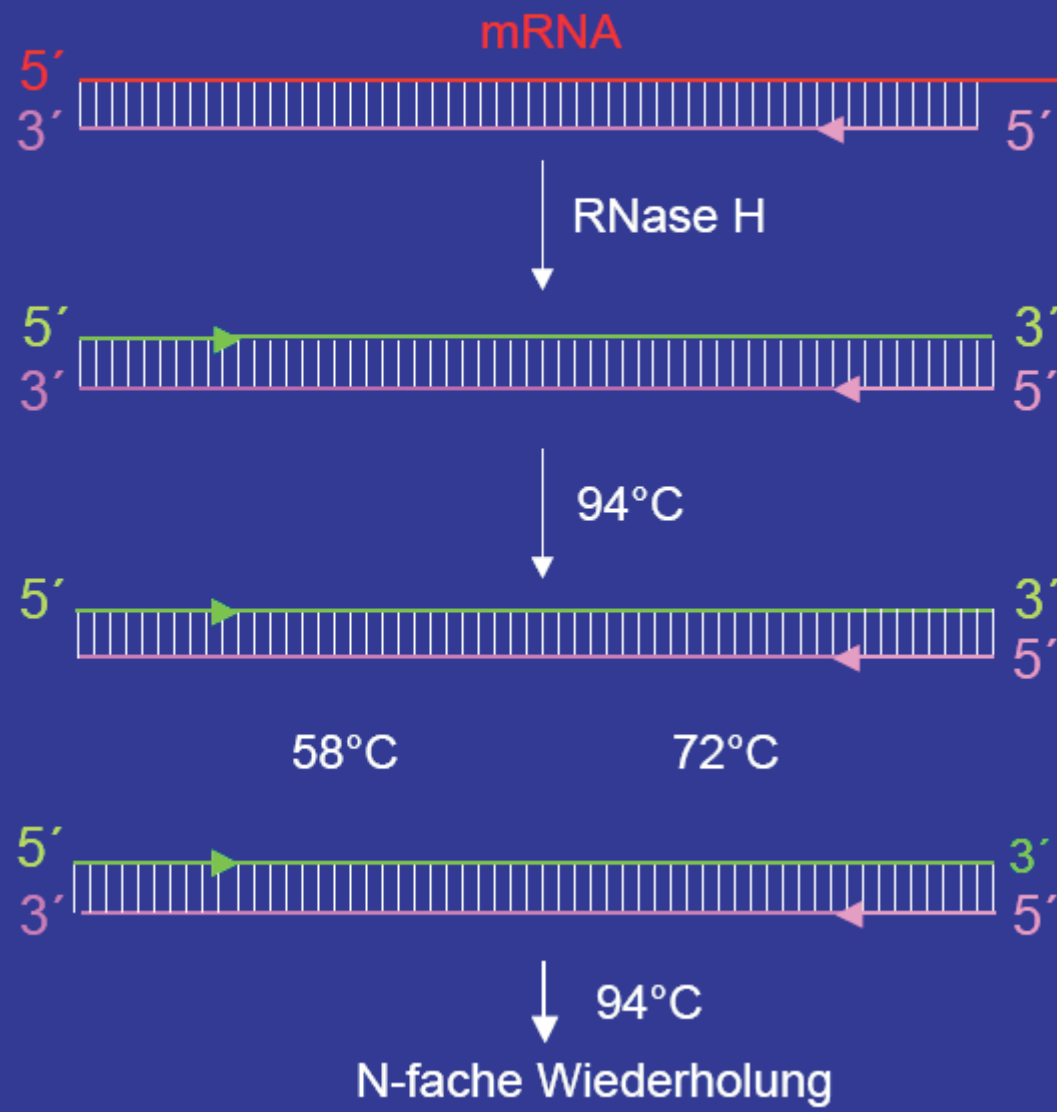
Nachweis und Quantifizierung von RNA in Zellen und Geweben:

- Northern Blot
- Multiplex RT-PCR
- Real-time RT-PCR

Northern Blotting



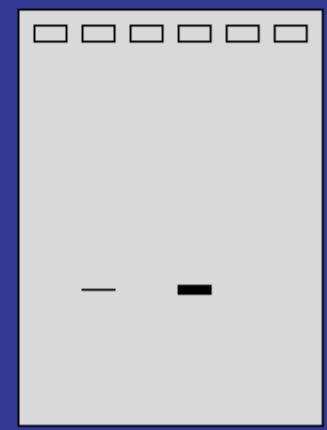
RT-PCR



Reverse Transkriptase
(MMLV, AMV)

PCR
(Taq, Pwo, Pfu)

Vorsicht!
DNA-Kontamination



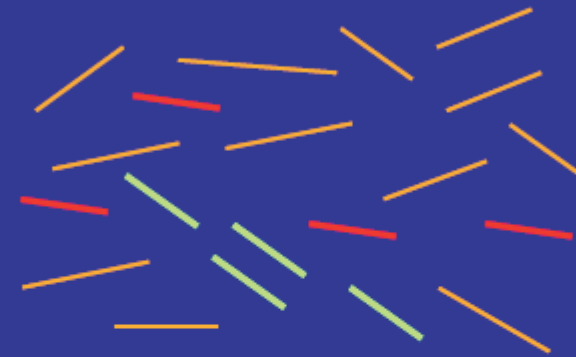
Zelle A Zelle B

Multiplex RT-PCR

Zelle A



Zelle B



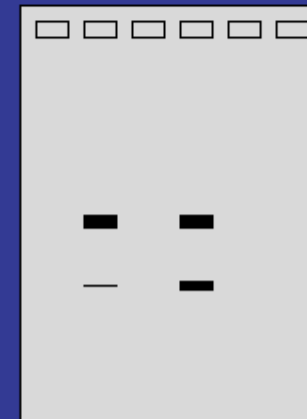
RT-PCR mit 2 Primer-Paaren:



Haushaltsgen



Gen von Interesse



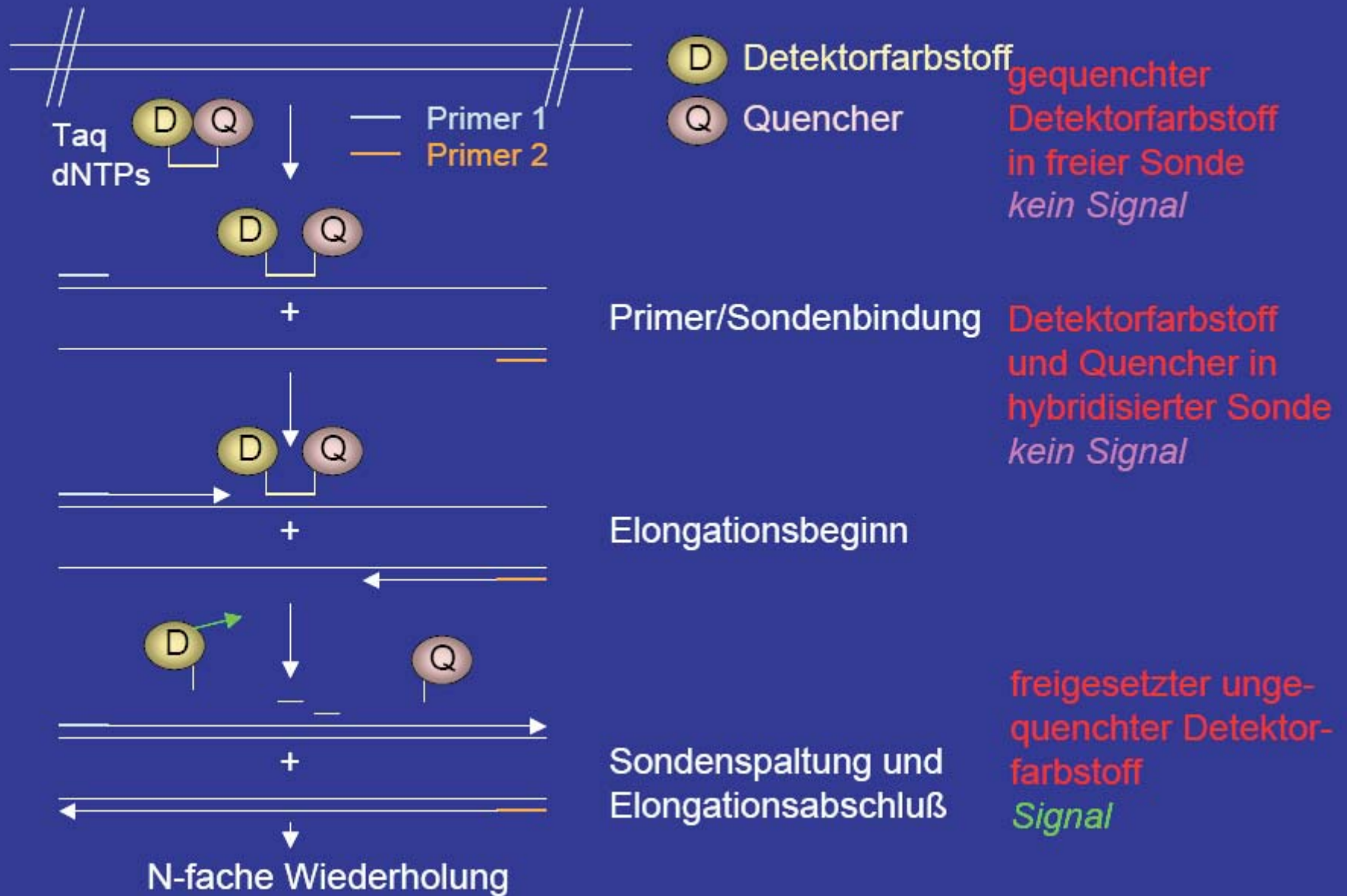
Zelle A Zelle B

Real-time RT-PCR

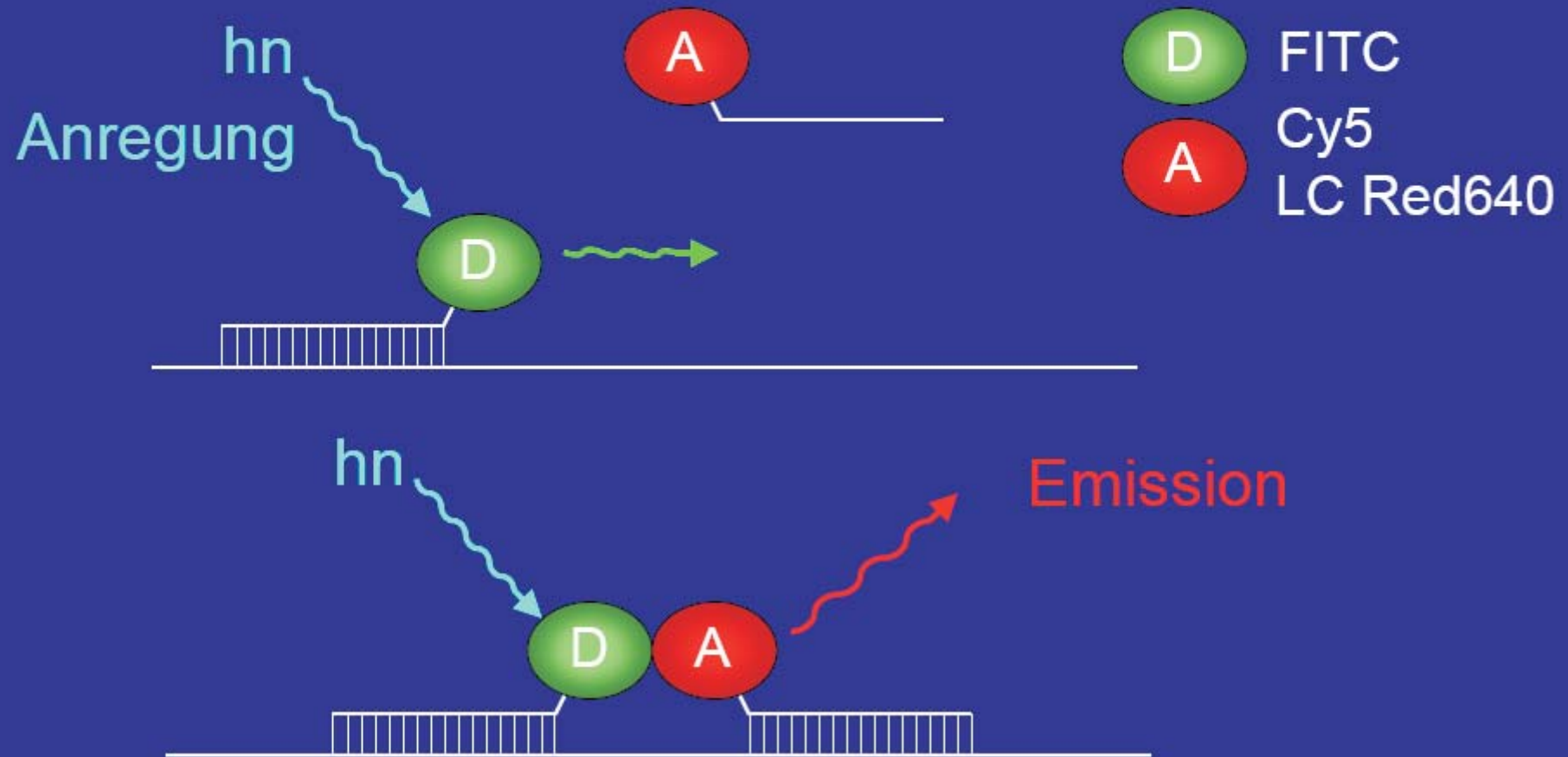
Quantitative PCR Reaktion, bei der kontinuierlich die Menge des entstandenen PCR-Produkts bestimmt werden kann.

- Fluorescence dequenching (FD)
(Taqman Technologie)
- Fluorescence resonance engery transfer (FRET)
(LightCycler Technologie)

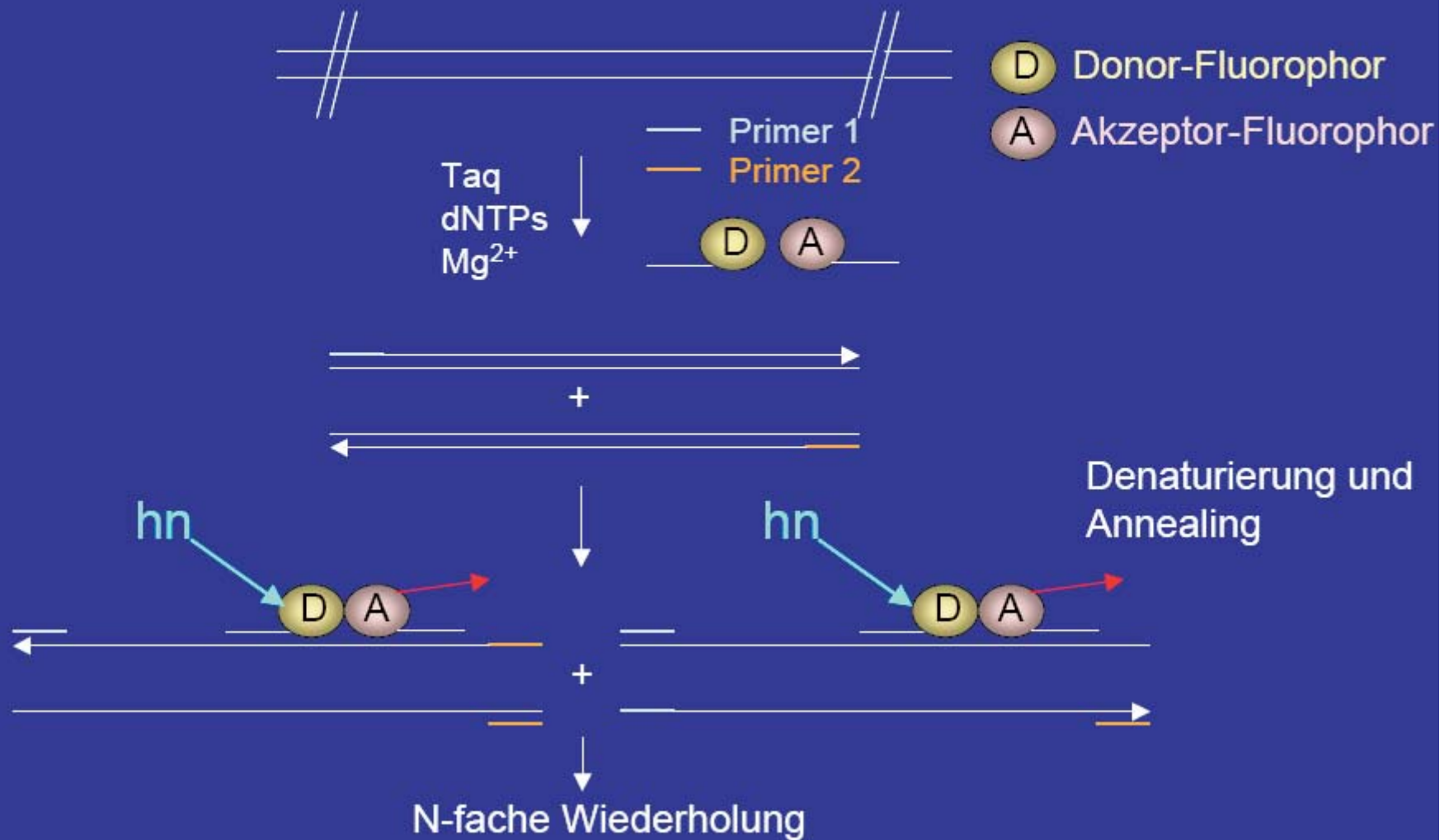
Die Taqman-Technologie



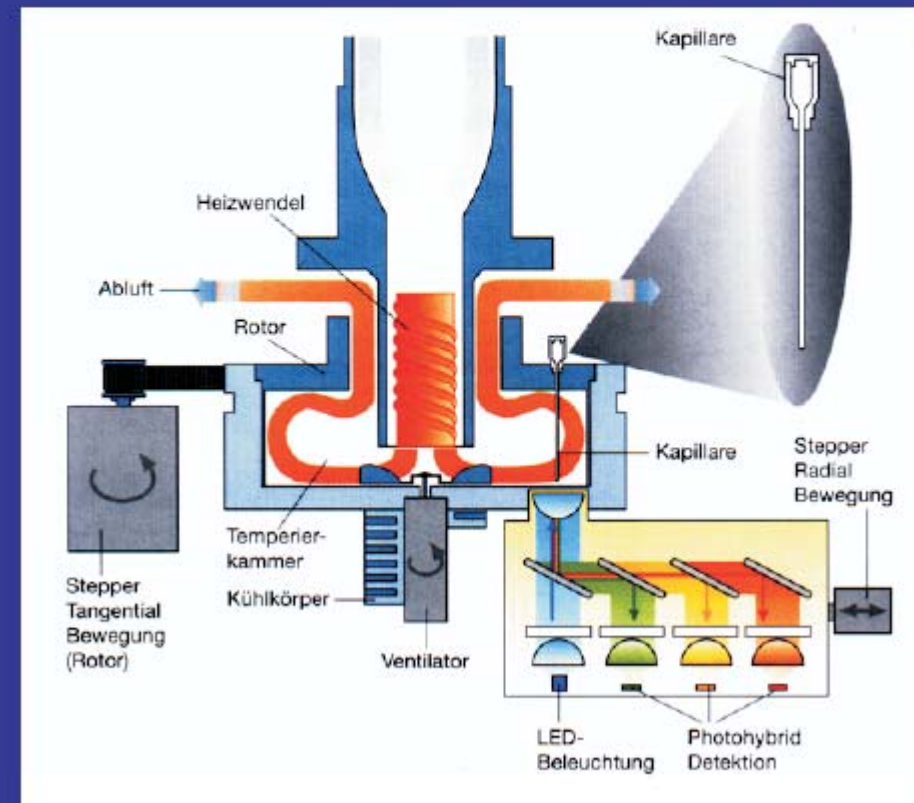
Fluorescence Resonance Energy Transfer (FRET)



Die LightCycler Technologie



Das LightCycler-System

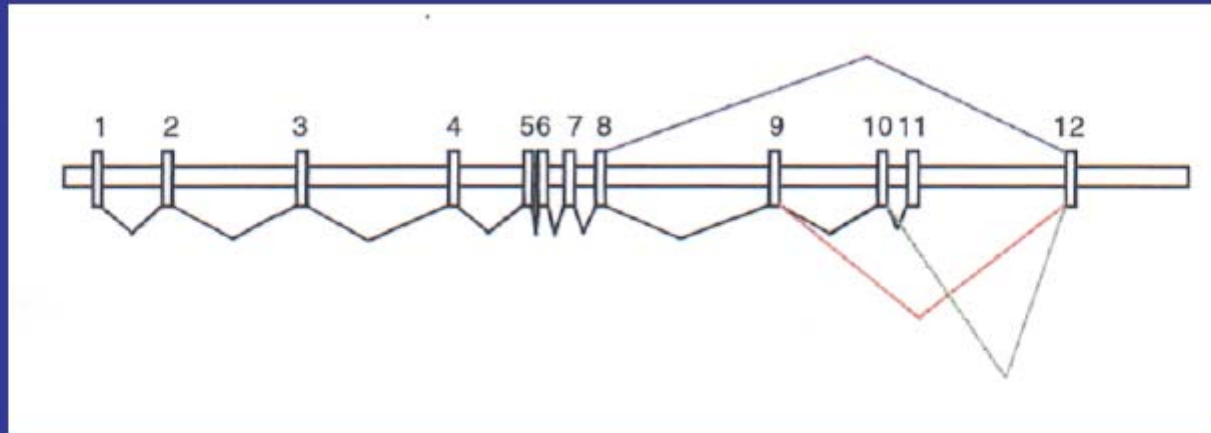


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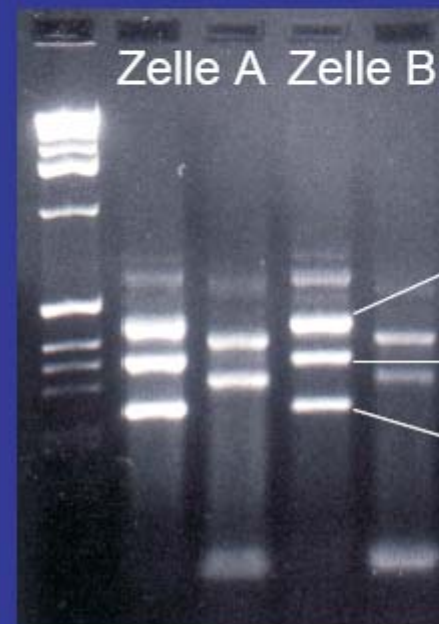
Untersuchungen zur:

- RNA-Stabilität
- Transkriptionsinitiation
- Transkriptionselongation
- RNA-Prozessierung

Detektion alternativer Spliceprodukte



- Northern Blot
- RT-PCR

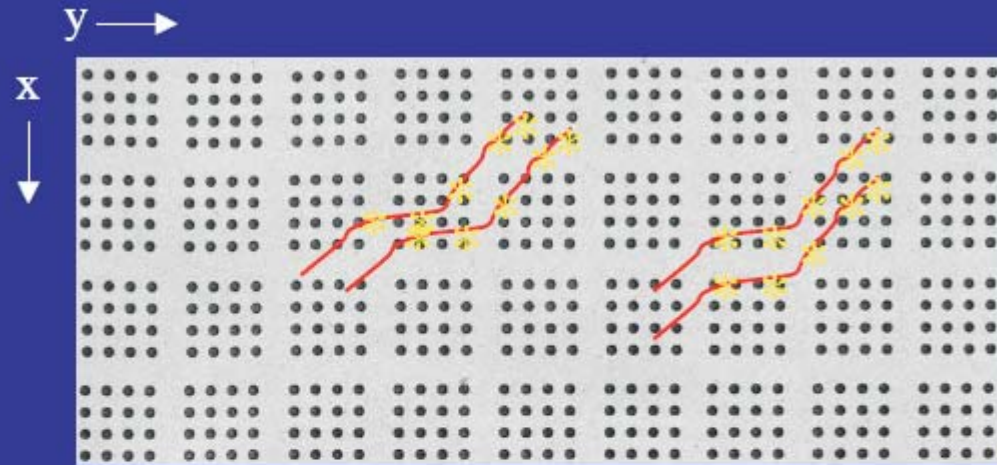


Ex 8-9-10-12

Ex 8-9-12

Ex 8-12

Isolierung von Promoter DNA



Hybridisierung eines
high density Filters mit
markierter Sonde



Autoradiographie bzw.
Chemolumineszenzdetektion

Methoden zum Studium der Genregulation

Bestimmung von Transkriptionsstartstellen:

- Primer Extension
- RNase Protection Assay
- S1 Nuclease Verdau
- 5' RACE (Rapid Amplification of cDNA Ends)

5'-RACE (Rapid Amplification of cDNA Ends)

SMART Oligonukleotid

5'—GGG 5'—AAAAAAAA 3'

Synthese des ersten
cDNA Stranges

reverse Transkriptase
dNTPs

←
genspezifischer Primer

5'—GGG 5'—AAAAAAAA 3'
← CCC ← Template Switching

Taq-Polymerase, dNTPs

Synthese des zweiten
cDNA Stranges

→ CCC →

→ SMART PCR Primer

←

+

→ CCC →

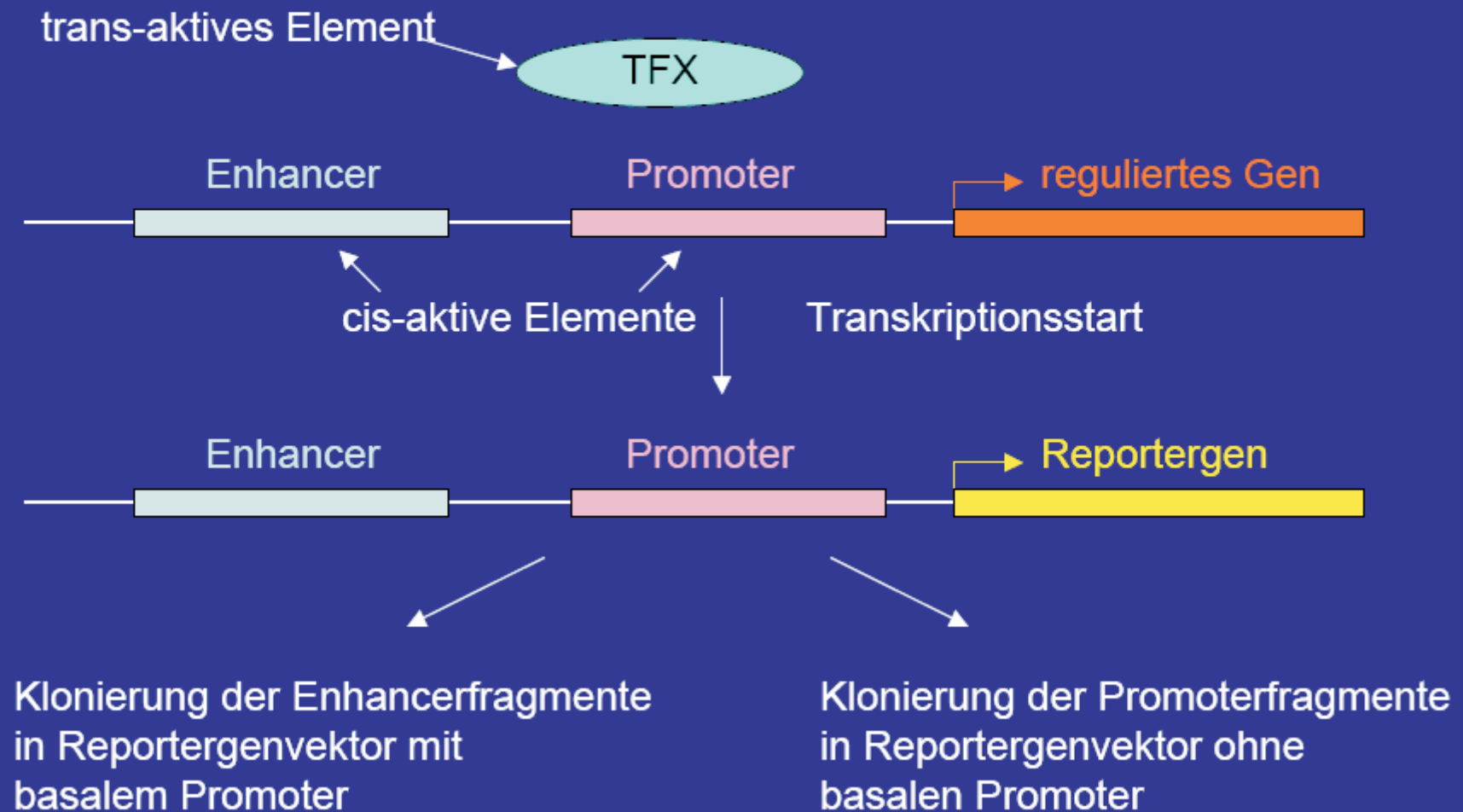
SMART Switching Mechanism At 5' end of RNA Transcript

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Quantifizierung der Promoteraktivität in Zellen:

Reportergenassays

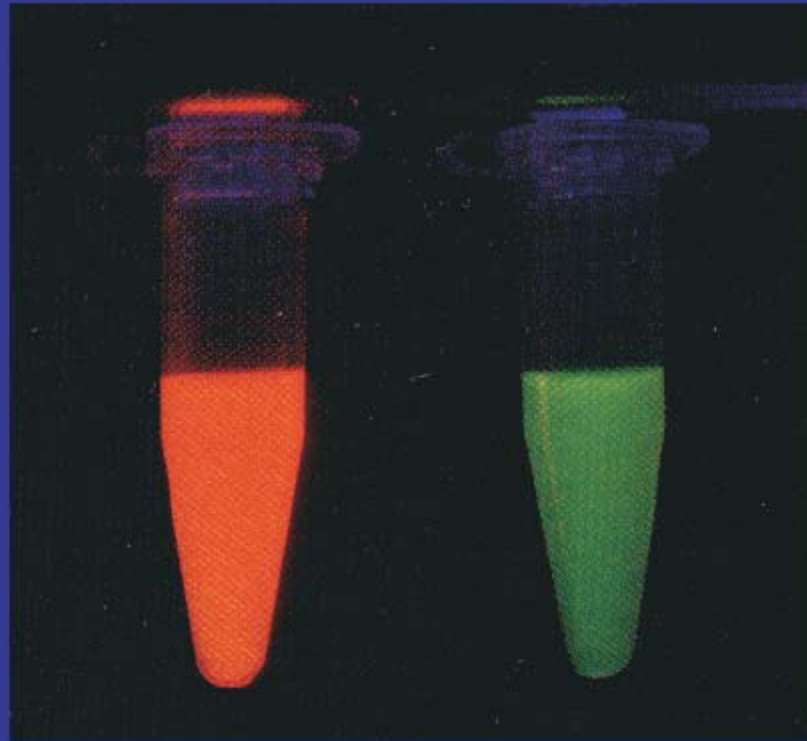
Prinzip eines Reportergenassays



Die gebräuchlichsten Reportersysteme

- Chloramphenicol-Acetyltransferase
- Luciferase
- β -Galactosidase
- Green fluorescent protein (GFP)

Green fluorescent protein (GFP)



EGFP: Enhanced green fluorescent protein

EGFP: Enhanced cyan fluorescent protein

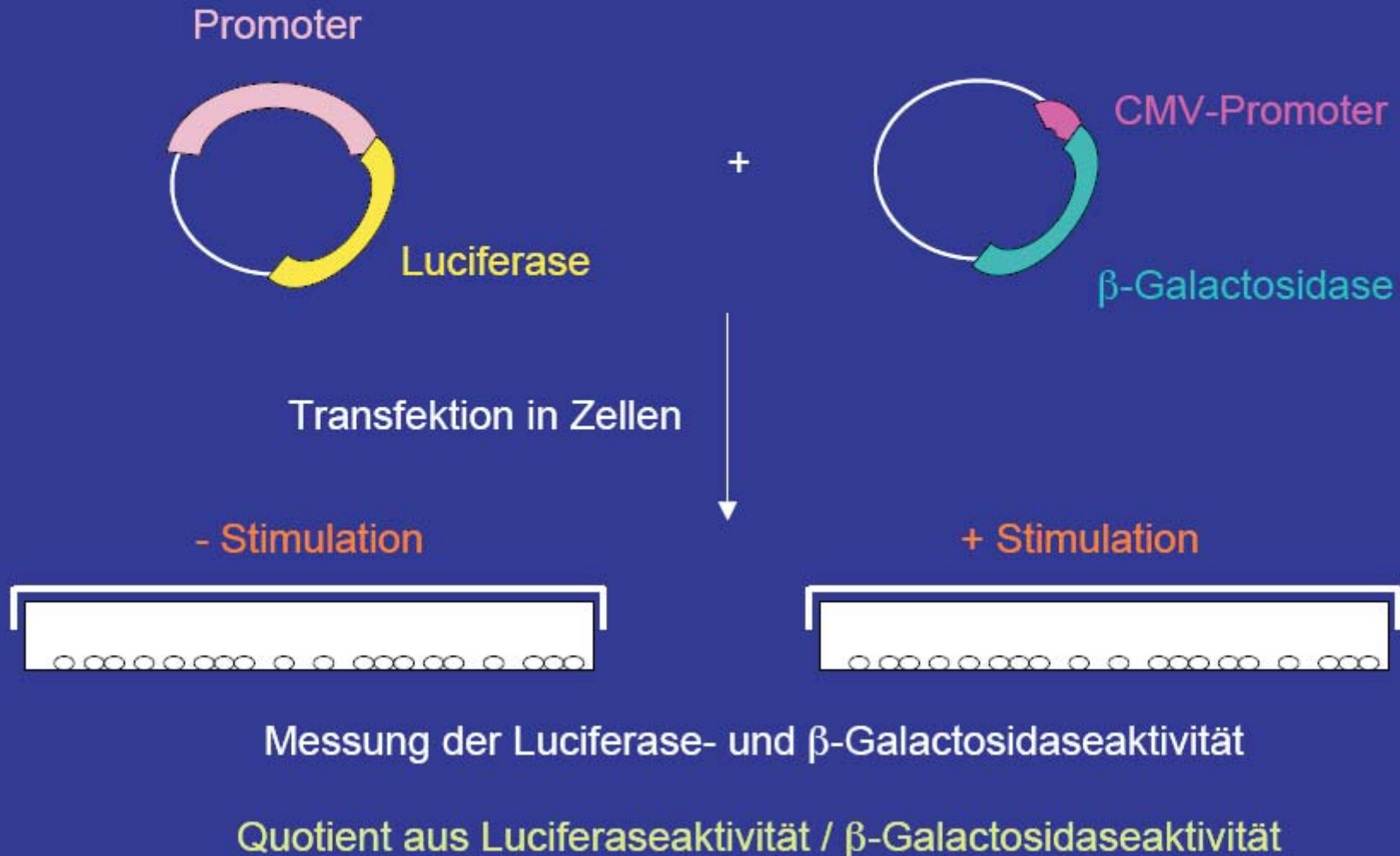
EYFP: Enhanced yellow fluorescent protein

DsRed: DsRed fluorescent protein

Aequoria victoria

Dicosoma

Durchführung eines Reporter-genassays



Identifizierung von regulatorischen Elementen

