

Fundamentals of Biology II (551-0104-01)

Plant Physiology Part

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Group of Plant Biochemistry

Prof. Sam Zeeman

Department of Biology, ETH Zurich



Molecular biology of gene silencing (Analysis of transgenic and silenced *Nicotiana benthamiana* plants)

- DNA extraction from leaf tissue
- Reverse transcription (cDNA synthesis)
- PCR

Chromatographic separation of photosynthetic pigments

- Pigments extraction
- Pigments separation
- Identification of the pigments

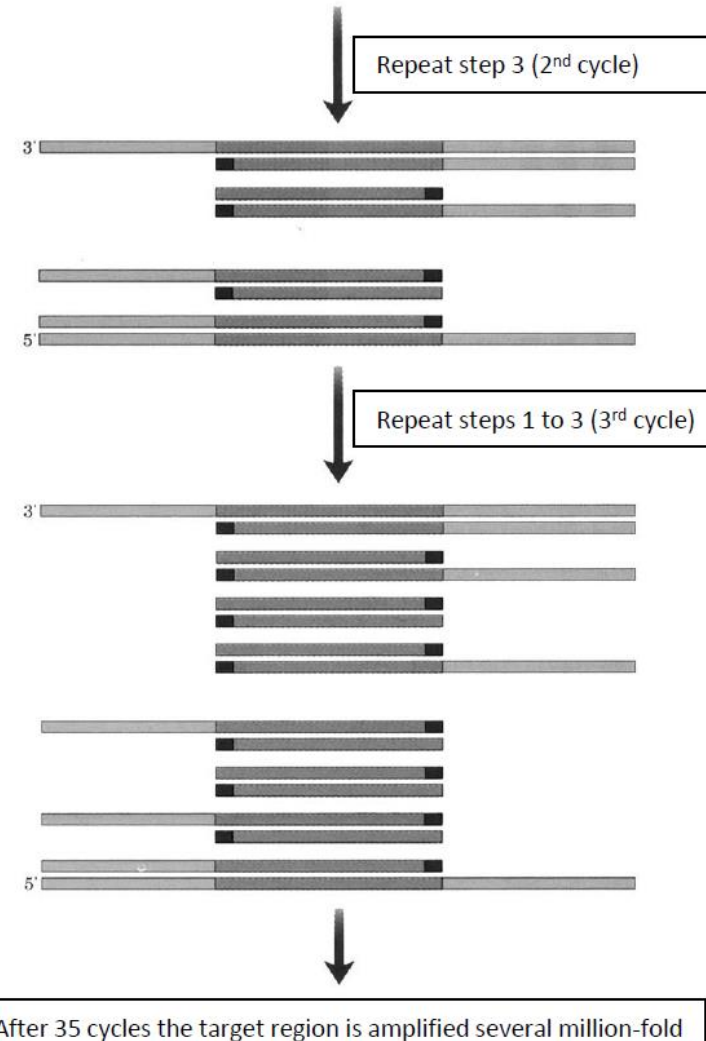
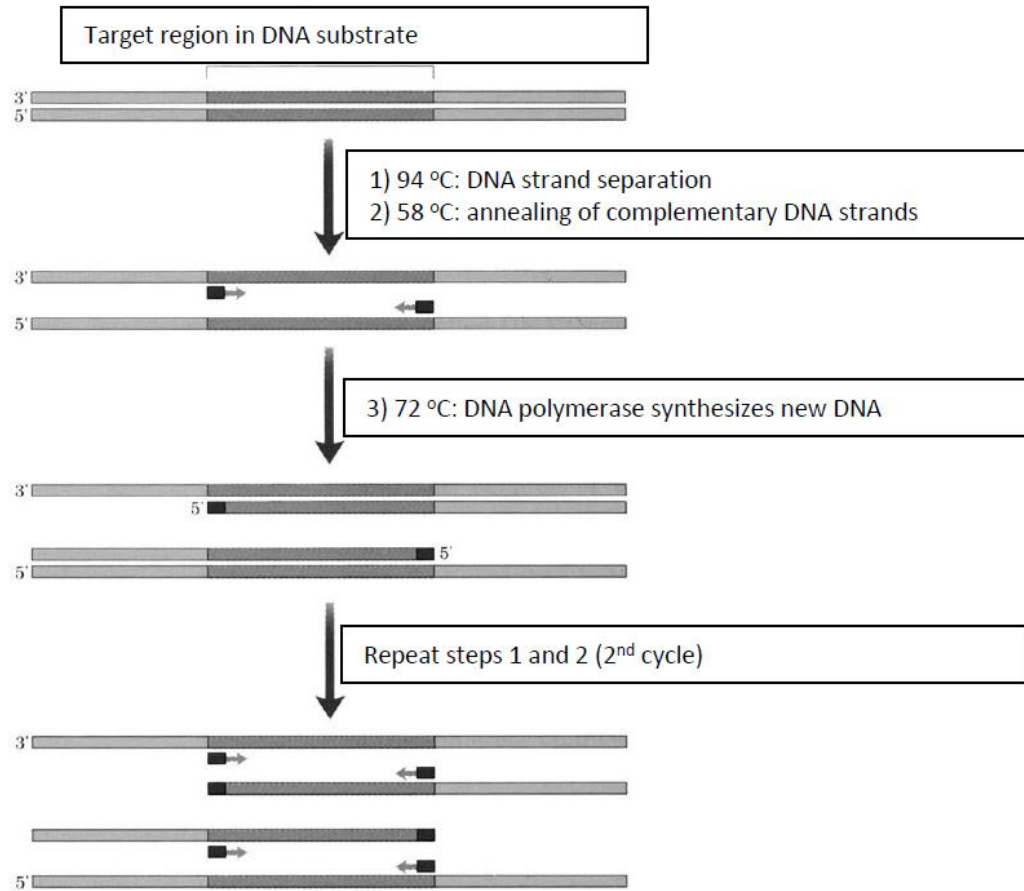
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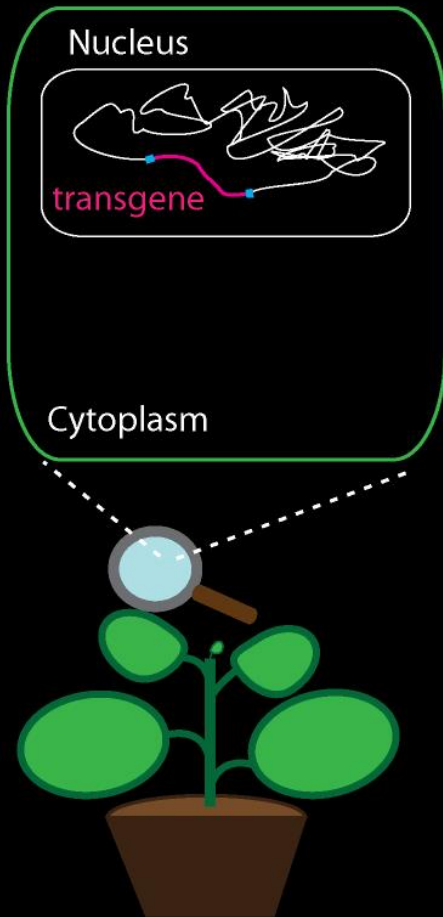
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Detecting a transgene by PCR (*Polymerase Chain Reaction*):



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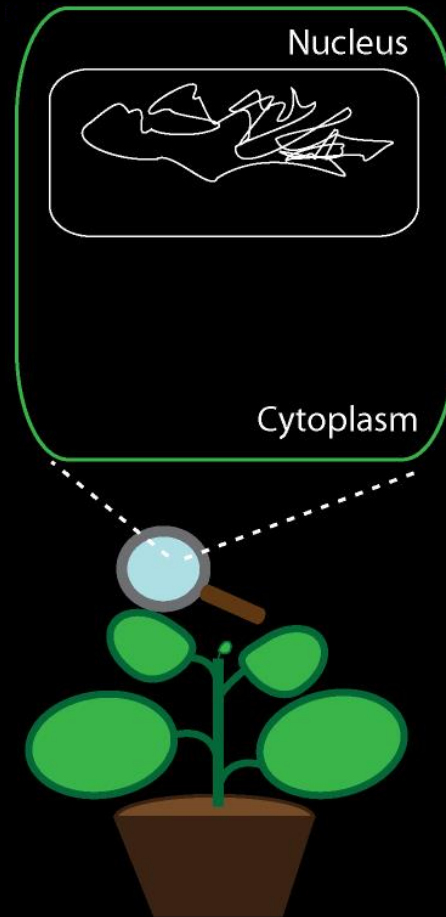
Plant cell



transgenic

Nicotiana benthamiana

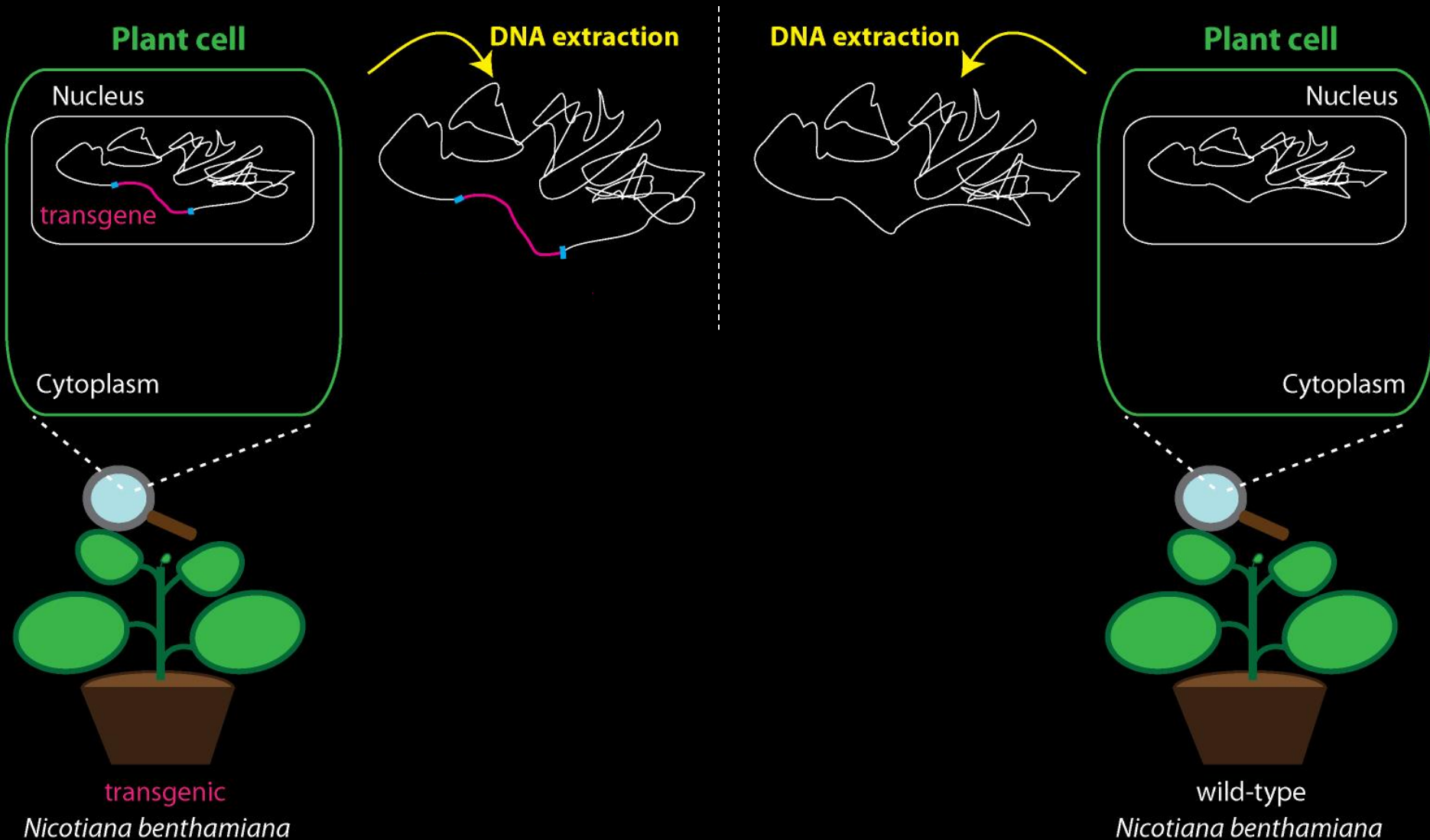
Plant cell



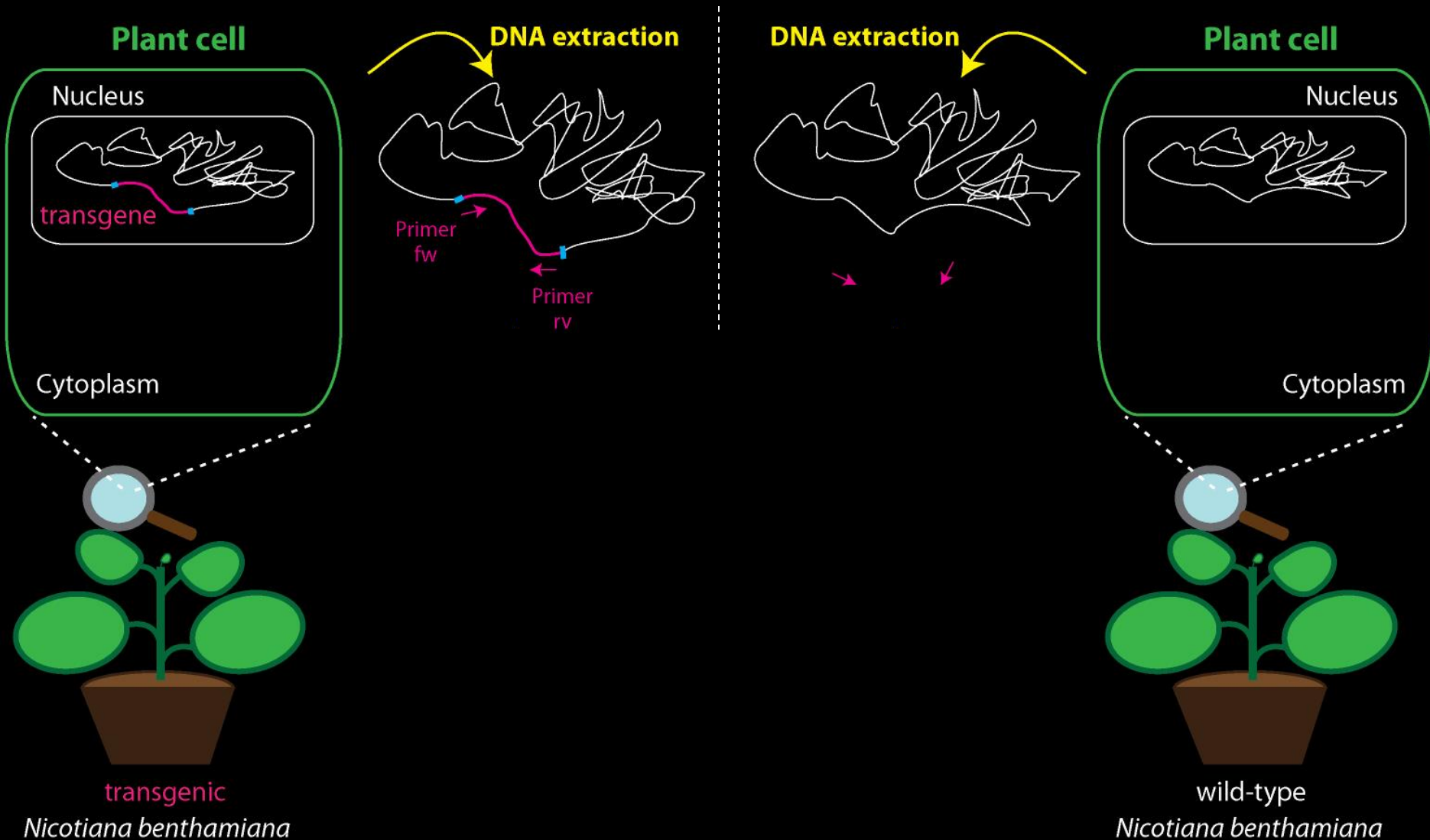
wild-type

Nicotiana benthamiana

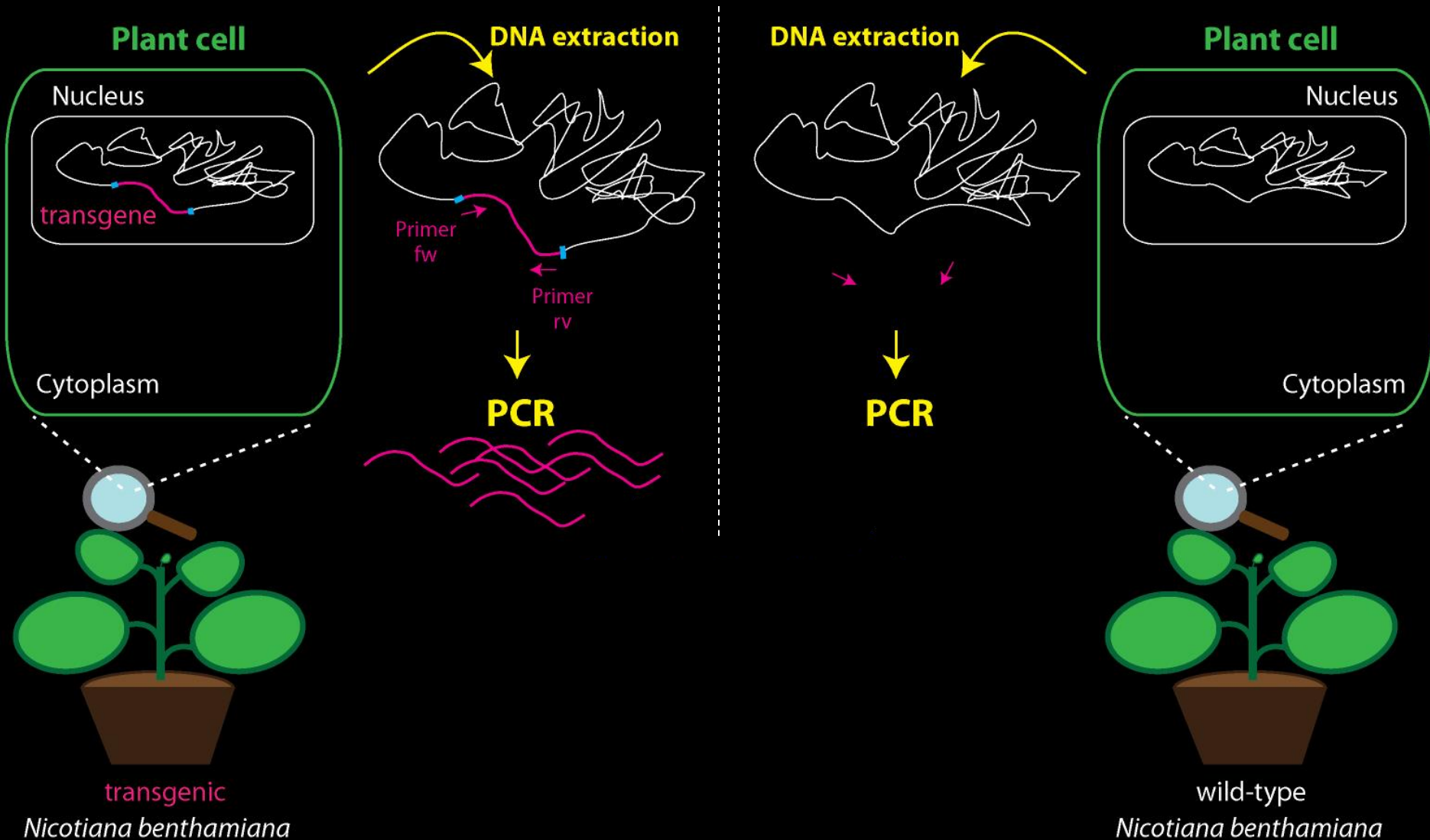
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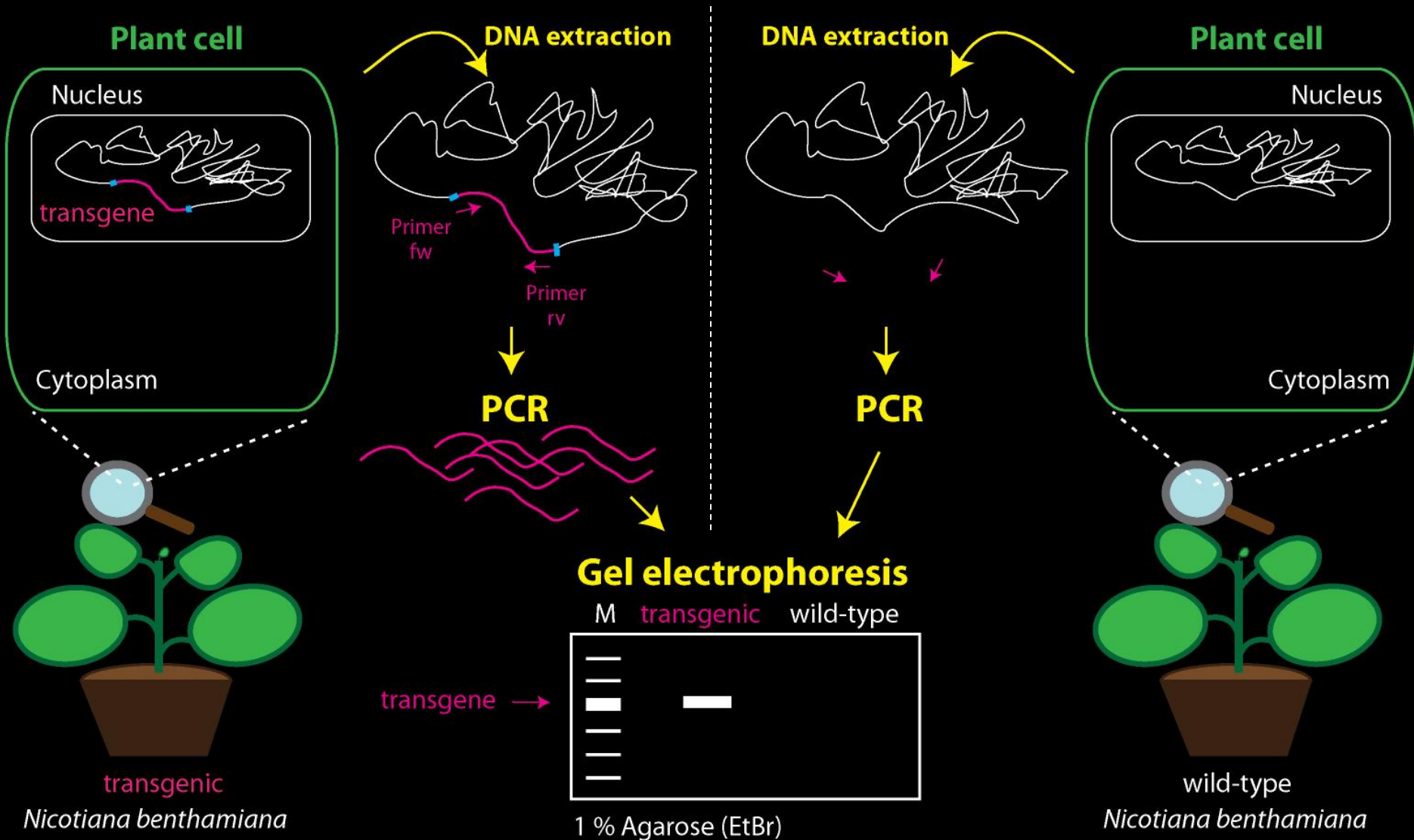
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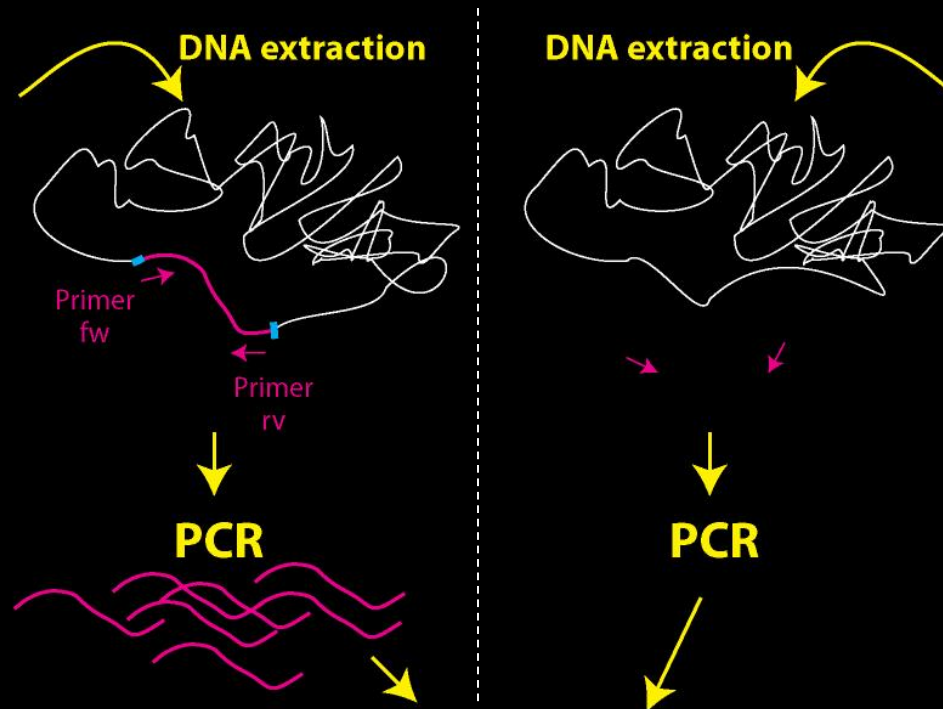


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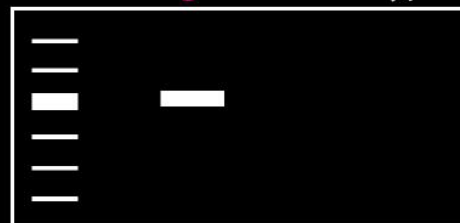
Detecting a transgene by PCR (*Polymerase Chain Reaction*):

Other reasons why band could be present or not?

transgene →

Gel electrophoresis

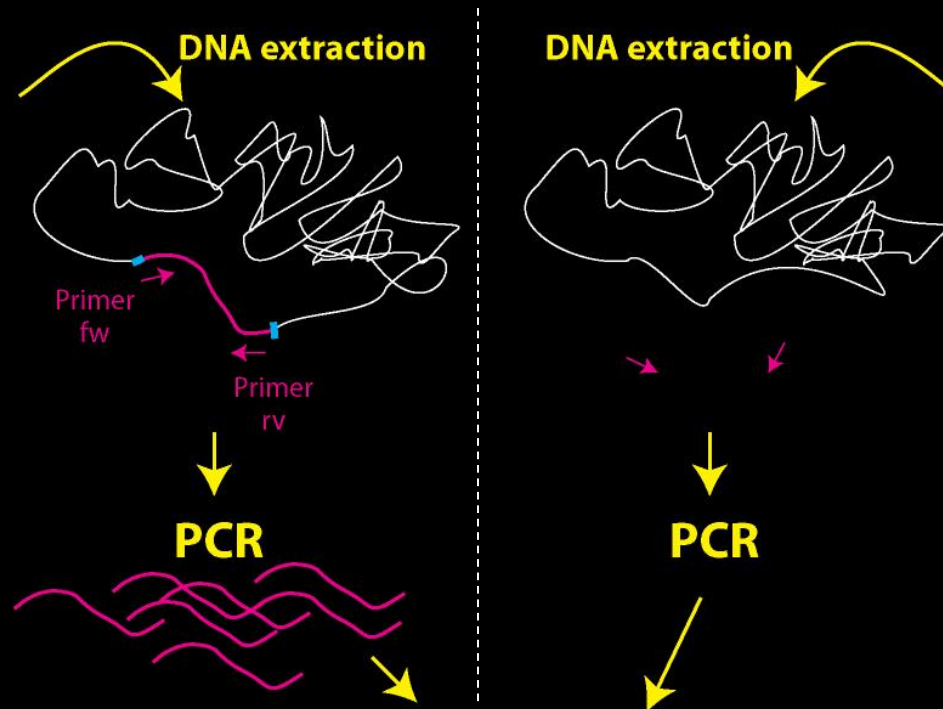
M transgenic wild-type



1 % Agarose (EtBr)

→ Controls needed

- 1) Water instead of DNA
- 2) Actin primers

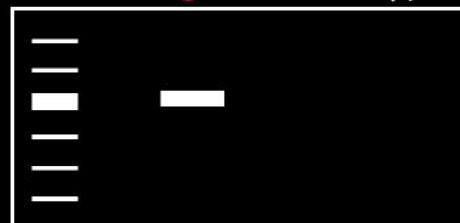
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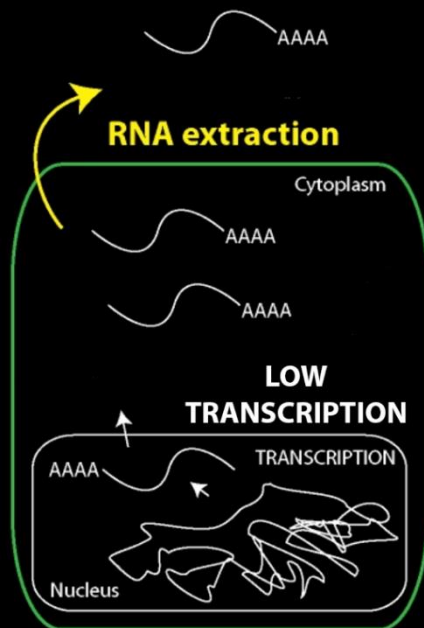
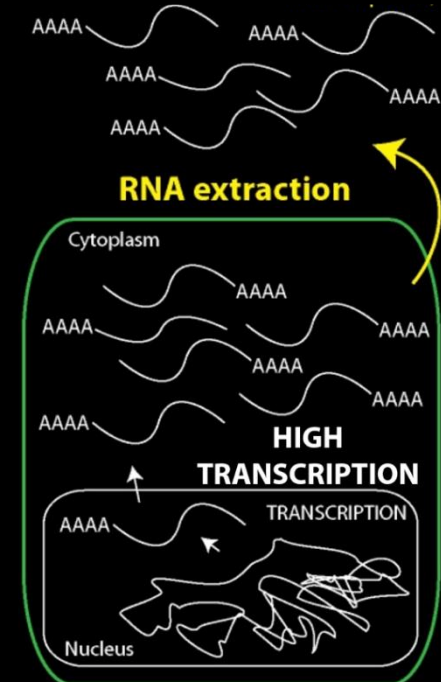
→ Controls needed

- 1) Water instead of DNA (contaminations?)
- 2) Actin primers (DNA quality?)

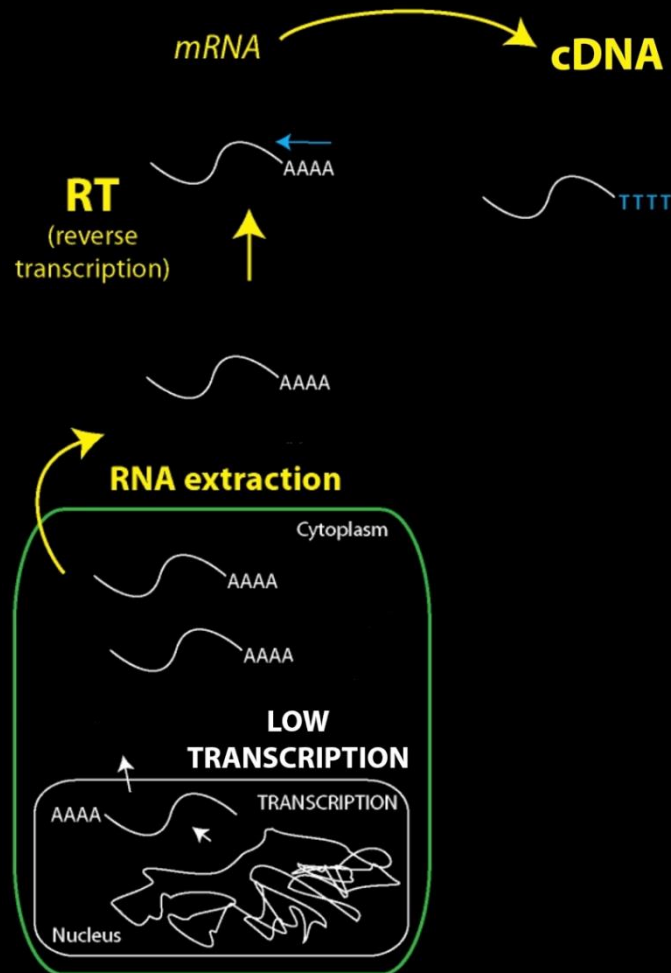
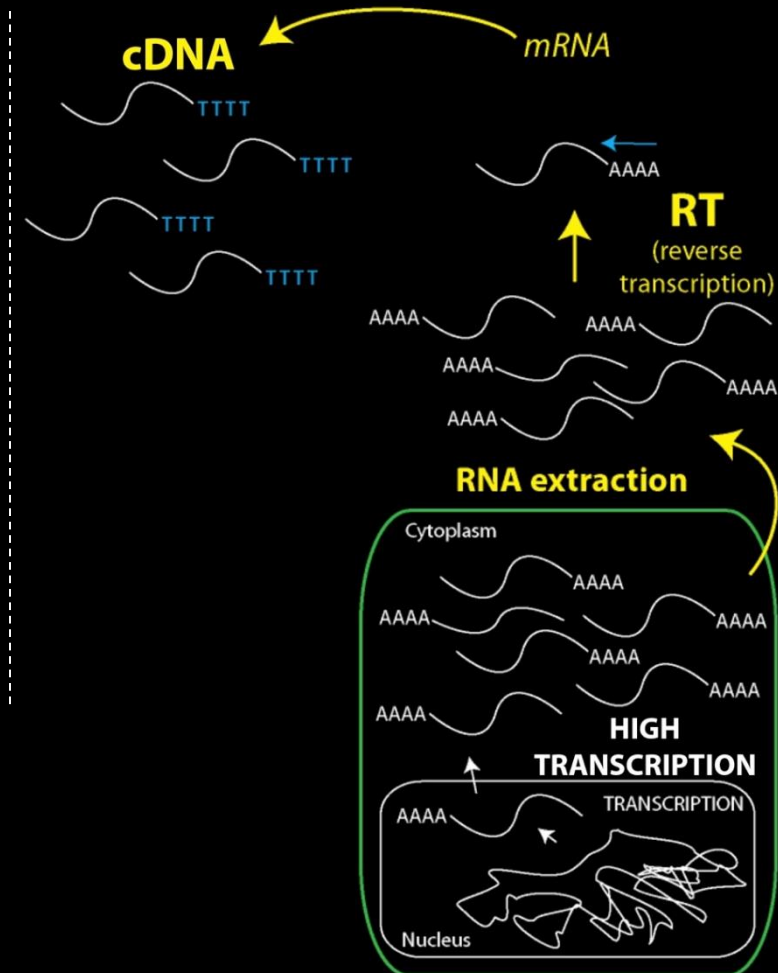
Detecting a transgene by PCR (*Polymerase Chain Reaction*):

- Allows us to distinguish between WT and GFP-expressing tobacco
- But how can we assess the gene silencing?

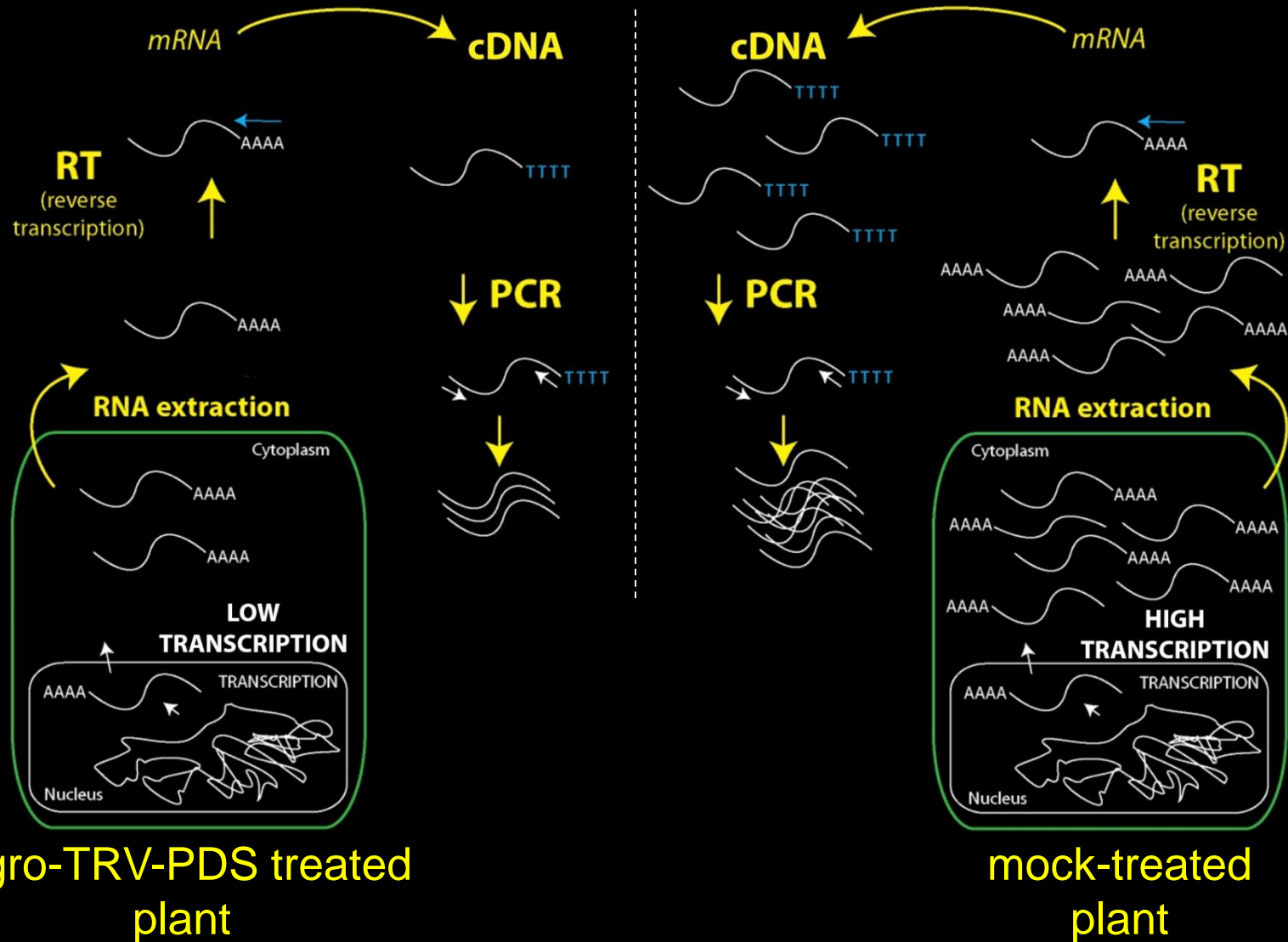
Measuring gene expression by RT-PCR:

Agro-TRV-PDS treated
plantmock-treated
plant

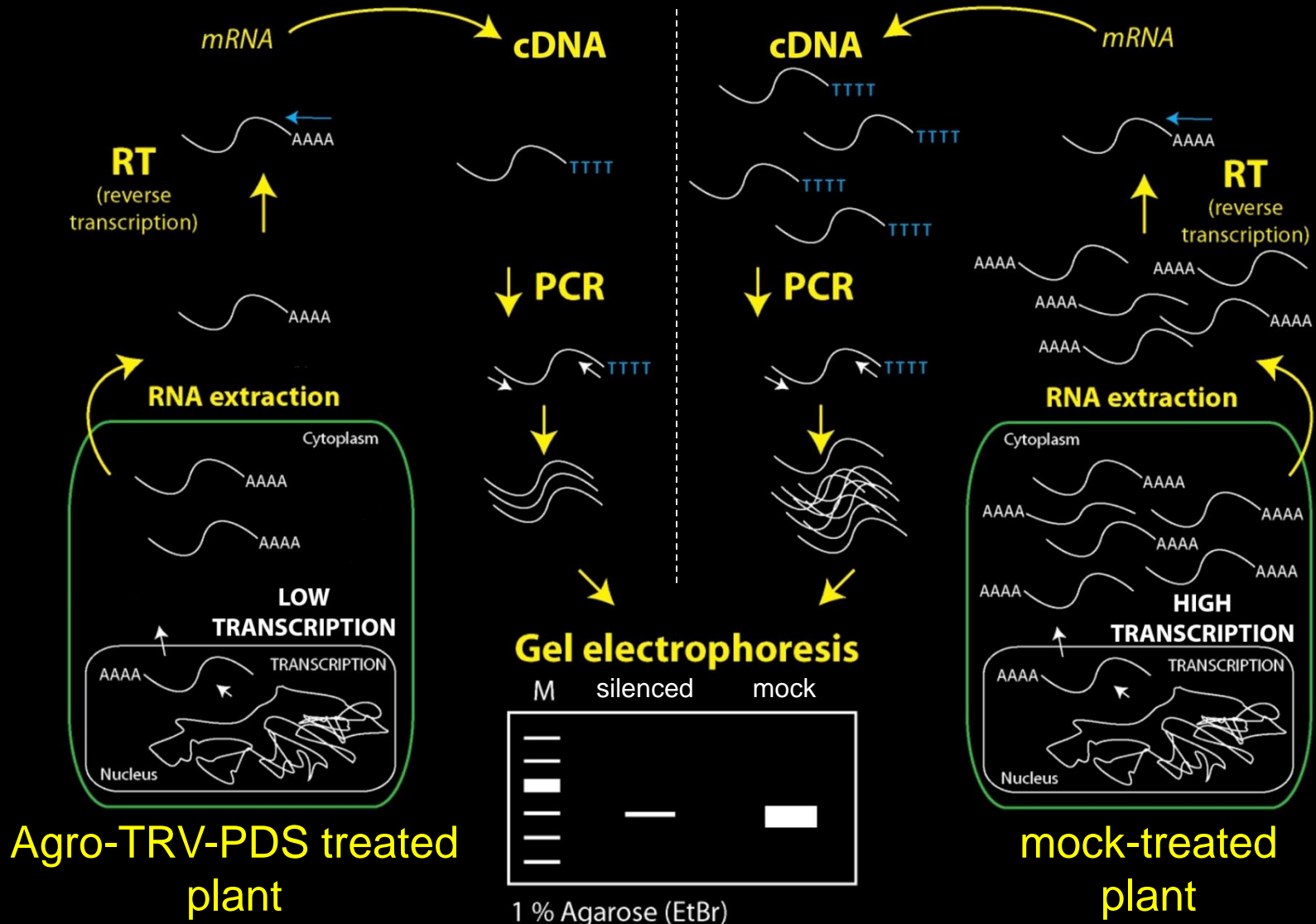
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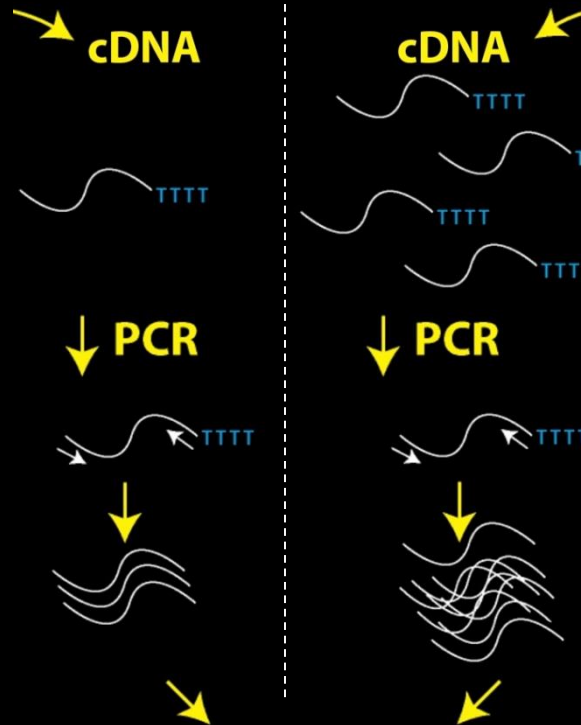
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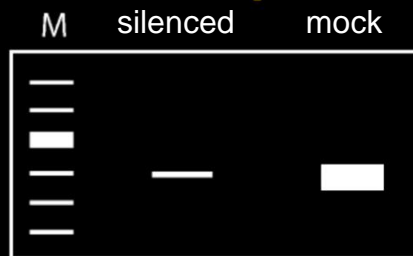
Measuring gene expression by RT-PCR:



Controls?

- 1) Water instead of cDNA
- 2) Actin primers

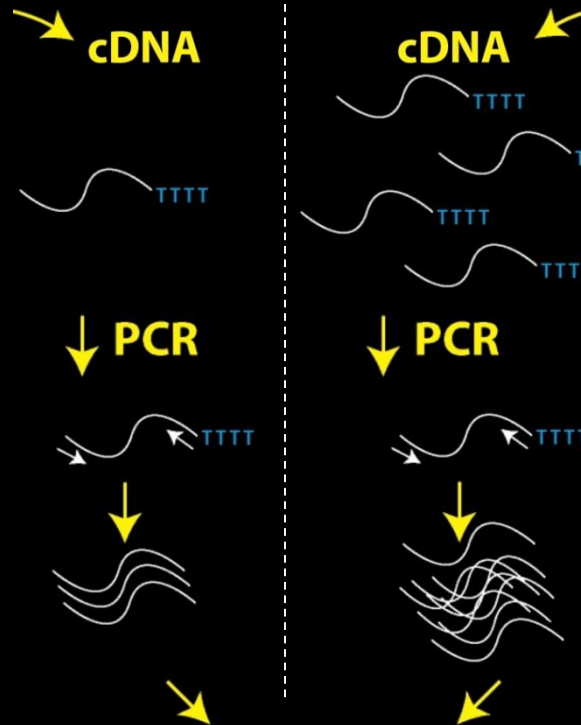
Gel electrophoresis



1 % Agarose (EtBr)

Actin is “house-keeping gene” →
mRNA levels *should* be stable under
diverse conditions

Measuring gene expression by RT-PCR:



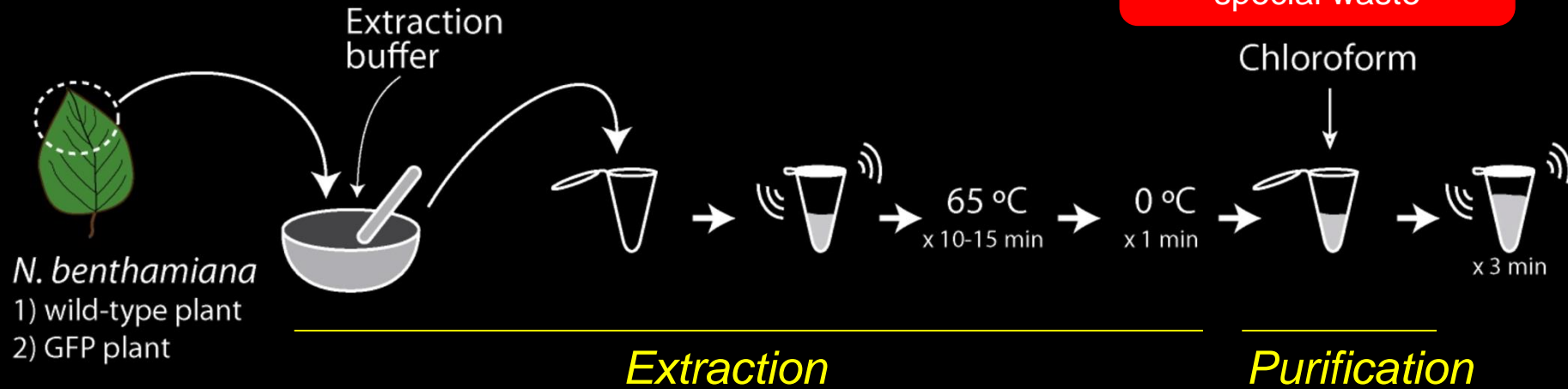
Controls?

- 1) Water instead of cDNA
- 2) Actin primers (cDNA quality, equal amounts of template)

Actin is “house-keeping gene” → mRNA levels should be stable under diverse conditions

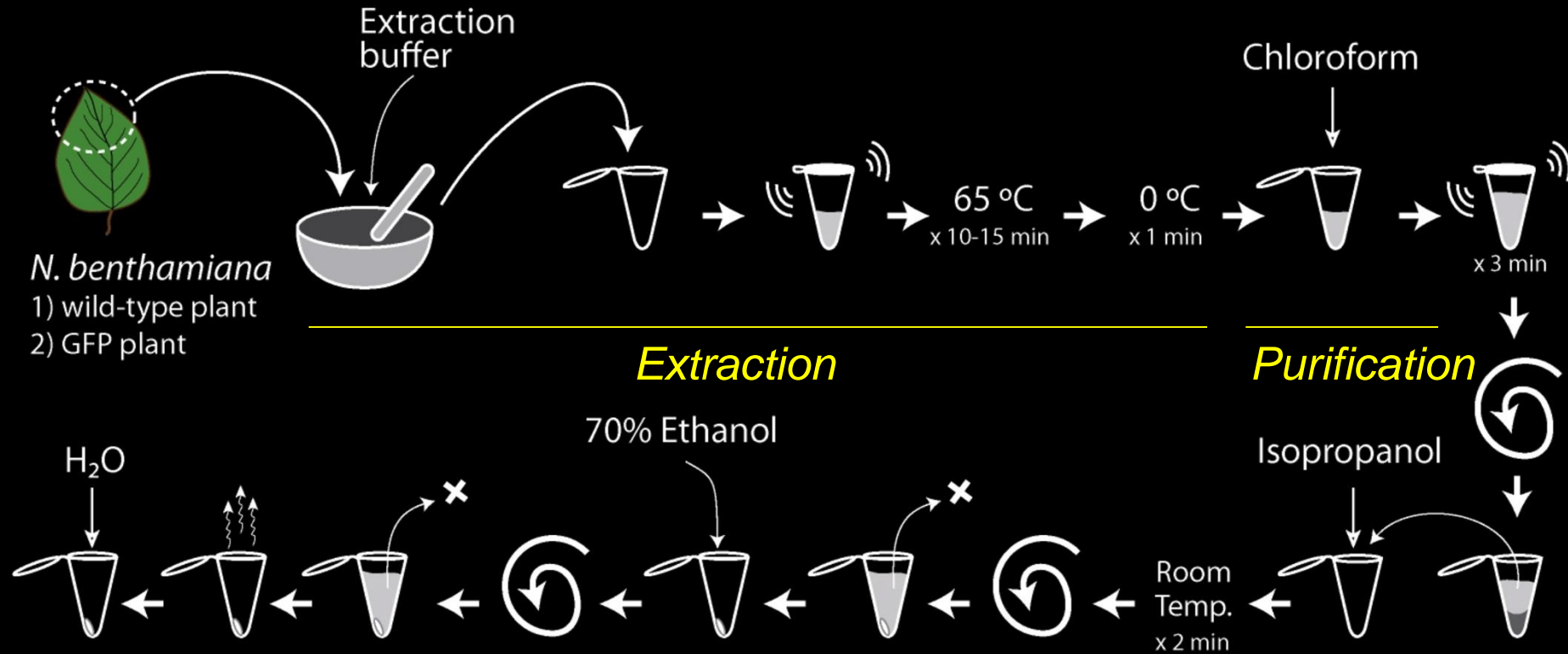
DNA extraction experiment

1) DNA extraction



DNA extraction experiment

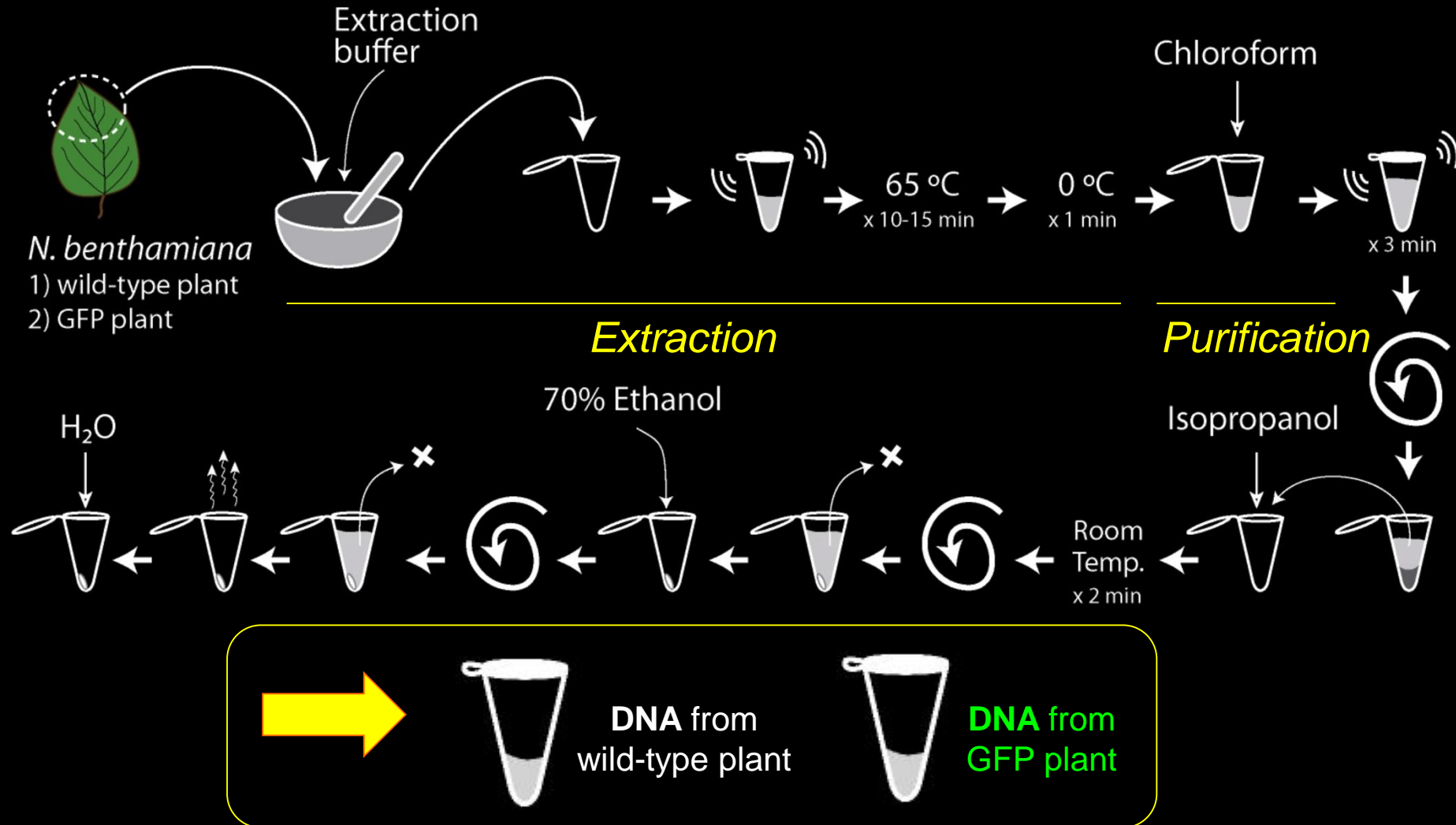
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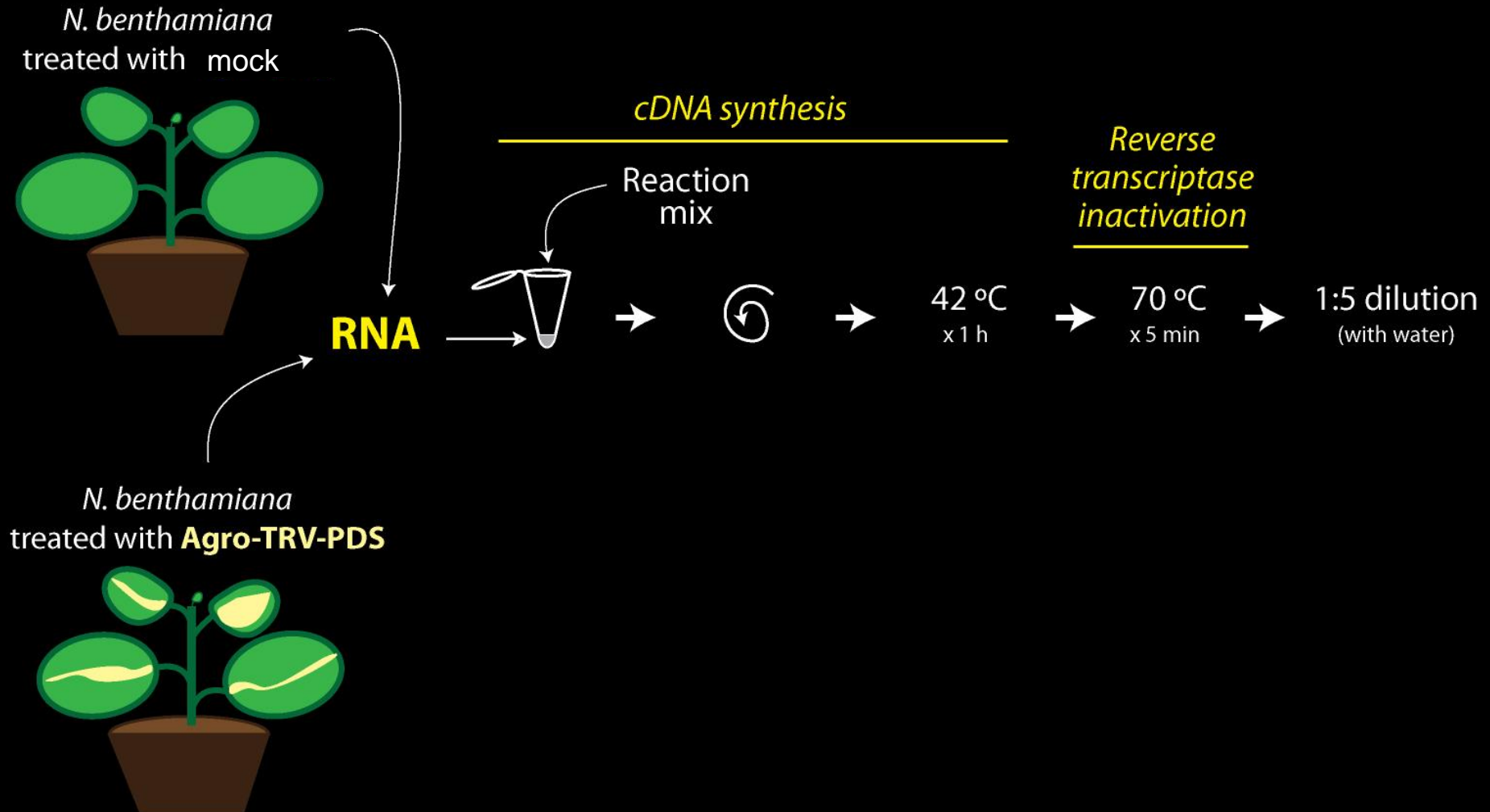
Alcohols:
precipitation of DNA

DNA extraction experiment

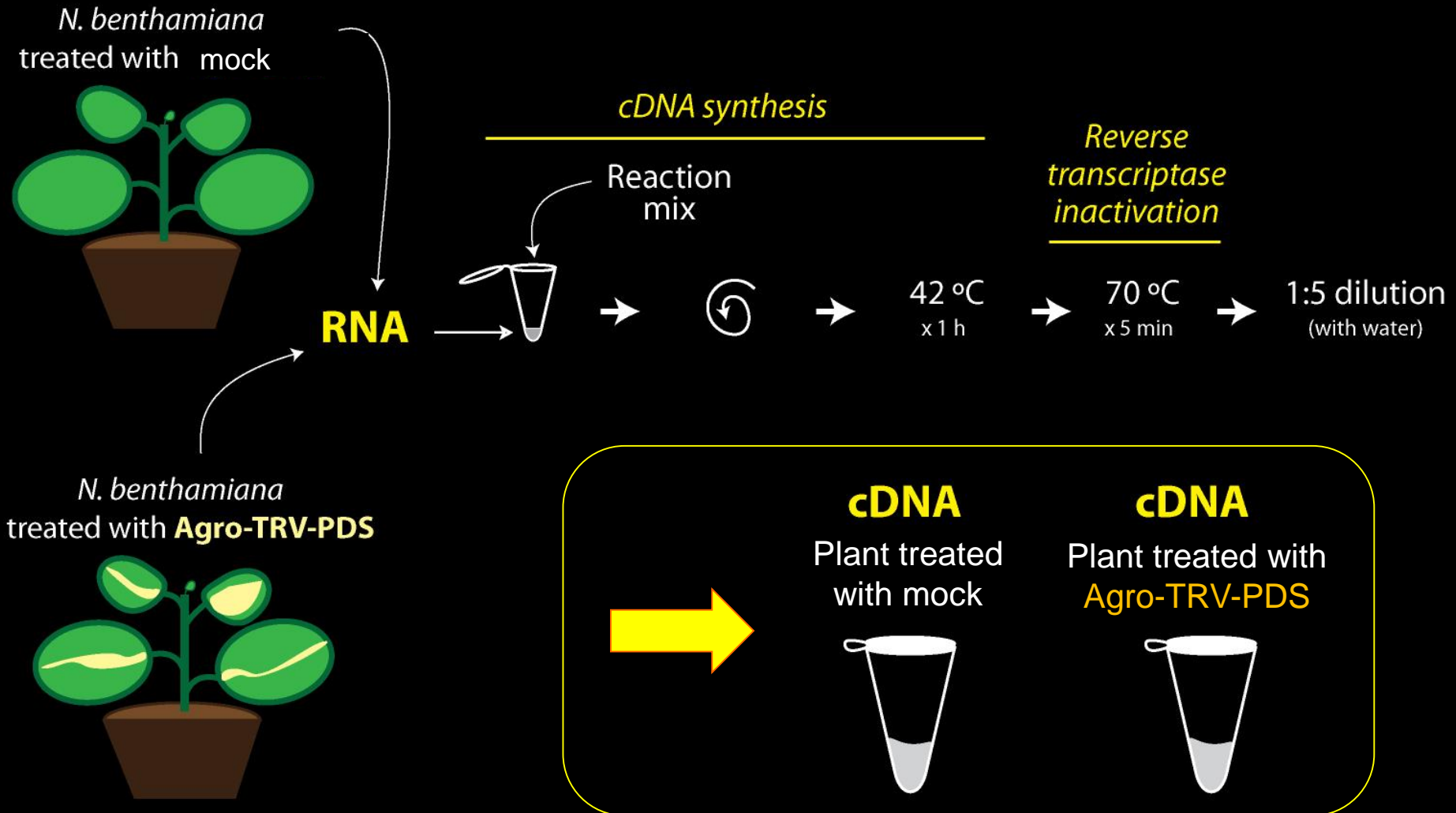
1) DNA extraction



2) cDNA synthesis



2) cDNA synthesis



3) PCR

DNA

PCR program with 35 cycles

PCR1 – Detection of GFP transgene

Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6	Tube 7	Tube 8
5 µl WT DNA	5 µl GFP DNA	5 µl H ₂ O	empty	5 µl WT DNA	5 µl GFP DNA	5 µl H ₂ O	empty
<ul style="list-style-type: none"> Add the PCR reaction mix to each tube (ask an assistant to pipette the mix in your prepared tubes). 							
GFP PCR mix	GFP PCR mix	GFP PCR mix		Actin PCR mix	Actin PCR mix	Actin PCR mix	

cDNA

PCR program with 25 cycles

PCR2 – Estimation of PDS mRNA levels

Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6	Tube 7	Tube 8
5 µl mock cDNA (control)	5 µl PDS- silenced cDNA	5 µl H ₂ O	empty	5 µl mock cDNA control	5 µl PDS- silenced cDNA	5 µl H ₂ O	empty
<ul style="list-style-type: none"> Add the PCR reaction mix to each tube (ask an assistant to pipette the mix in your prepared tubes). 							
PDS PCR mix	PDS PCR mix	PDS PCR mix		Actin PCR mix	Actin PCR mix	Actin PCR mix	

3) PCR

DNA

PCR program with 35 cycles

PCR1 (program: GFP35)

- Step 1. 95°C for 3 min (*initial denaturation*)
- Step 2. 95°C for 30 sec (*denaturation*)
- Step 3. 55°C for 30 sec (*annealing*)
- Step 4. 72°C for 30 sec (*elongation*)
- Step 5. Go back to step #2 34 times (*exponential amplification*)
- Step 6. 72°C for 1 min (*final elongation*)
- Step 7. 14 °C for ever (*storage*)

cDNA

PCR program with 25 cycles

PCR2 (program: PDS25)

- Step 1. 95°C for 3 min (*initial denaturation*)
- Step 2. 95°C for 30 sec (*denaturation*)
- Step 3. 55°C for 30 sec (*annealing*)
- Step 4. 72°C for 30 sec (*elongation*)
- Step 5. Go back to step #2 24 times (*exponential amplification*)
- Step 6. 72°C for 1 min (*final elongation*)
- Step 7. 14 °C for ever (*storage*)

Molecular biology of gene silencing (Analysis of transgenic and silenced *Nicotiana benthamiana* plants):

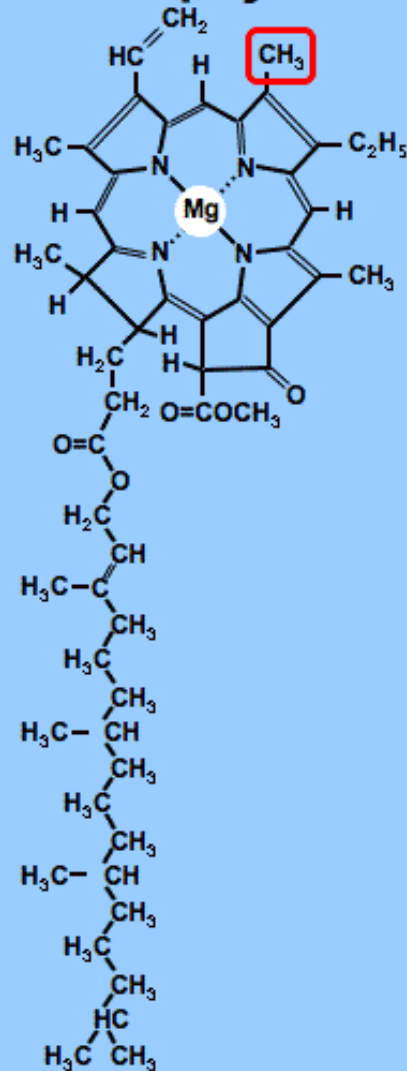
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Chromatographic separation of photosynthetic pigments

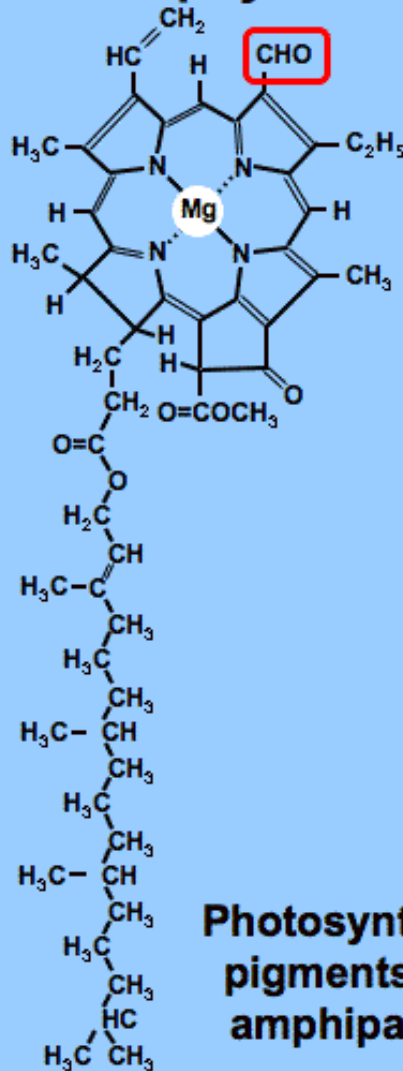
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About photosynthetic pigments

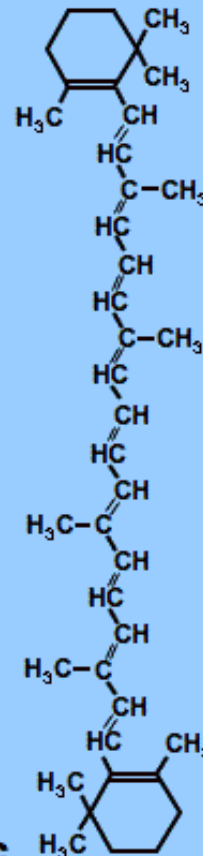
Chlorophyll a



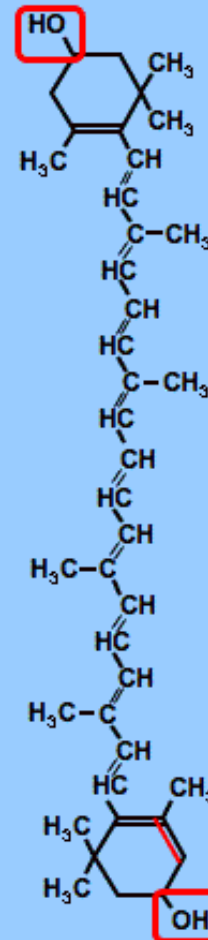
Chlorophyll b



β-Carotene



Zeaxanthin



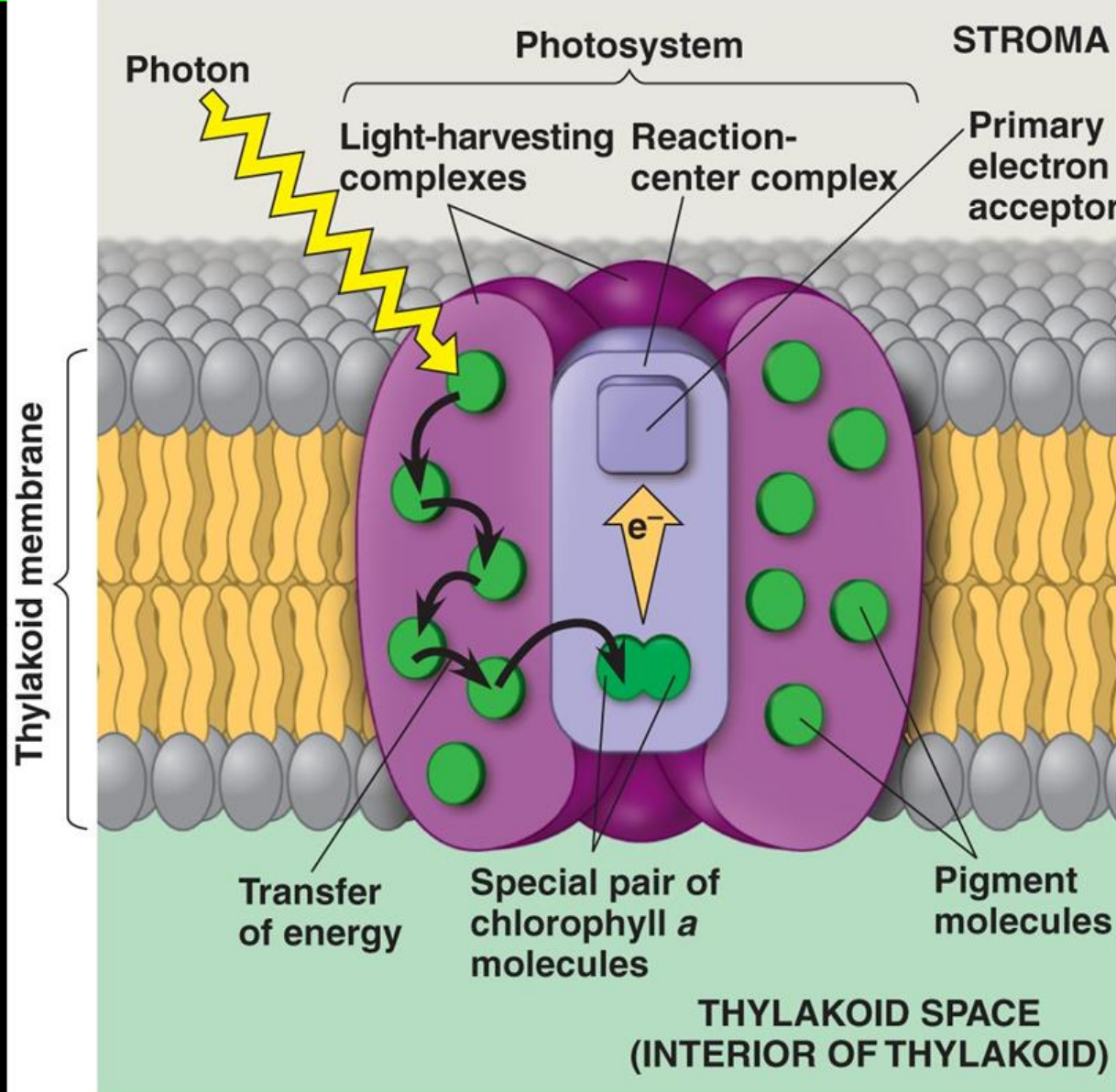
Photosynthetic pigments are amphipathic

Lutein

About photosynthetic pigments

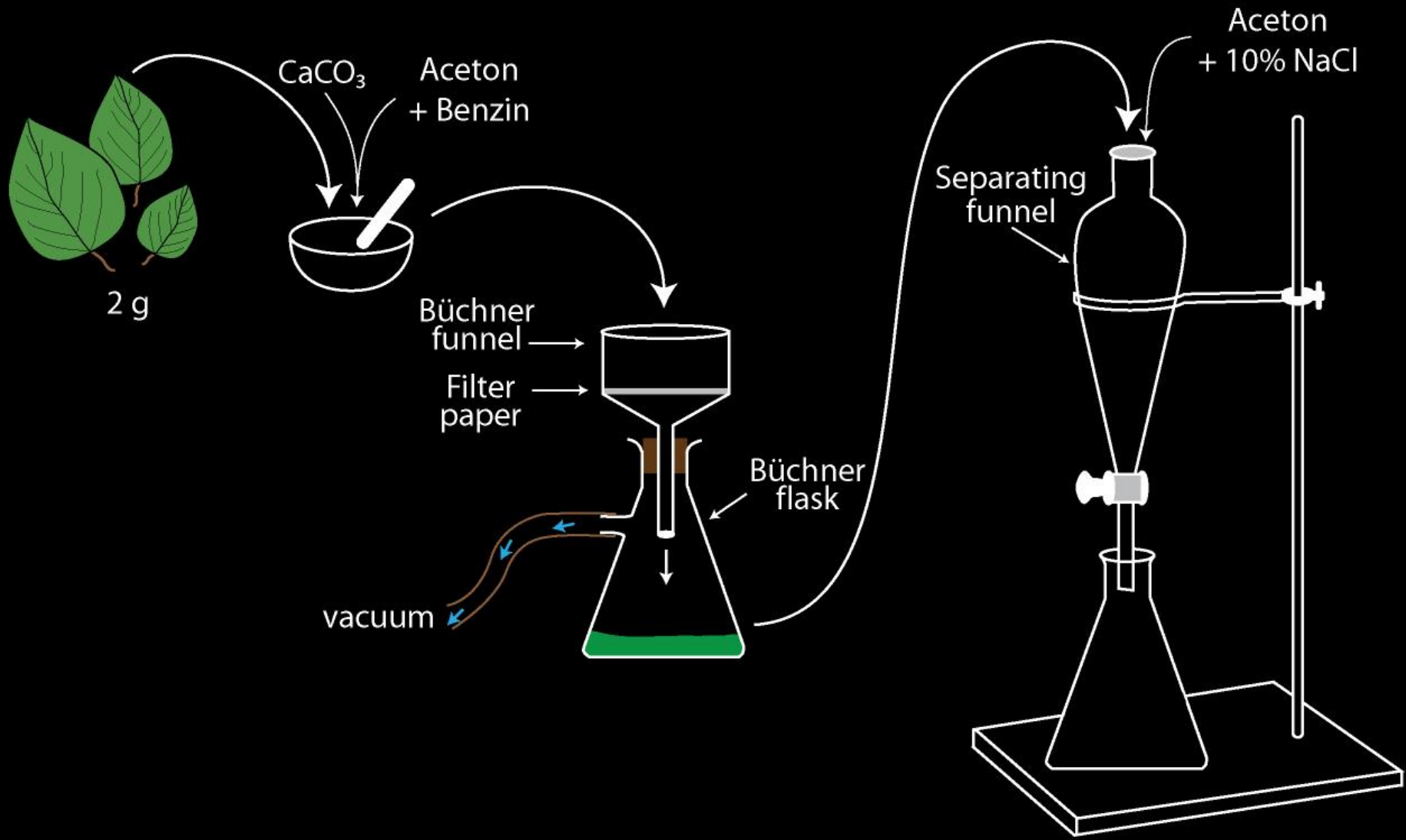
Photosynthetic pigments are associated to integral membrane proteins.

How can we extract them?



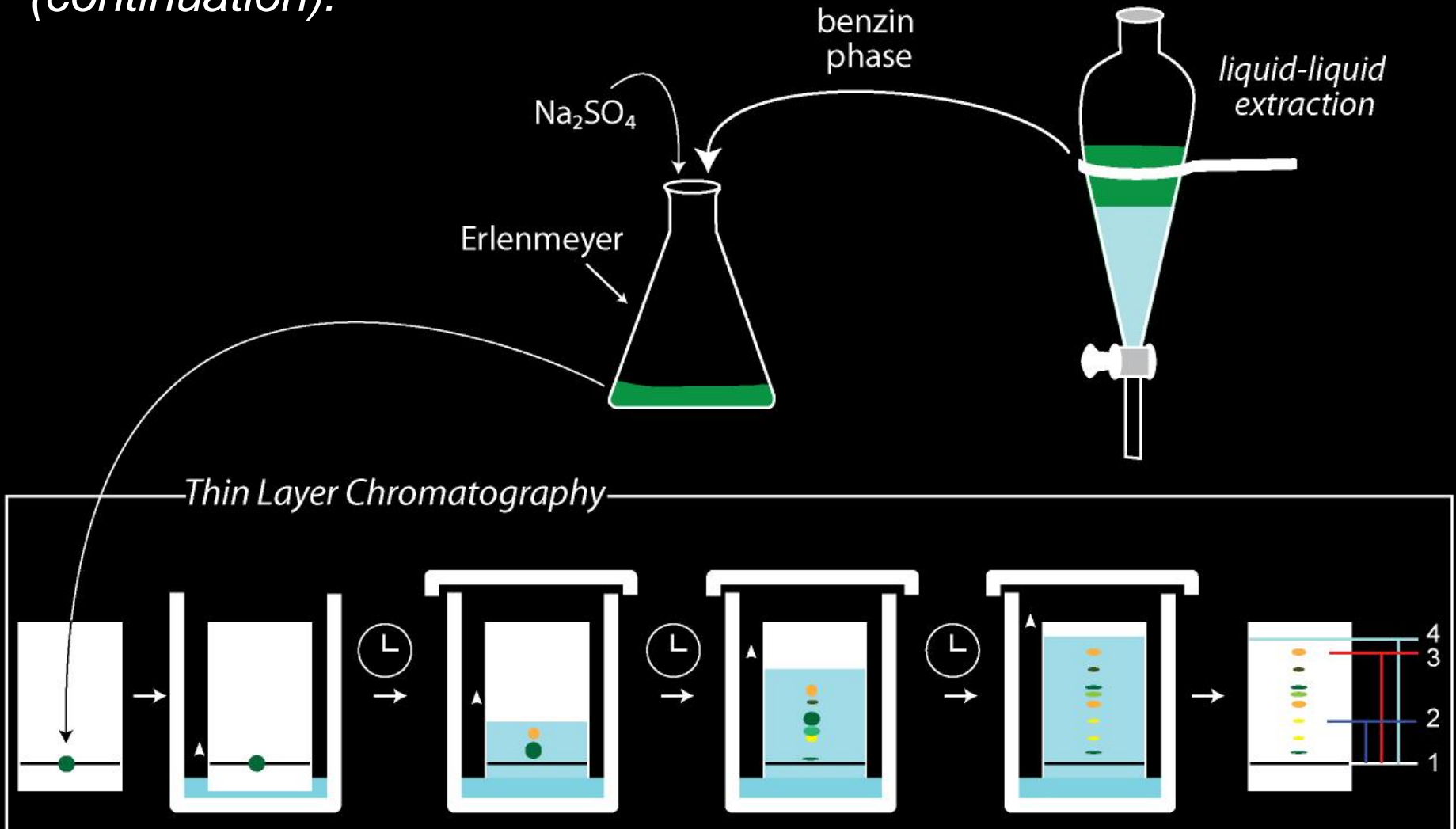
Chloroplast pigments experiment

Chromatographic separation of the photosynthetic pigments.



Chloroplast pigments experiment

Chromatographic separation of the photosynthetic pigments (continuation).

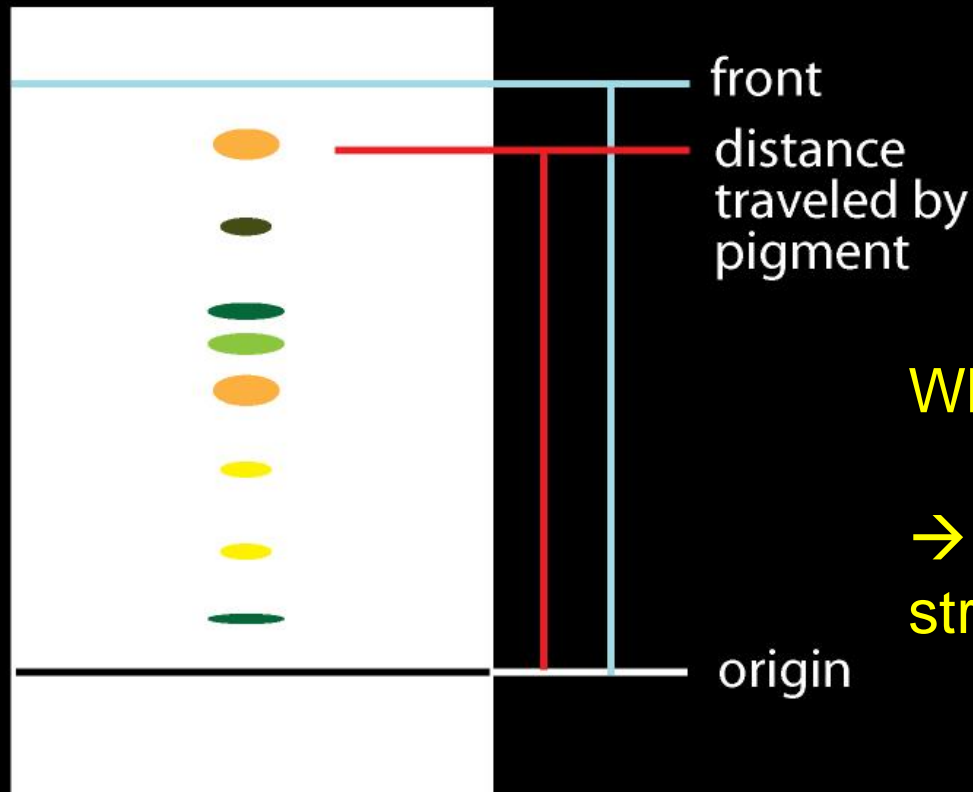


Chloroplast pigments experiment

Chromatographic separation of the photosynthetic pigments (continuation).

Determination of R_f value:

$$R_f = \frac{\text{distance traveled}}{\text{front}}$$

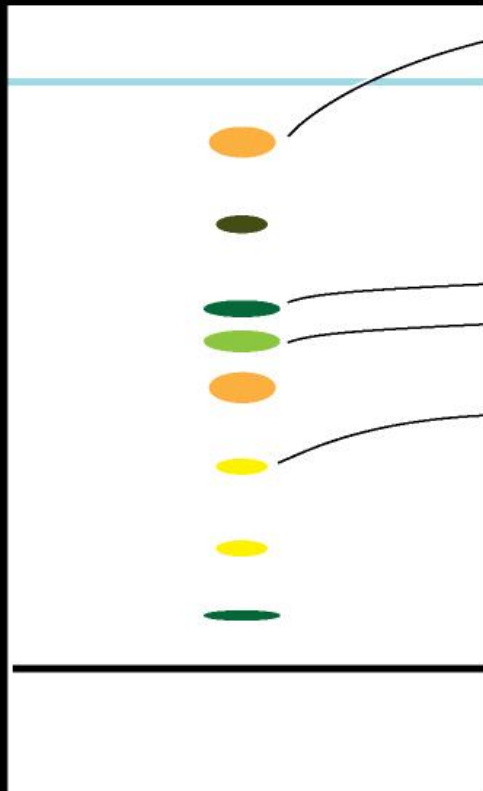


What is the separation based on?

→ Relate R_f values to molecular structure of pigments

Chloroplast pigments experiment

Chromatographic separation of the photosynthetic pigments (continuation).



*Each group will
analyze
only **one** band.*

Carotin (with hexane)

Chlorophyll a (with ether)

Chlorophyll b (with ether)

Lutein (with ethanol)

Spectroscopy

Absorption spectrum of photosynthetic pigments:

