Identification of the Polycomb Repressive Complex 2 in Fungi

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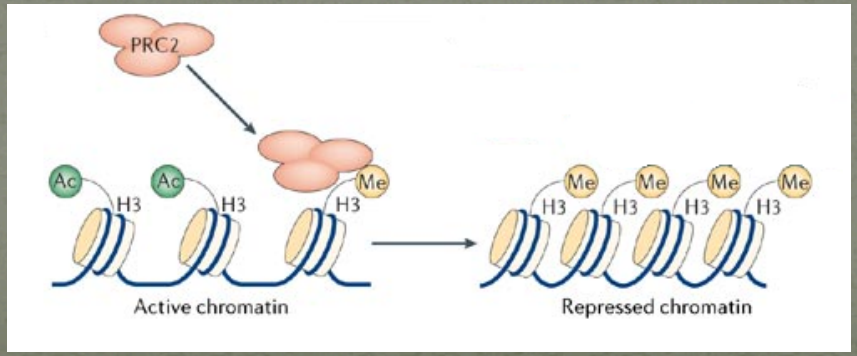
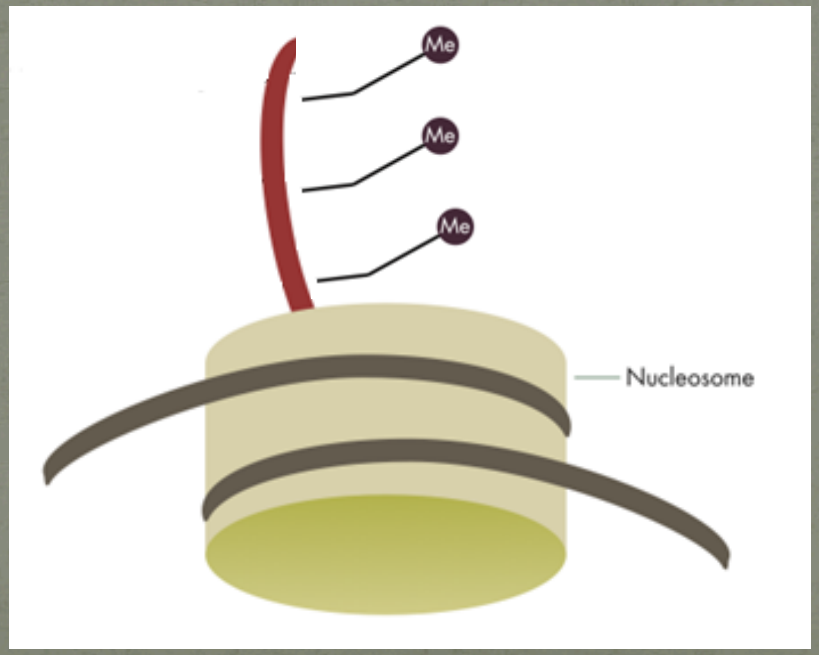


http://www.nature.com/nrm/journal/v7/n9/box/nrm1987_BX4.html

Activating and silencing of genes

- Transcriptional "gene silencing"
- Genes can be repressed by methyl groups on histones



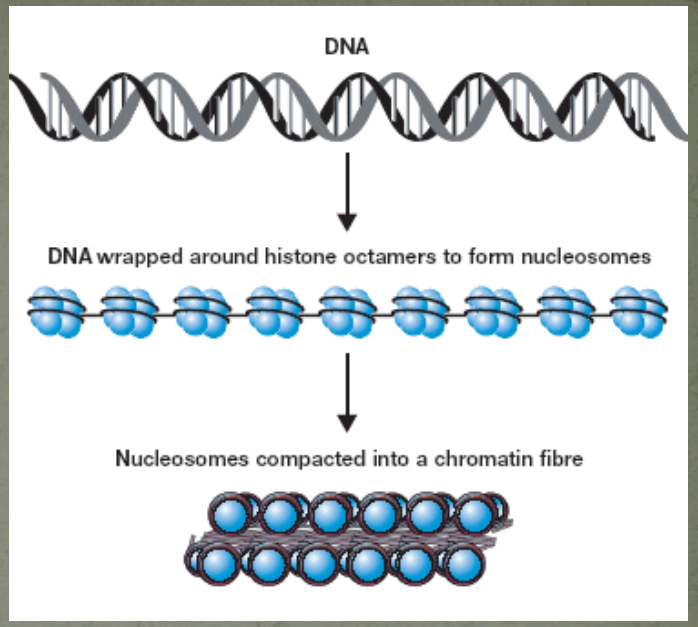
http://www.blogoup.com/blog/tag/epigenetics

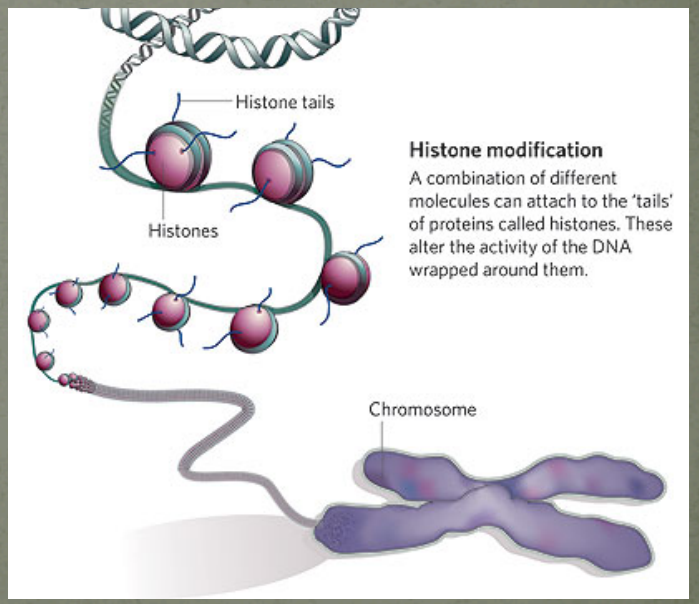
Function of gene silencing

- Regulate genes expression
- Normal development in stem cells
- Protect against viruses and transposons

Histone methylation

- Histones bind to DNA
- Makes up “chromatin”
- Histone 3:
  - methyl-lysine 4 (K4me2): active
  - methyl-lysine 27 (K27me3): silent





http://www.abcam.com/index.html?pageconfig=resource&rid=9

http://idv.sinica.edu.tw/ckao/Research%20interests_c.html

Organism

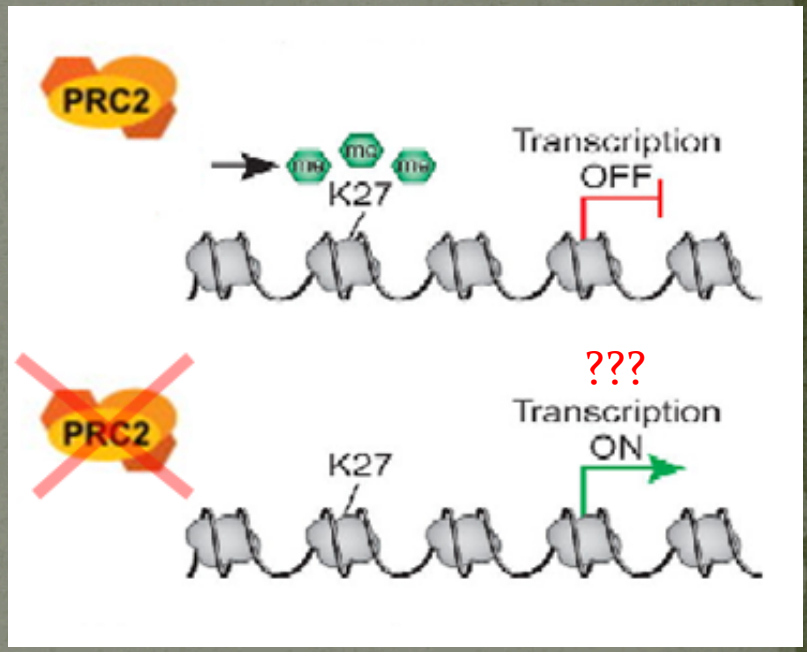
- H3K27me3 first found in fly
- *Fusarium graminearum*
- PRESENT: gene expression **OFF**
- ABSENT: gene expression **ON**
- Primary & secondary metabolites

http://en.wikipedia.org/wiki/File:F.graminearum.JPG
http://www.ag.ndsu.edu/pubs/plantsci/smgrains/pp894w.htm

Enzyme Responsible for H3K27me3

- Polycomb Repressive Complex 2 (PRC2)
- PRC2 includes 4 protein subunits:
  - *Set7, Caf1-3, Suz12, Eed*
  - (Subunits 1, 2, 3, 4)
- Deletion of subunit 1 removes H3K27me3

  → infertility, slow growing, orange
- Developmental effects due to misregulation



http://www.nature.com/ng/journal/v42/n2/full/ng0210-100.html

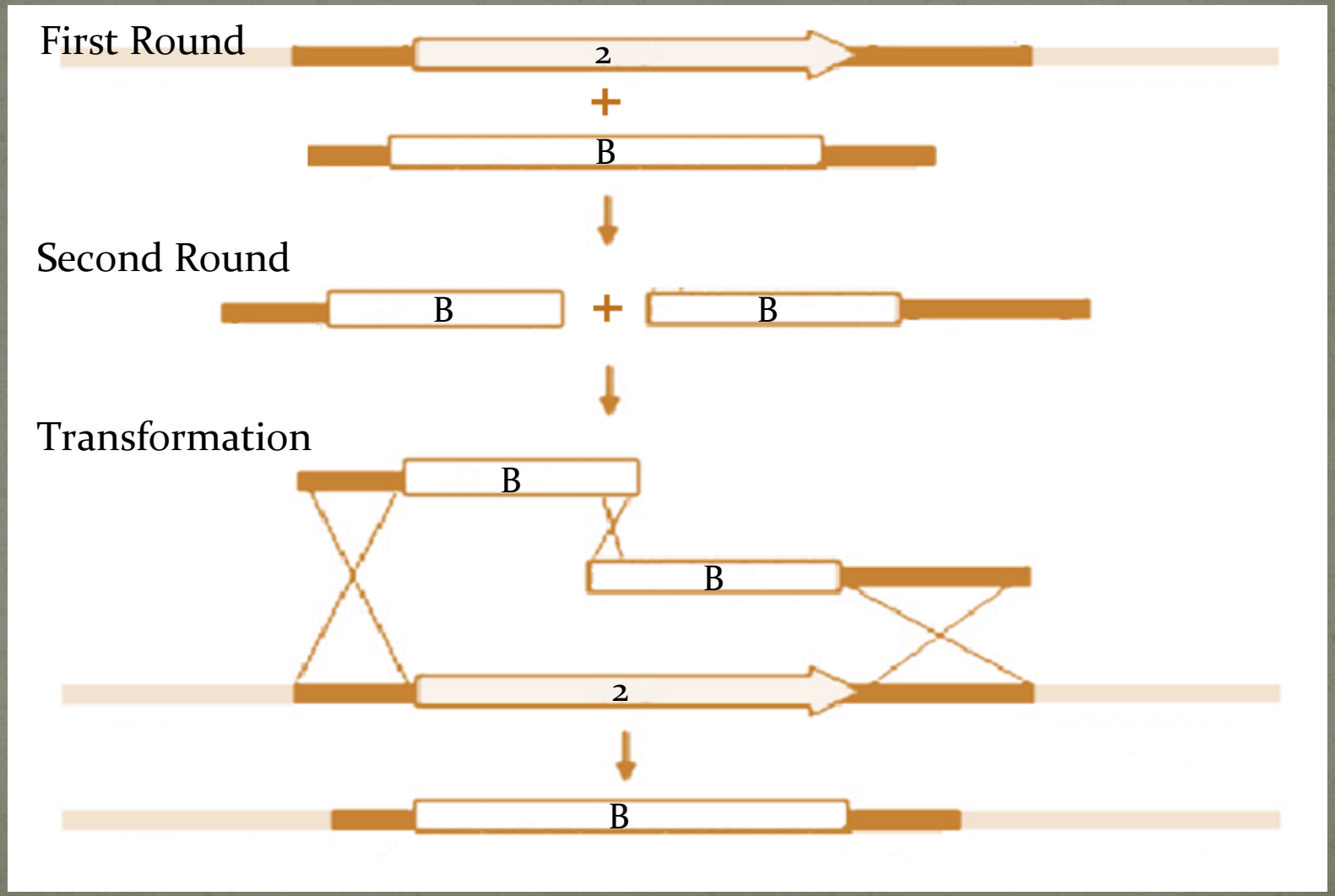
Aims of this study

- Test if deletion of subunits 2, 3, and 4 will show similar effects as deletion of subunit 1
- Study interactions between PRC2 subunits
- Determine the composition of fungal PRC2

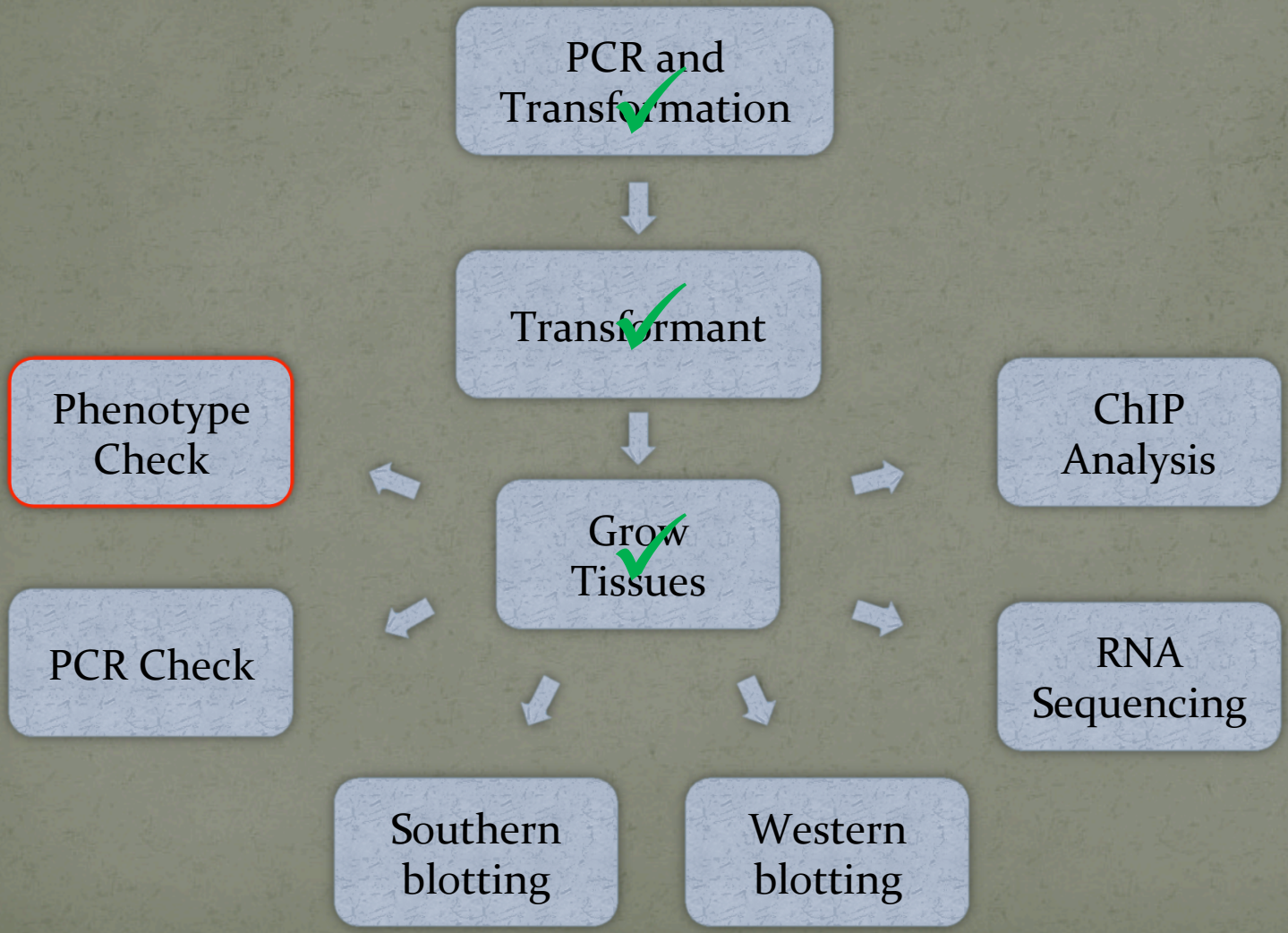
Deletion by Split Marker PCR

- First round: Generated fragments
  - *Cafi-3, Suz12, Eed* (2-4)
  - *neo* and *hph* – genes encoding for antibiotic resistance (A and B)
- Second round: Generated split marker fragments (flank and ½ of A or B)
- Transformation: Replaced genomic 2, 3, or 4 with A or B
  - *Δ2* (*Δ2*::A and *Δ2*::B)
  - *Δ3* (*Δ3*::A and *Δ3*::B)
  - *Δ4* (*Δ4*::A and *Δ4*::B)
- Only proceeded with *Δ2*::A, *Δ3*::B, and *Δ4*::B

Split Marker PCR



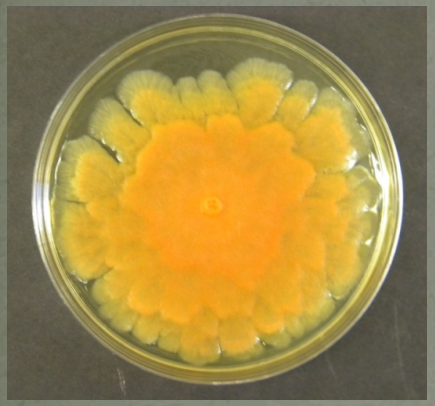
Methods: Analysis of deletion strains



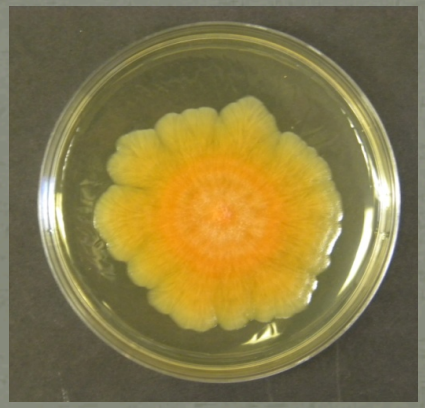
Phenotype Check

Wild Type (WT)

Δ1



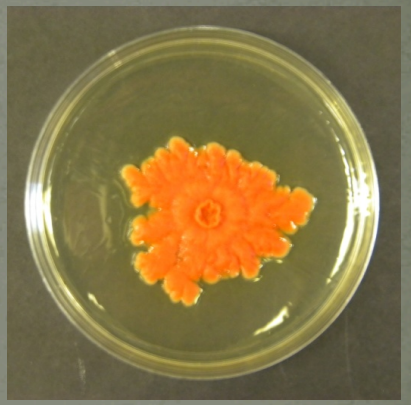
Wild Type (WT)



Δ2

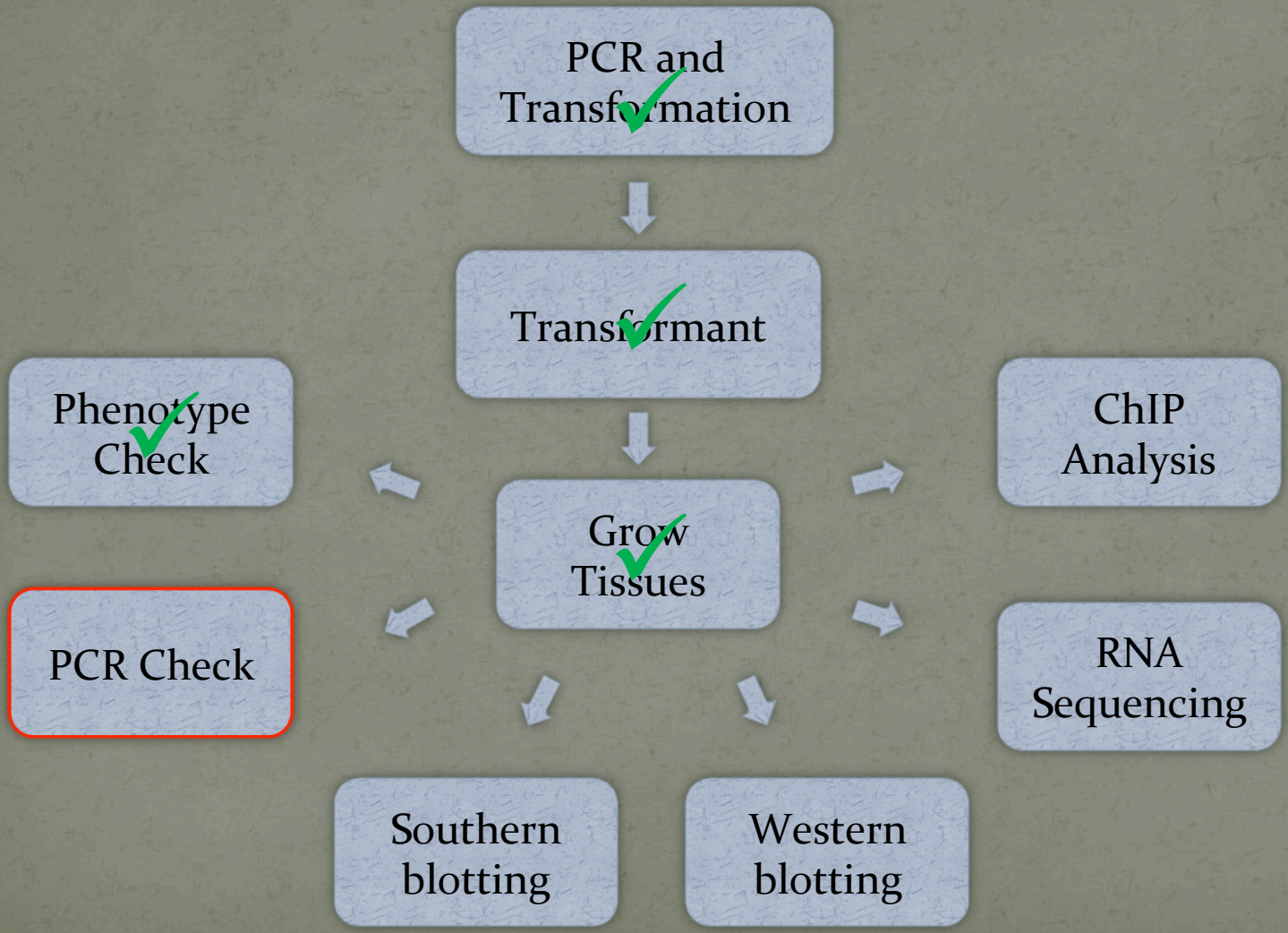
???

Δ3

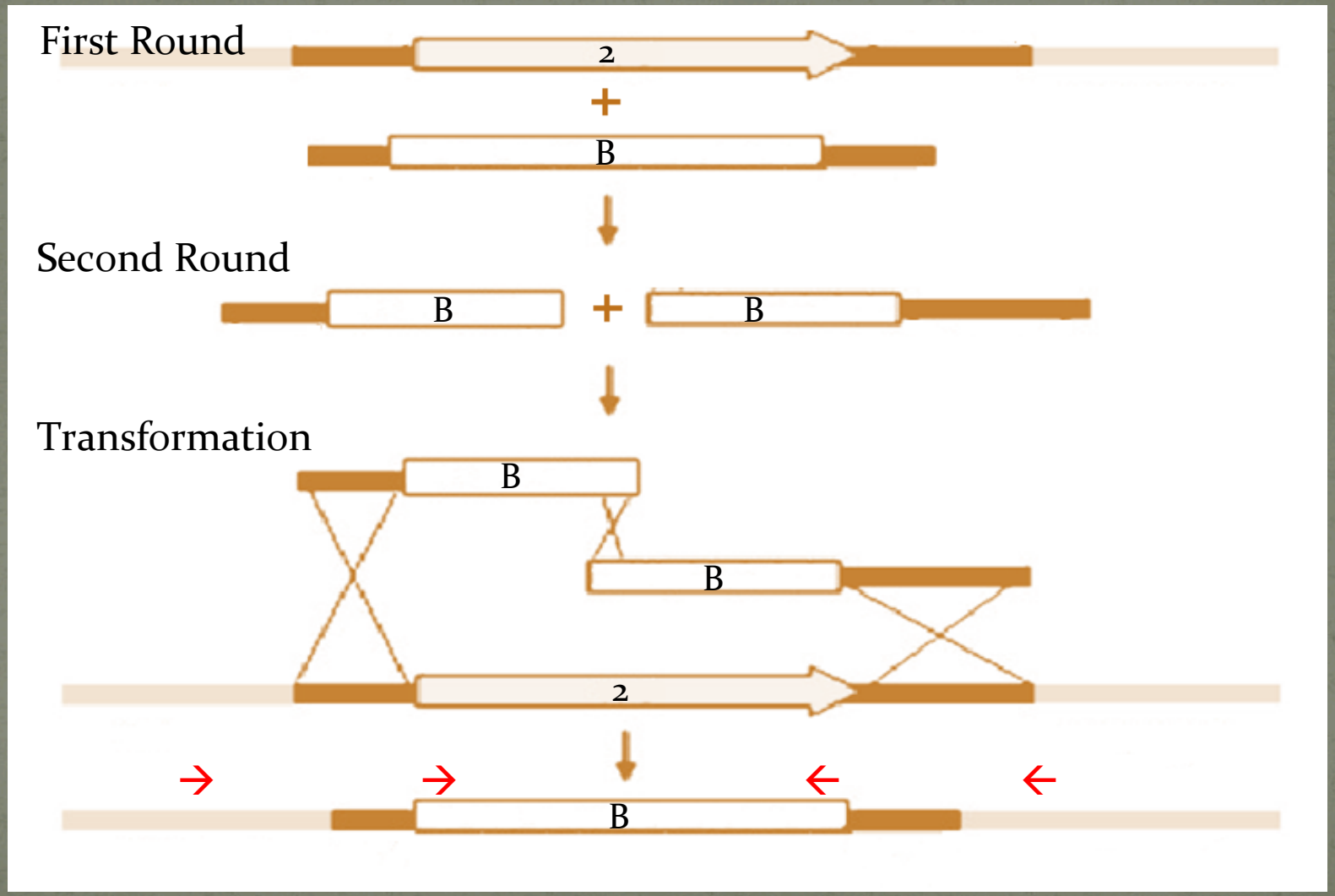


Δ4

Methods: Analysis of deletion strains

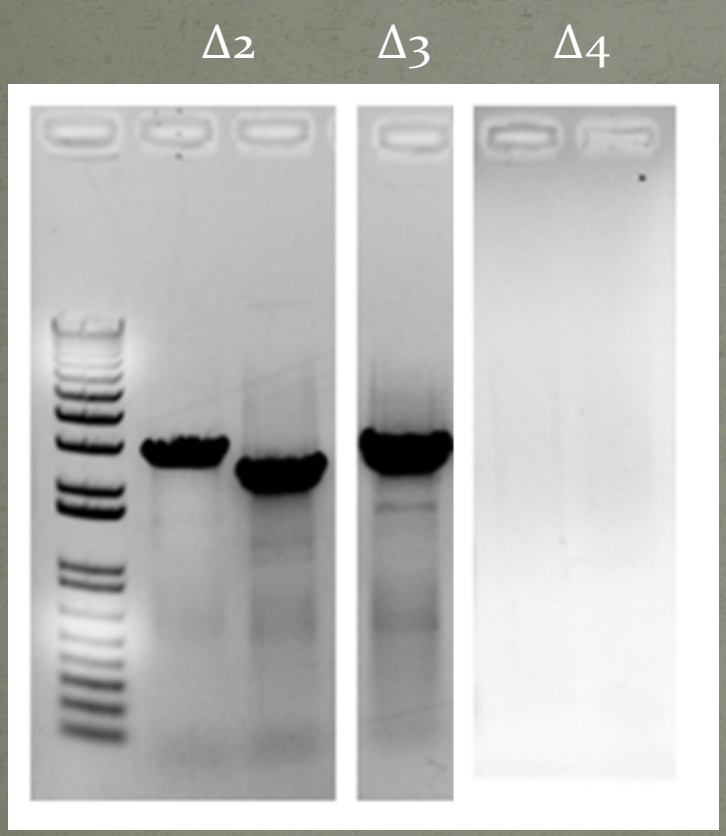


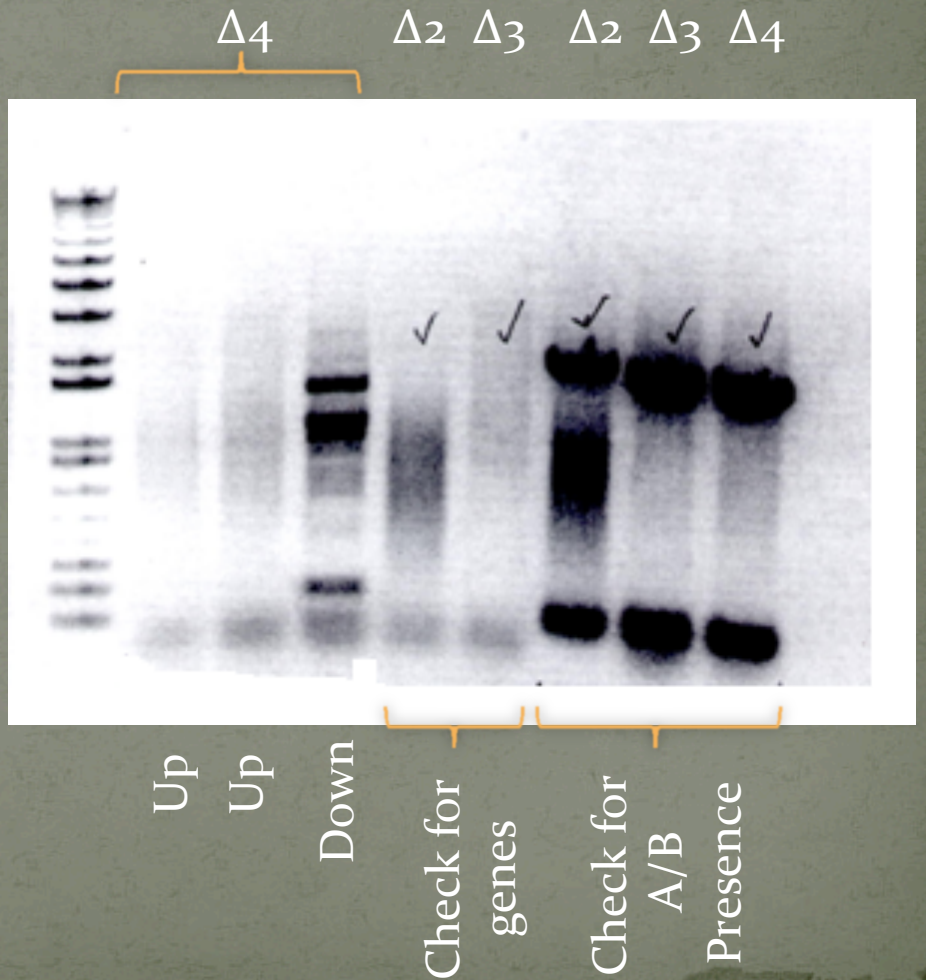
Split Marker PCR



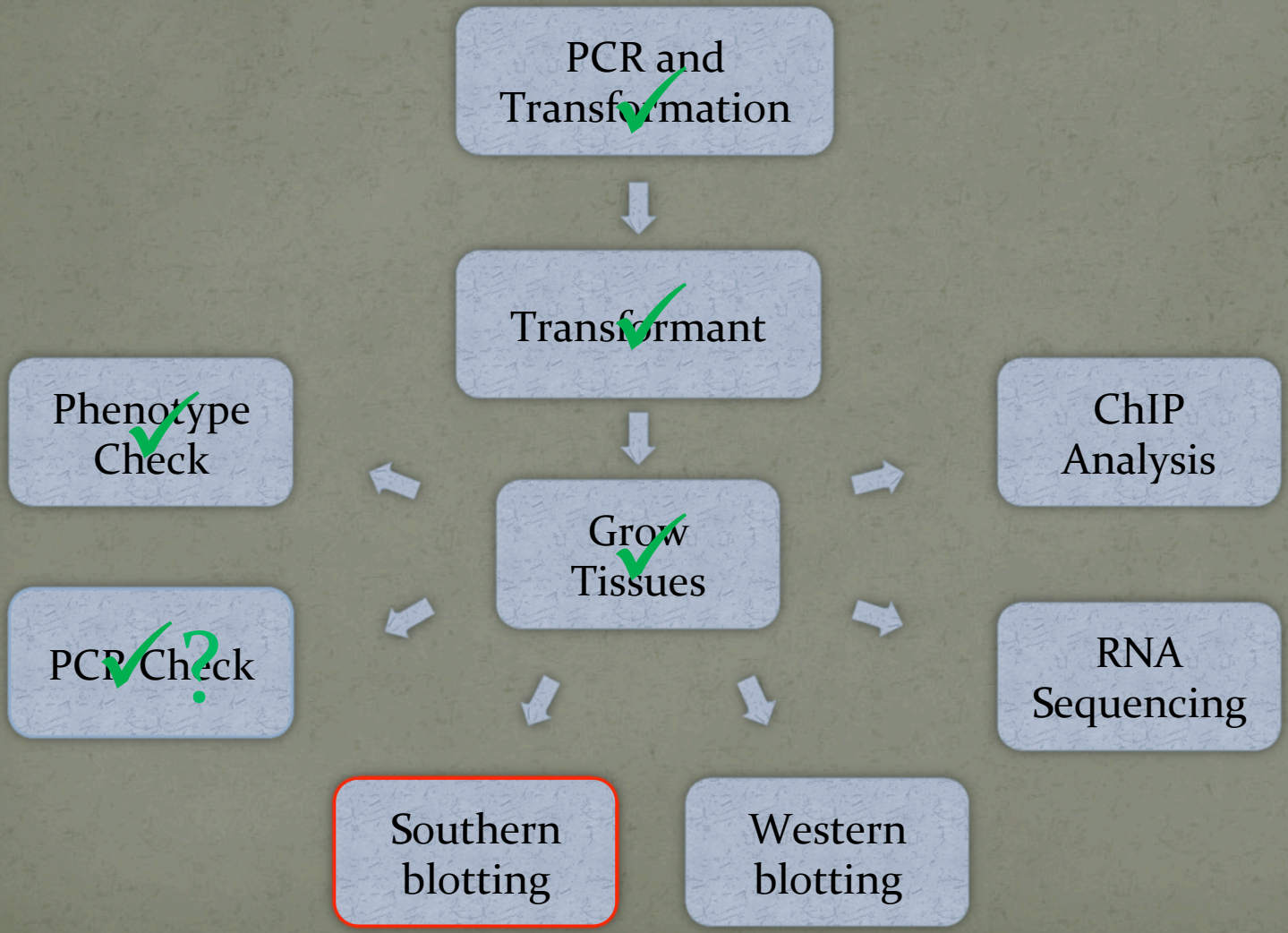
PCR Check

Check for A/B Up & Down

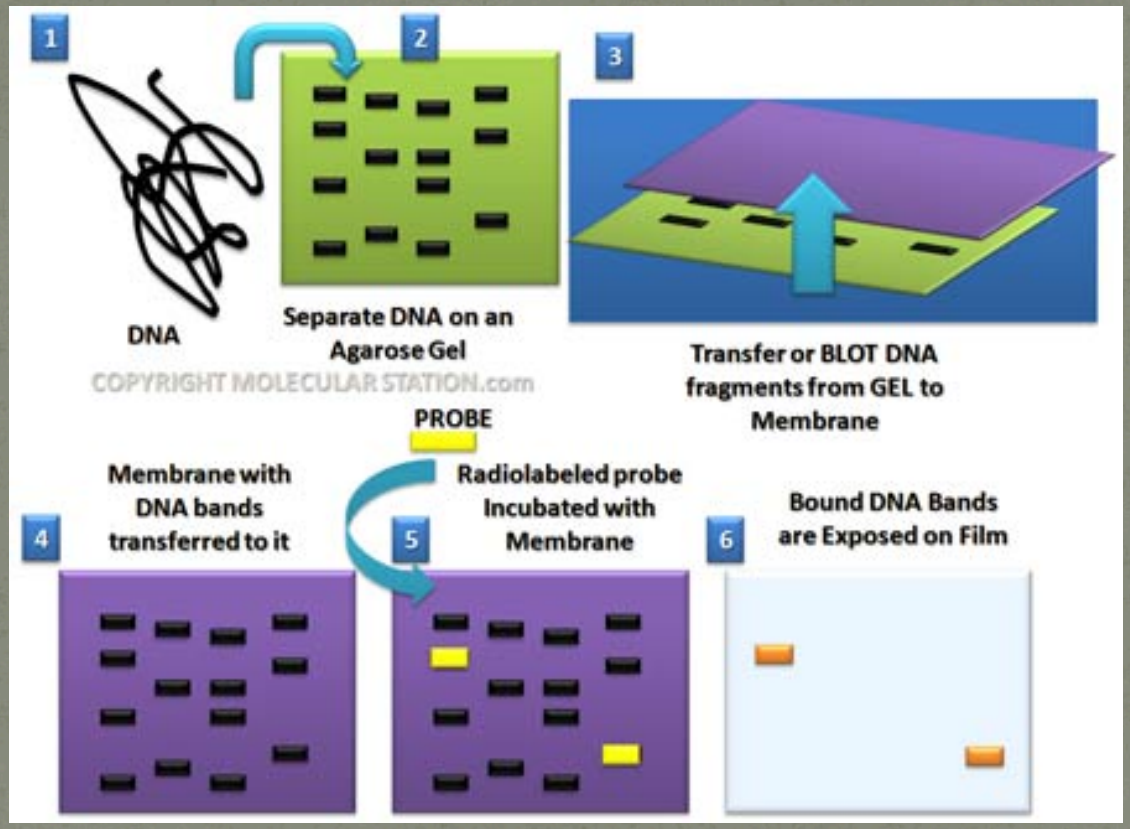




Methods: Analysis of deletion strains



Southern blotting

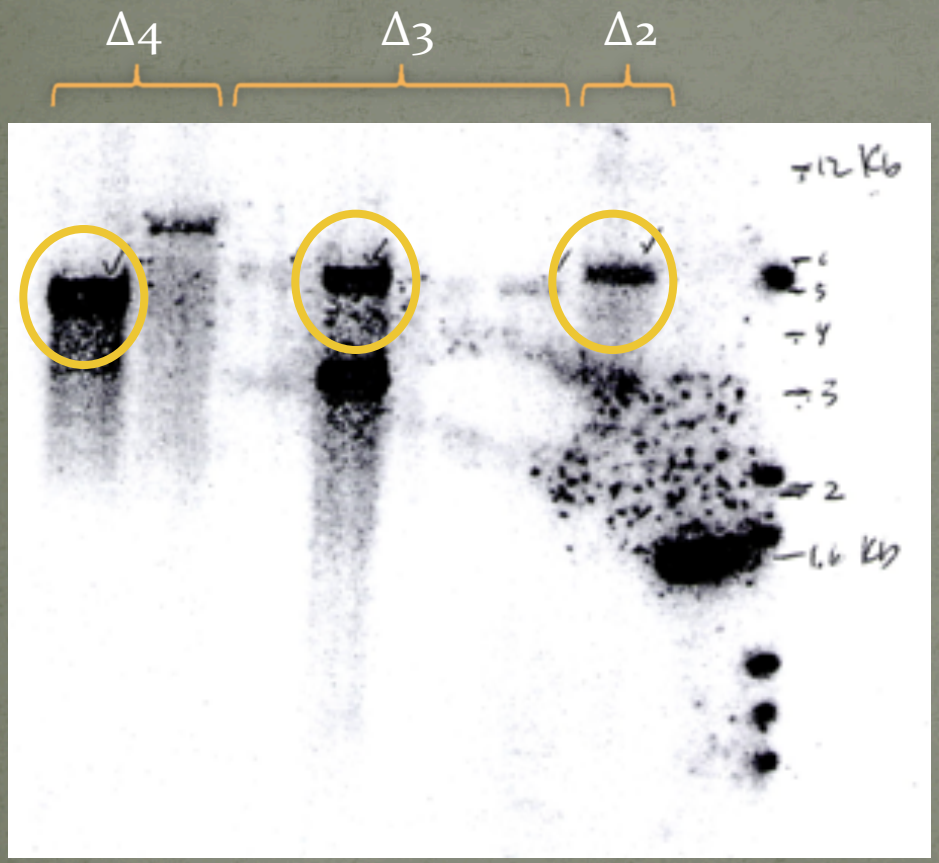


- A/B ✓ 2/3/4 ✗

http://enfo.agt.bme.hu/drupal/en/node/8926

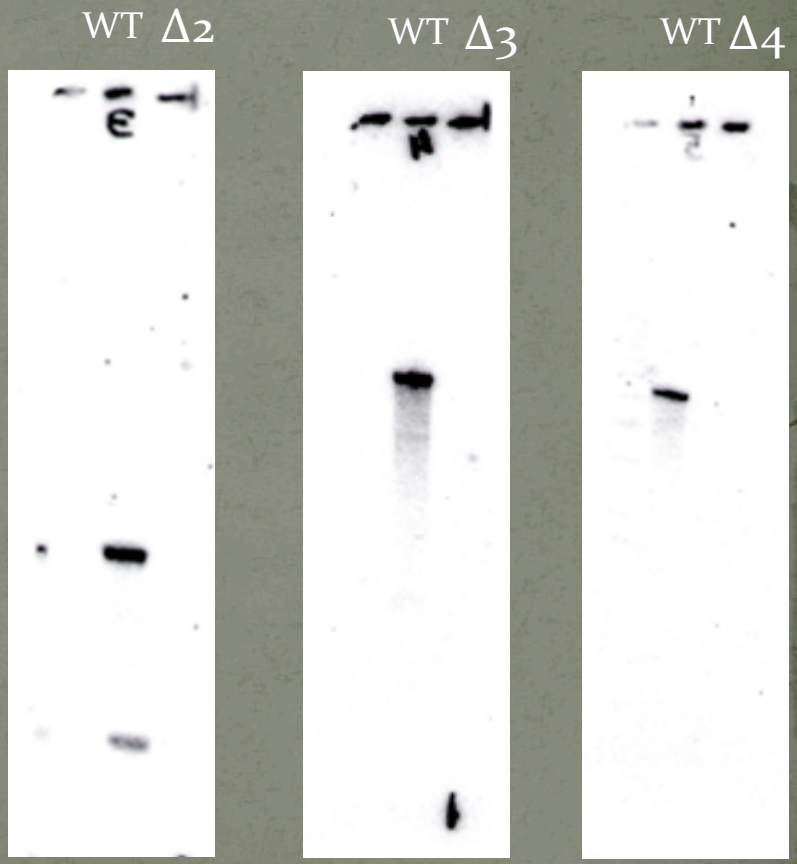
Southern blots

Check for A/B:



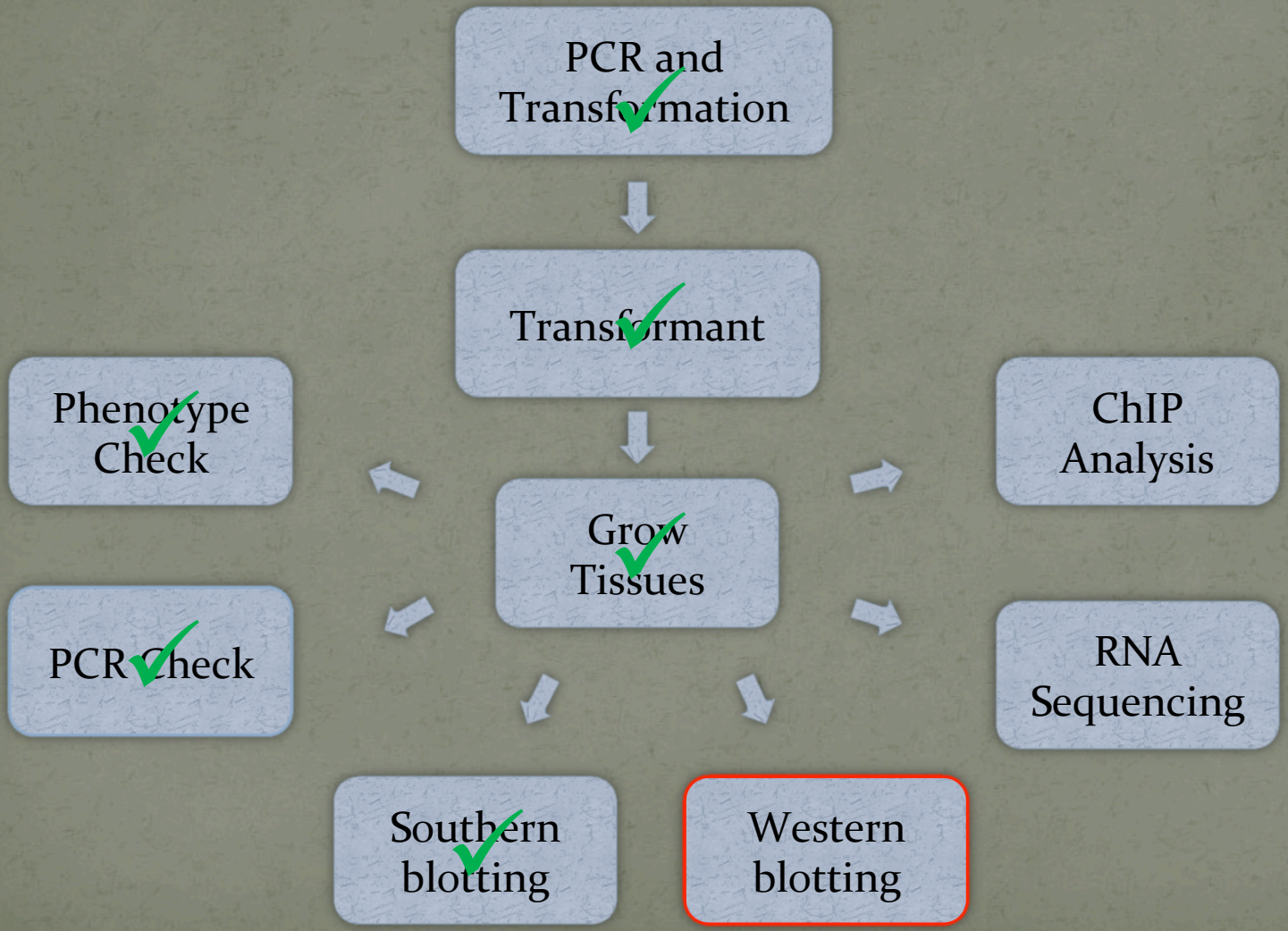
→ **Mutants possess A/B**

Check for genes:

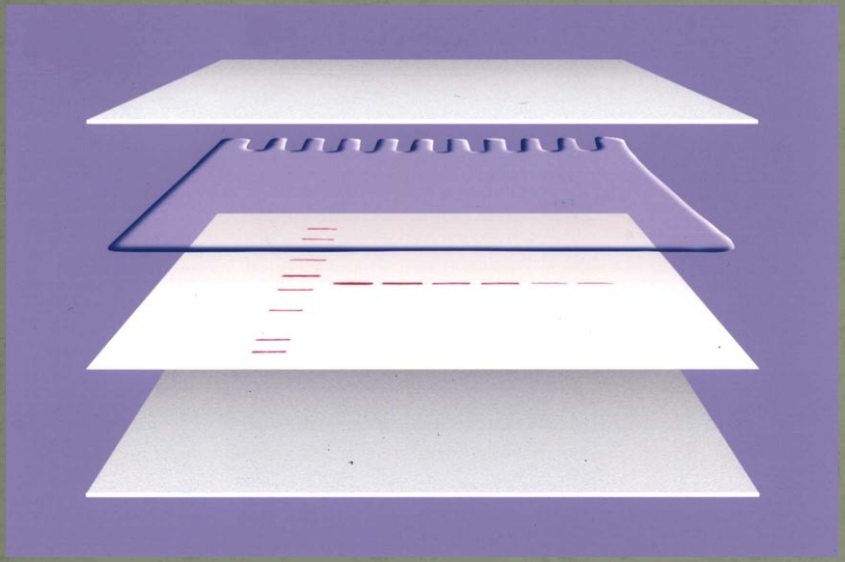


→ **Mutants lack KO genes**

Methods: Analysis of deletion strains



Western blotting



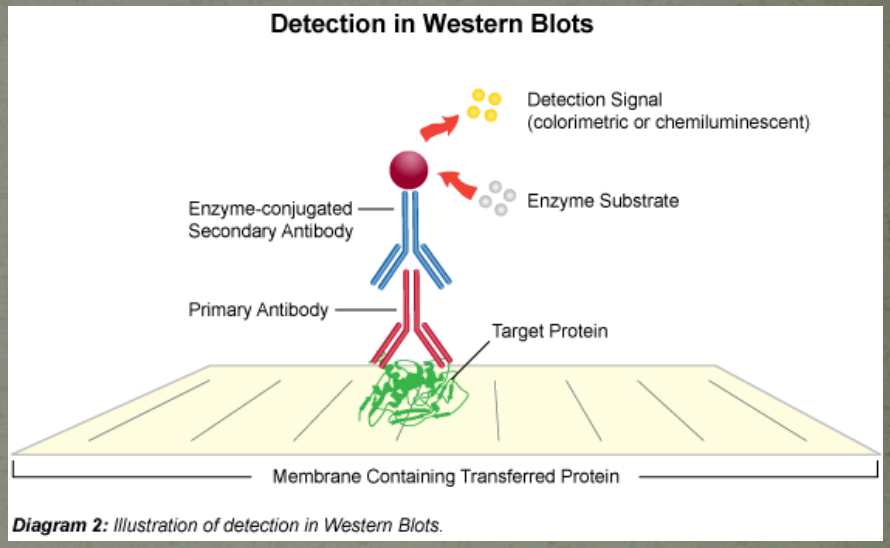


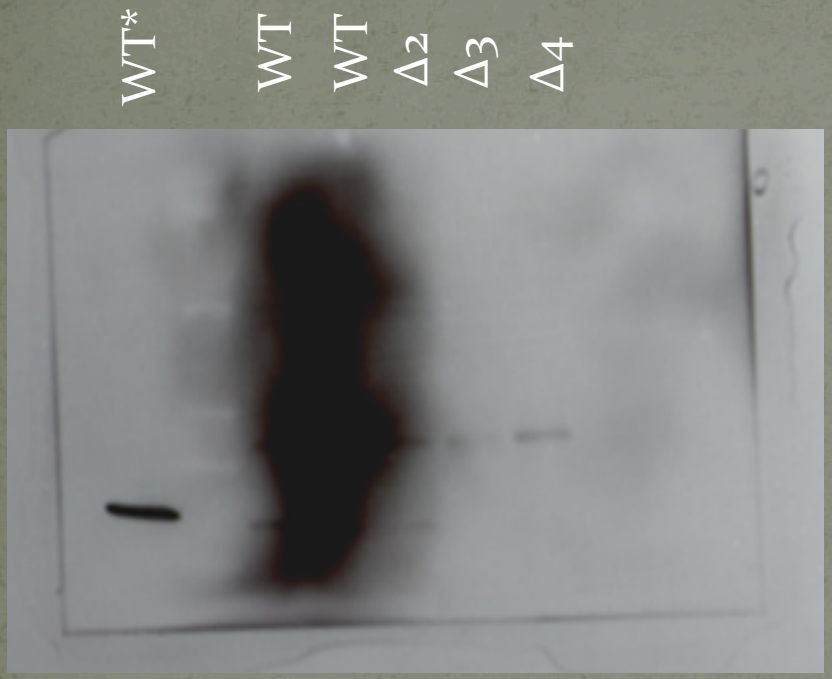
Diagram 2: *Illustration of detection in Western Blots.*

- Deletion mutants are predicted to have no H3K27me3 markers
  - Exceptions may be present in Δ2

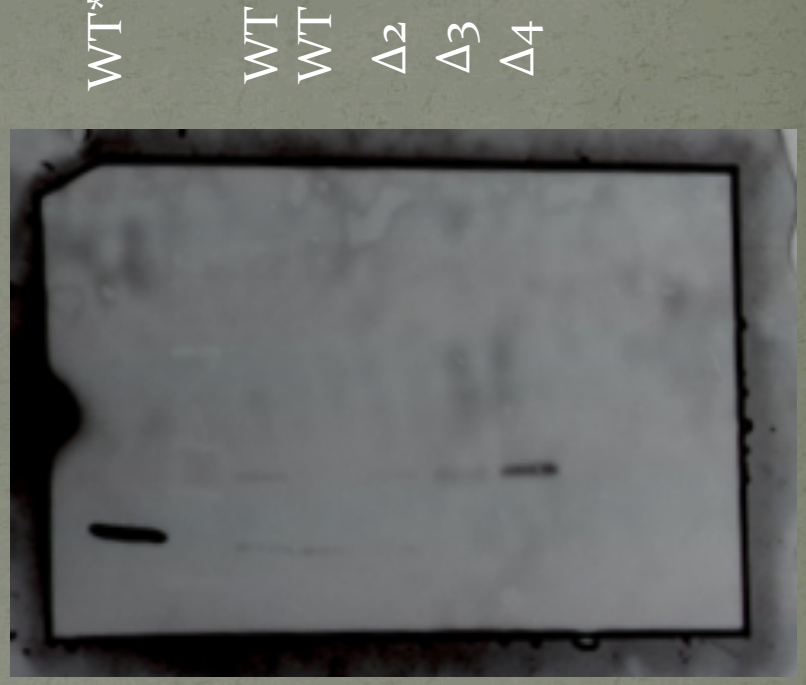
http://news.thomasnet.com/fullstory/Membrane-Sandwiches-make-western-blotting-easier-13151

http://www.leinco.com/general_wb

Western blotting



Before water wash



After water wash

- Δ3 and Δ4 do not have H3K27me3 marker
- Δ2 shows weak signal for the marker

Next…

- Investigate by ChIP technique
- Analyze using RNA sequencing method
- Perform gene tagging

Applications

- Learn how PRC2 operates as a protein complex
- Understand importance of each protein subset
- Recognize functions of PRC2 in cell development

Acknowledgments

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