# Exploring Chromodomain Genes in the Fungus *Fusarium graminearum* Through Targeted Genetic Manipulation

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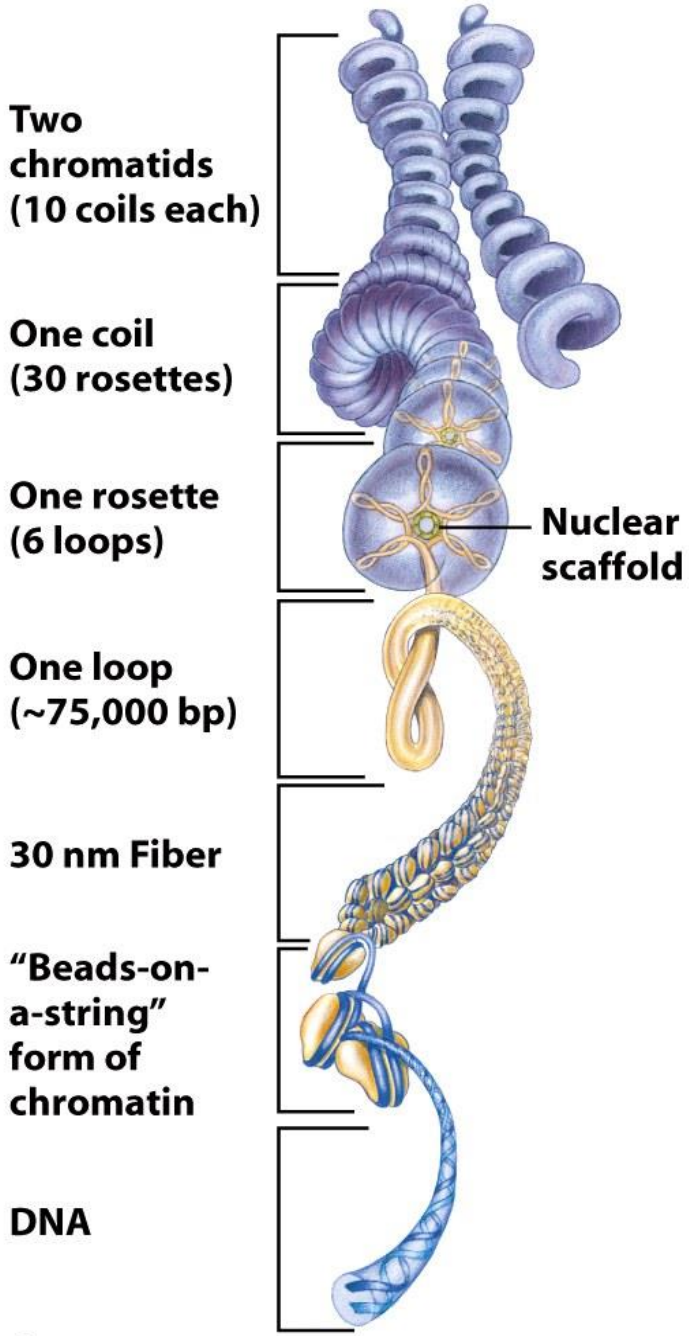



**Figure 24-33**
***Lehninger Principles of Biochemistry, Fifth Edition***


# DNA and Chromatin

- Genomic DNA exists as chromatin
  - Nucleosomes
- Chromatin structure alters gene expression
  - Heterochromatin is densely packed and inactive
  - Euchromatin is loosely packed and active

# Histones

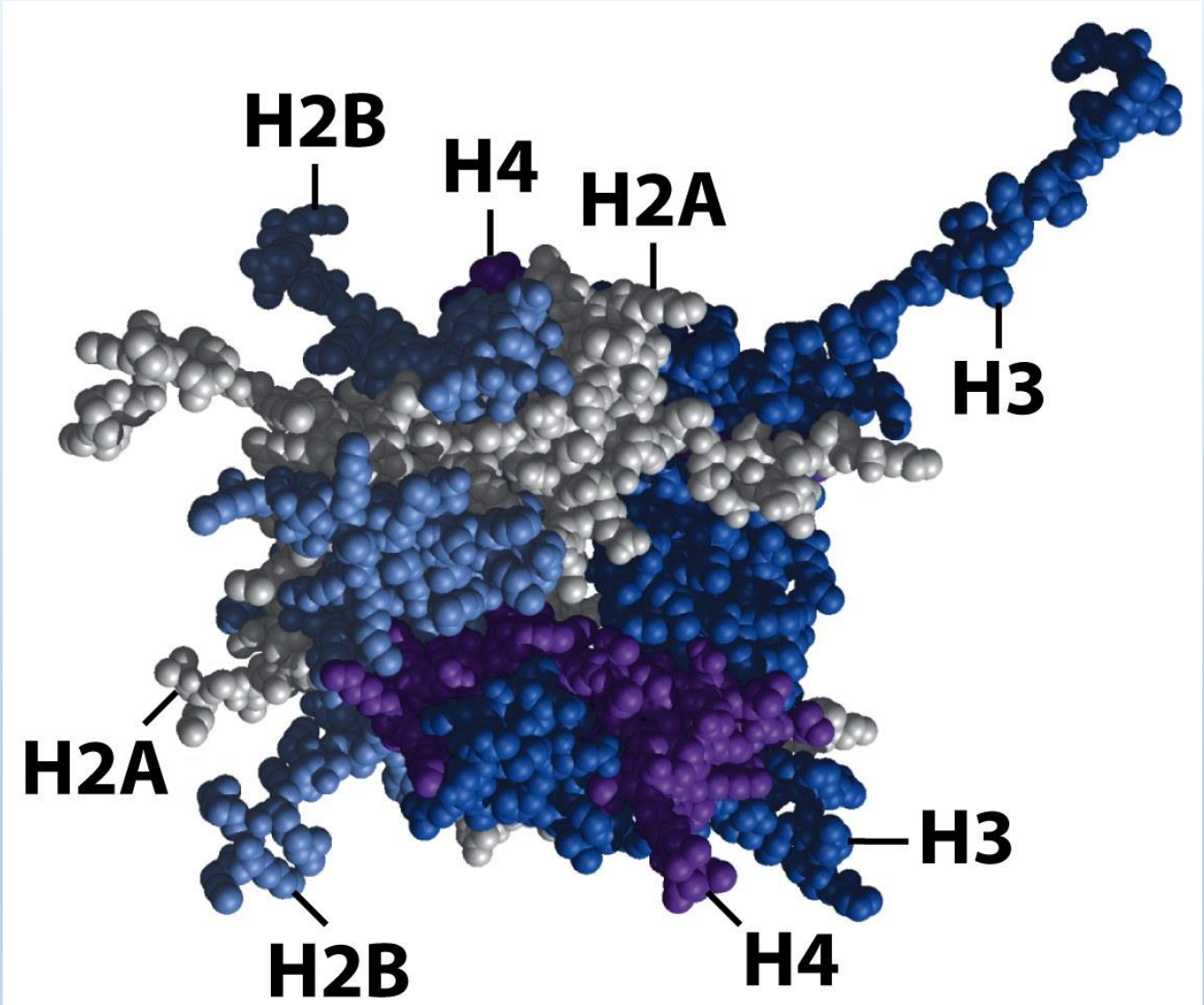
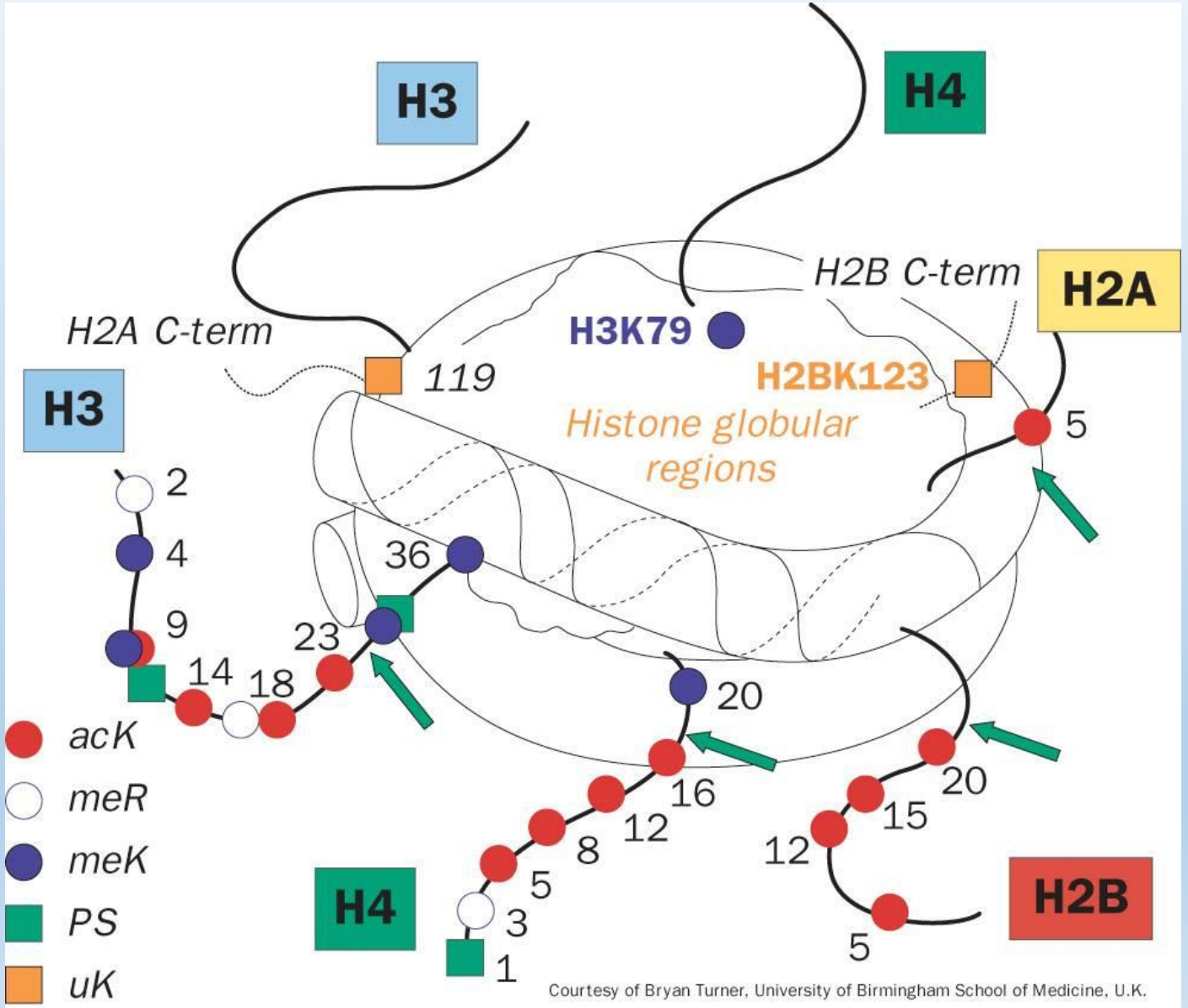


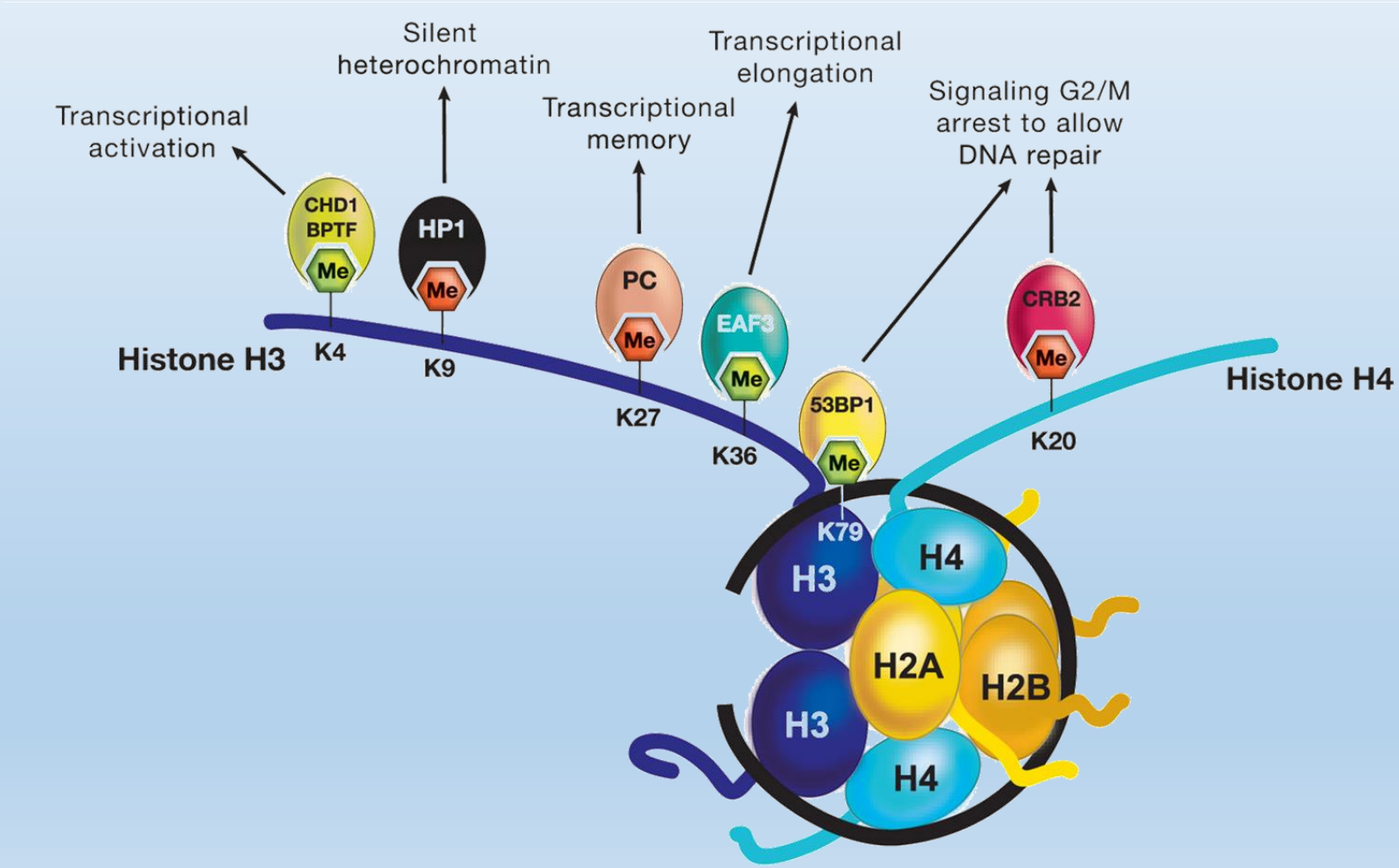

**Figure 24-27a**
***Lehninger Principles of Biochemistry, Fifth Edition***

# Histone Modification Alters Chromatin Structure



# Chromodomain Proteins

- CDPs
- Chromatin binding motif
  - Pc and HP1
- Nuclear Localization
  - GFP strains



# *Fusarium graminearum*

- Cause of *Fusarium* Head Blight (FHB)
  - Crop Damages
  - Food Contamination
- Model Organism
  - H3K27me3
  - Find possible PRC1 homolog

http://upload.wikimedia.org/wikipedia/commons/1/1e/Wheat_scab.jpg

# *F. graminearum* CDP Gene Candidates

- Sequence homology with CDPs
  - Focus on those with unknown functions

- 8 Gene Candidates
  - Gene "KOs" – gene is absent
  - GFP fusion – localization
  - Observe phenotypes to identify CDP genes and perhaps PRC1 homolog

| CDP Candidate | Fusarium Gene ID | N. crassa Gene ID | Proposed Function |
|---|---|---|---|
| **FgCDP1** | FGSG_01512 | NCU08362 | Centromere Regulation |
| **FgCDP2** | FGSG_05030 | NCU00738 | H3K9me Regulation |
| **FgCDP3** | FGSG_04328 | NCU01522 | Unclear Function |
| **FgCDP5** | FGSG_11309 | N/A | Unclear Function |
| **FgCDP6** | FGSG_14036 | NCU06788 | Histone Acetylation |
| **FgCDP8** | FGSG_03473 | N/A | Unclear Function |
| **FgCDP9** | FGSG_02144 | N/A | Unclear Function |
| **FgCHD3** | FGSG_07346 | NCU06696 | Histone Deacetylation |

# DNA Cassette Generation

- 5’ and 3’ flanking fragments
  - Flank targeted gene candidate

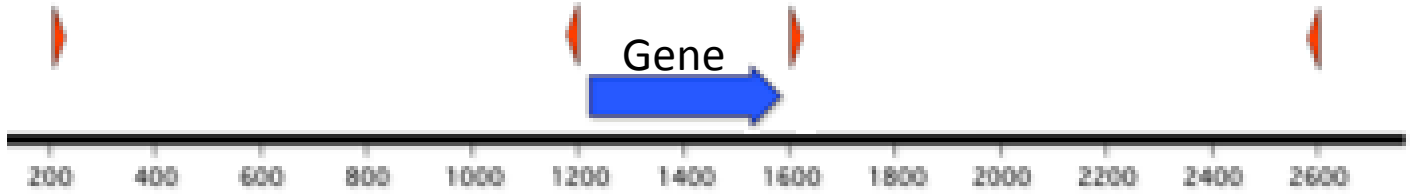
- GFP-S tag-*hph* or *neo* fragments
  - *neo* – neomycin antibiotic resistance gene
    - Confers resistance to G418

  - *hph* – hygromycin resistance resistance gene
    - Confers resistance to hygromygin

- Synthesized by PCR, gel-purified

# DNA Cassette Generation

1.

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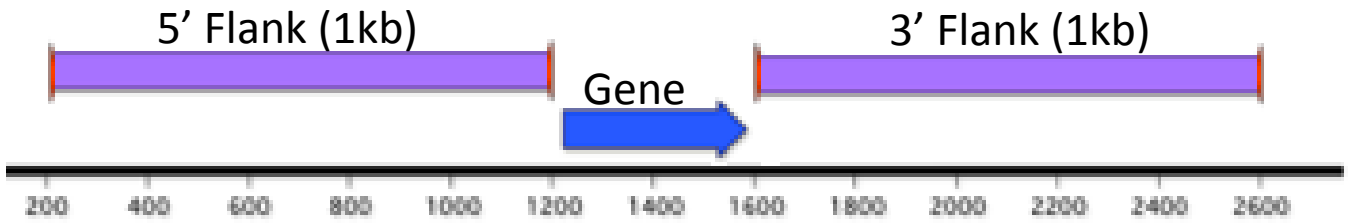


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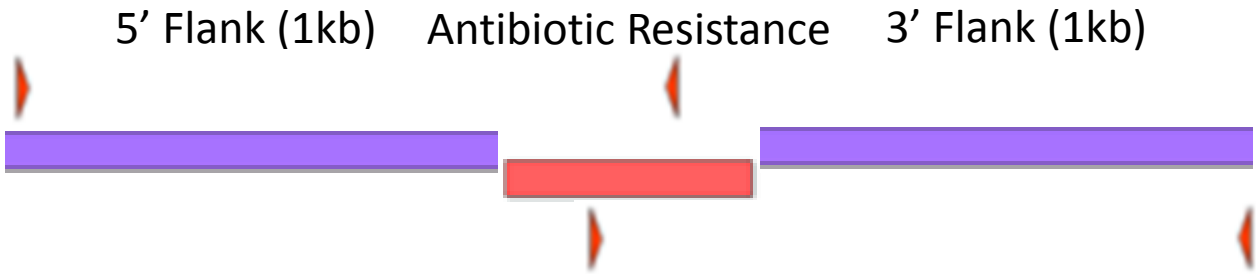


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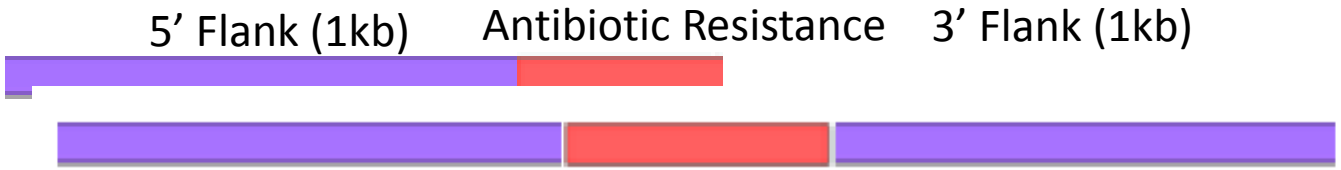


# Transformation and Selection

- DNA cassette is integrated into protoplast genome at target site
  - Selection:
    - G418 for strains with *neo* gene
    - Hygromycin for strains with *hph* gene
- Genomic DNA is prepared from strains
  - Used to verify targeted gene insertion
    - PCR and Southern Blots

# Verification of Transformant Strains

- PCR Verification
  - Primers:
    - Amplify gene segment
    - Amplify cassette
- Southern Blots
  - Probes:
    - Gene candidate specific
    - Antibiotic gene specific

# Phenotypic Assays

- Changes in appearance
  - Color, shape, etc.
- Growth rate changes
- Protein localization
  - GFP tagged strains

7 days, YPD

WT *ΔKmt-6*

Photo taken by Lanelle Connolly, Freitag Lab

# Growth Assay/Westerns

- Growth Assay
  - Conidia grown, single spores picked
  - Linear growth on agar plate
  - YPD (rich) and FMM (poor) media

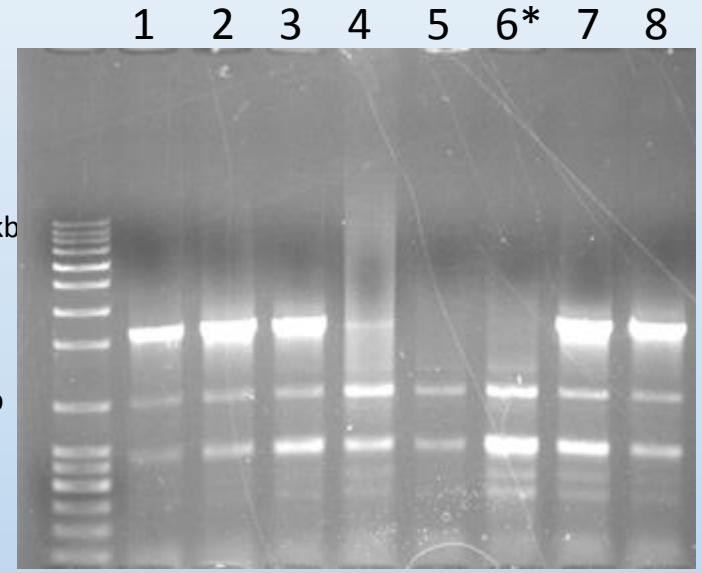
- Western Blots
  - Histone isolation
  - Coomassie blue stain
  - Western
    - Antibodies specific for histone modifications

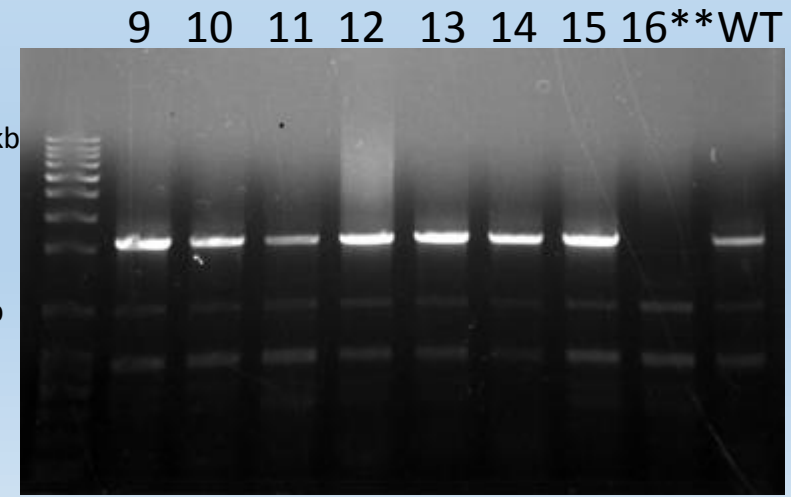
# Verified Transformants

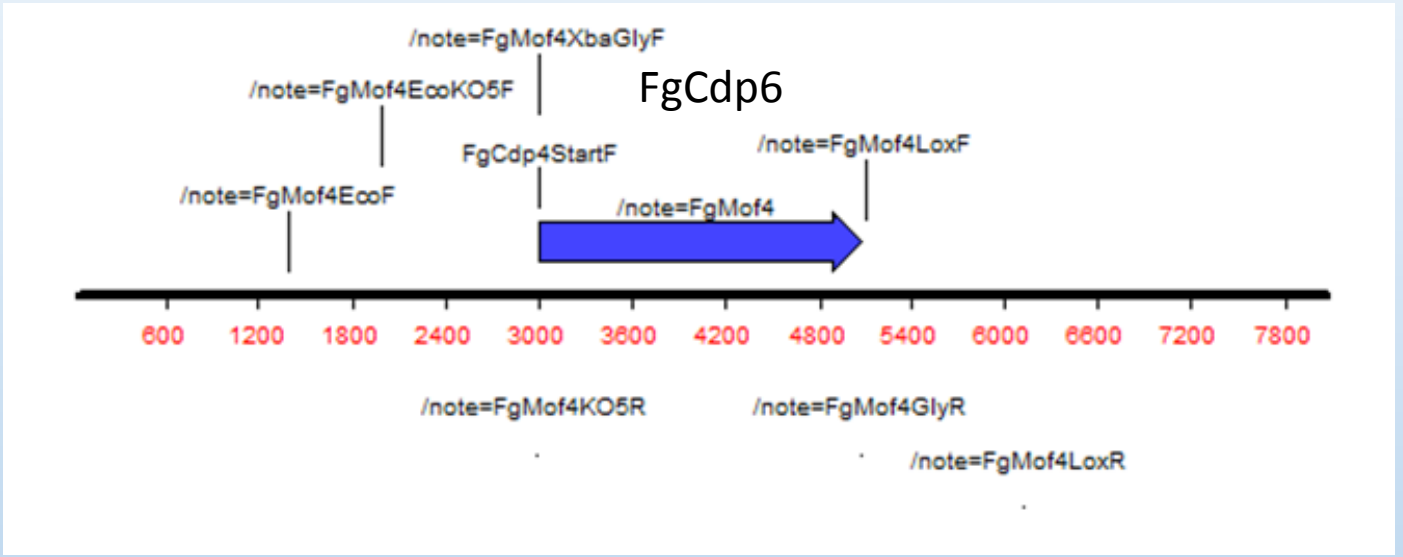
| Transformation | Obtained Verified Strains |
|---|---|
| ***ΔFgCdp1::neo*** | 1 |
| ***FgCdp1-GFP-S tag-hph*** | 0 |
| ***ΔFgCdp2::neo*** | 2 |
| ***FgCdp2-GFP-S tag-hph*** | 1 |
| ***ΔFgCdp3::neo*** | 1 |
| ***FgCdp3-GFP-S tag-hph*** | 4 |
| ***ΔFgCdp5::neo*** | 1 |
| ***ΔFgCdp6::neo*** | 2 |
| ***FgCdp6-GFP-S tag-hph*** | 1 |
| ***ΔFgCdp8::neo*** | 0 |
| ***ΔFgCdp9::neo*** | 1 |
| ***ΔFgChd3::neo*** | 1 |

# Verification of Transformants via PCR

*ΔFgCdp6::neo* KO

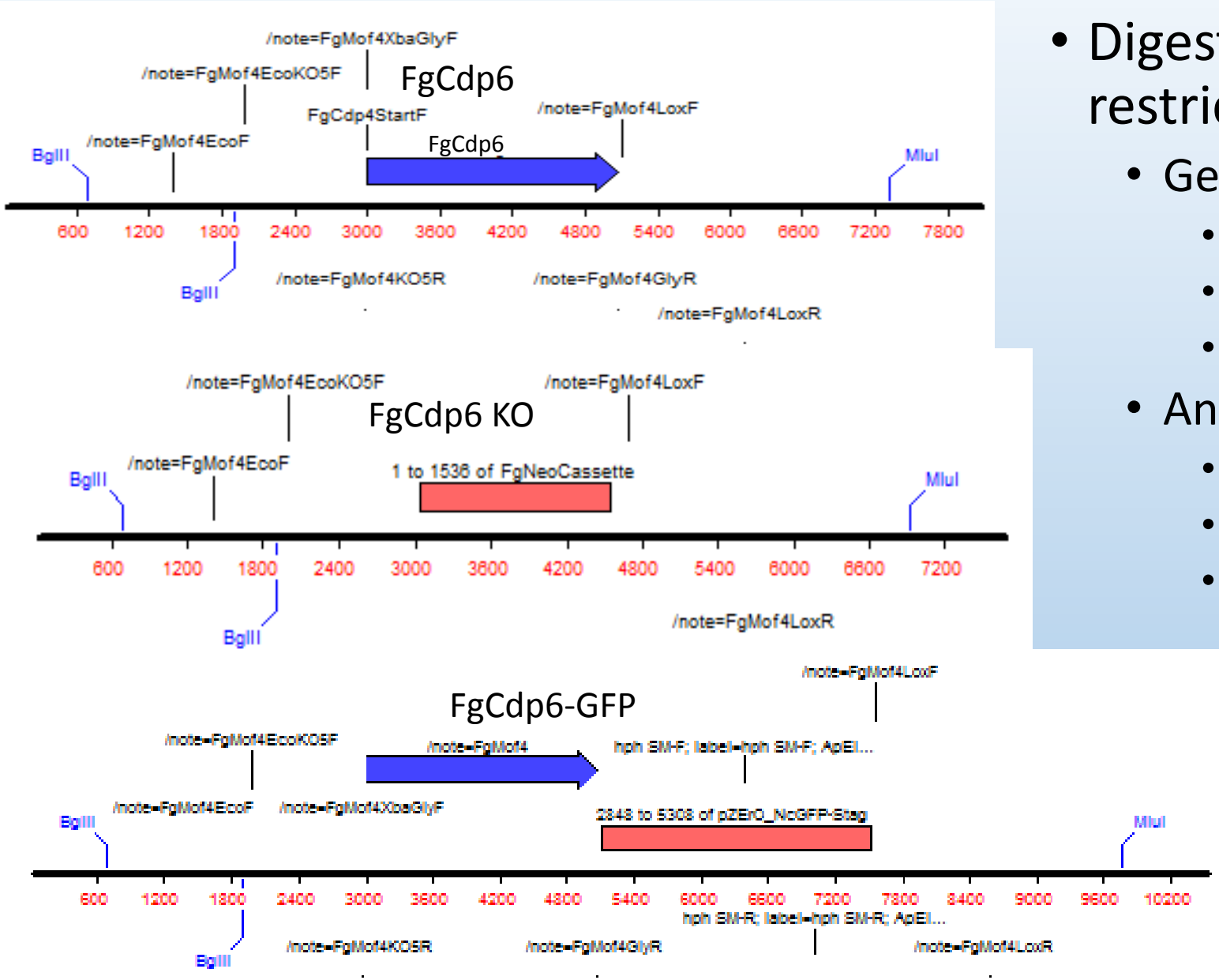


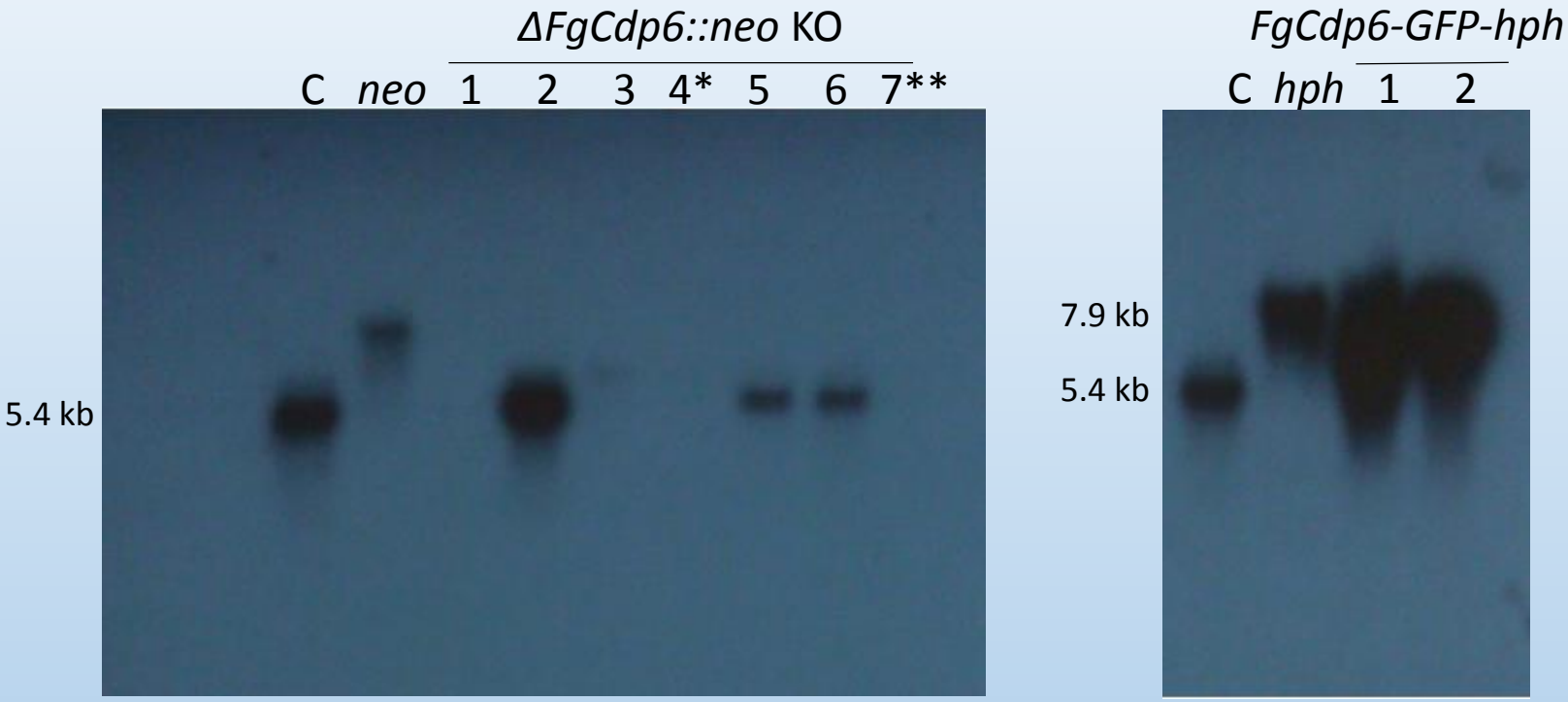

- Primers – StartF – GlyR
  - WT – 2.0 kb
  - KO – 0 kb

# Verification of Transformants via Southern Blot



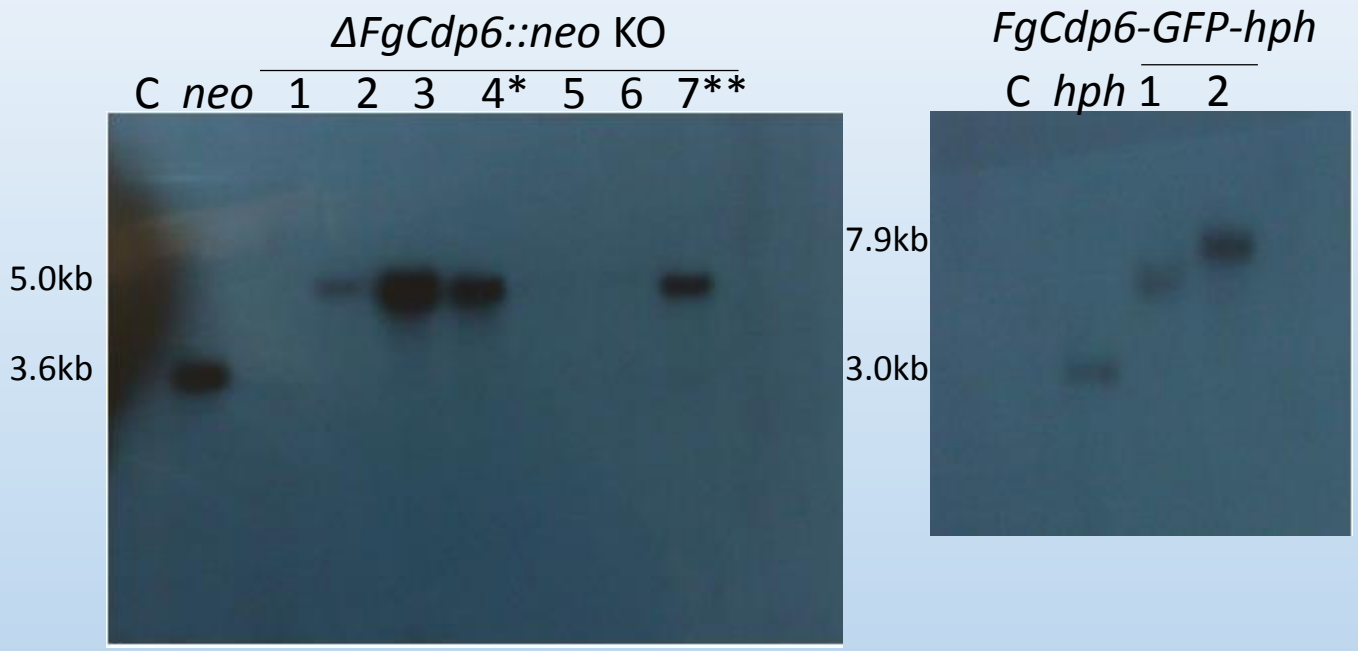

- Digested with *Mlu*I and *Bgl*II restriction enzymes
  - Gene Probe
    - WT control – 5.4 kb
    - KO strains – 0 kb
    - GFP-*hph* strains – 7.9 kb
  - Antibiotic Probe
    - WT control (both probes) – 0 kb
    - KO strains (*neo*)– 5.0 kb
    - GFP-*hph* strains (*hph*) – 7.9 kb

# Verification of Transformants via Southern Blot




- *FgCdp6* gene probe
  - WT control – 5.4 kb
  - KO strains – 0 kb
  - GFP-*hph* strains – 7.9 kb

# Verification of Transformants via Southern Blot




- Antibiotic gene probe
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  - GFP-*hph* strains (*hph*) – 7.9 kb

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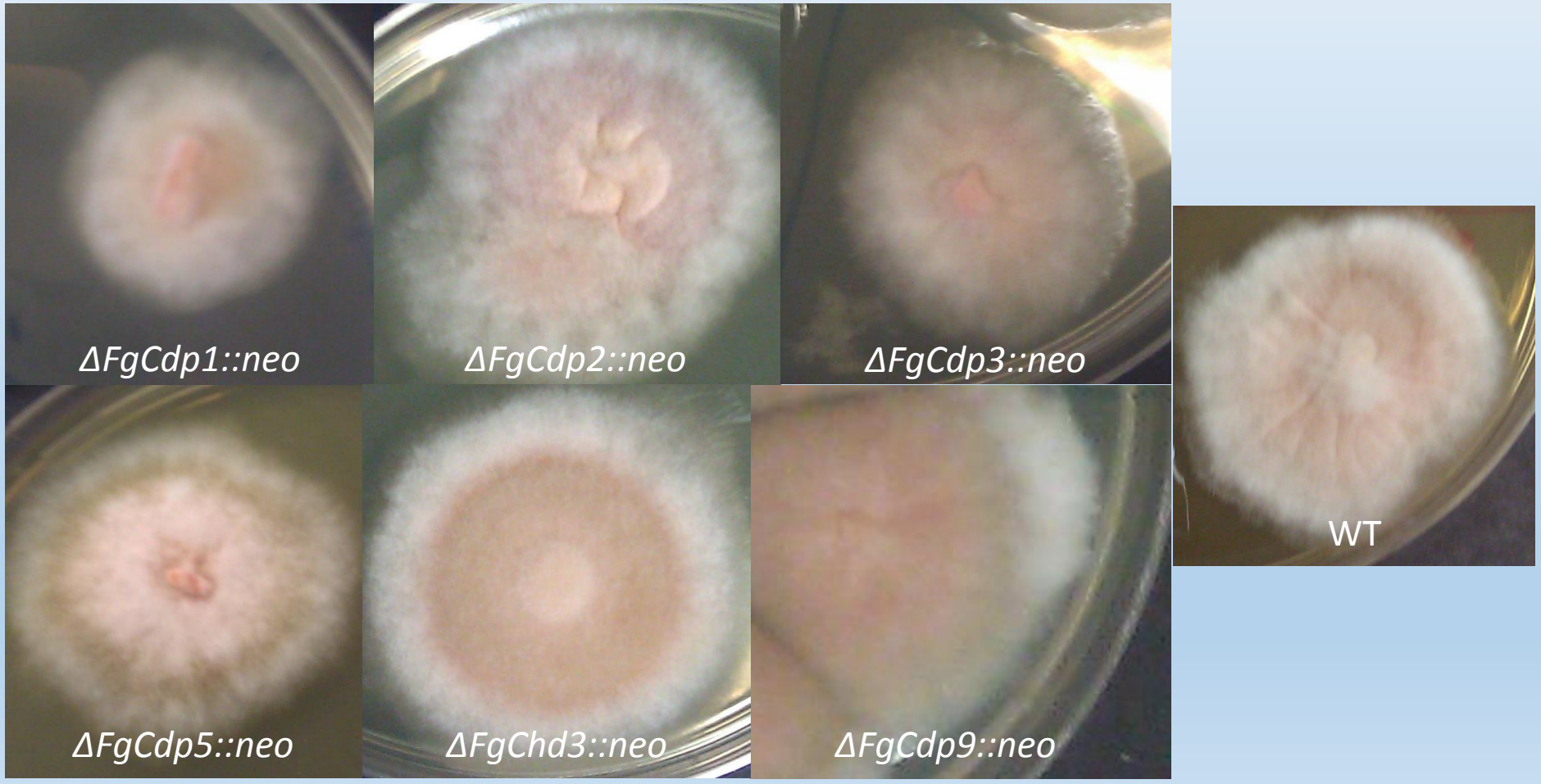
| ***ΔFgCdp5::neo*** | 1 |
| ***ΔFgCdp6::neo*** | 2 |
| ***FgCdp6-GFP-S tag-hph*** | 1 |
| ***ΔFgCdp8::neo*** | 0 |
| ***ΔFgCdp9::neo*** | 1 |
| ***ΔFgChd3::neo*** | 1 |

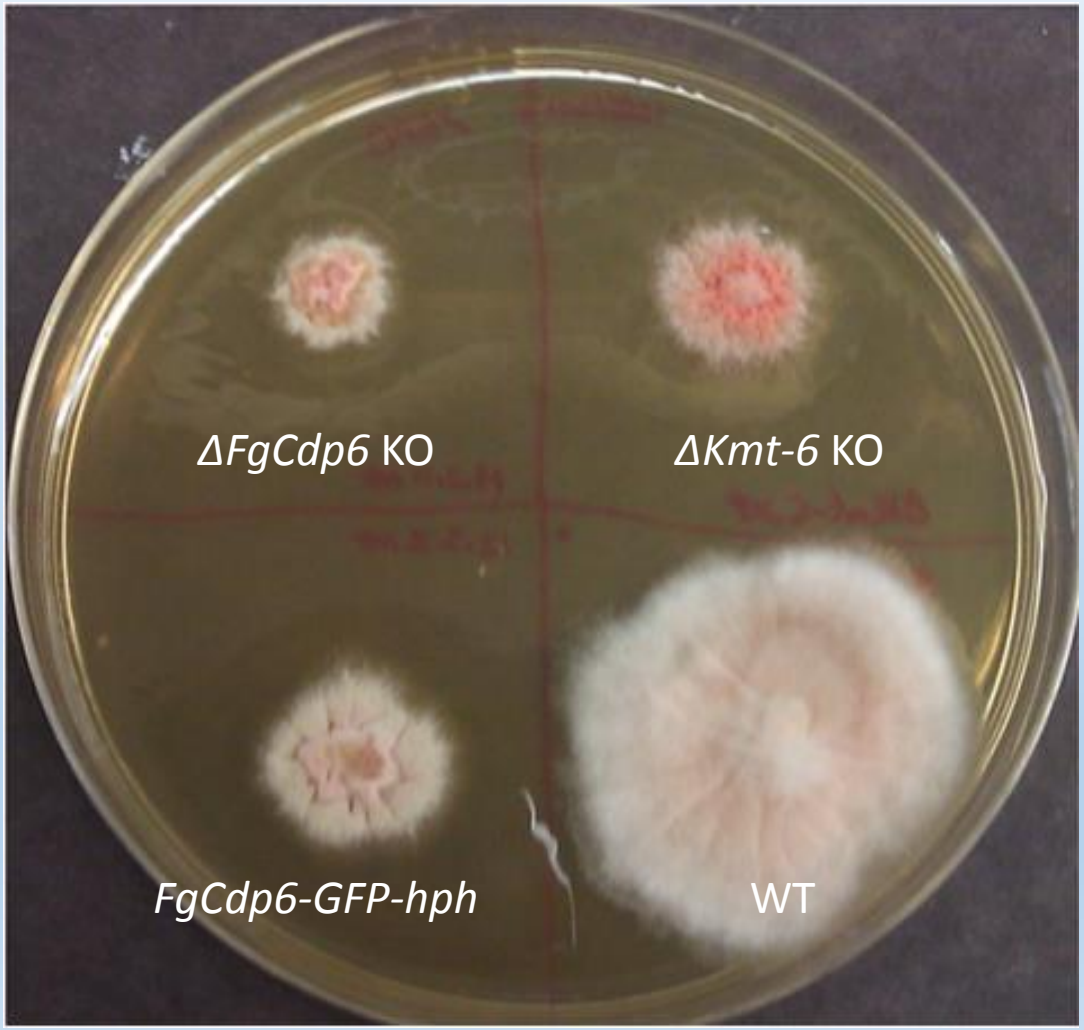
# Phenotypic Assays

- Most strains had no difference in phenotype

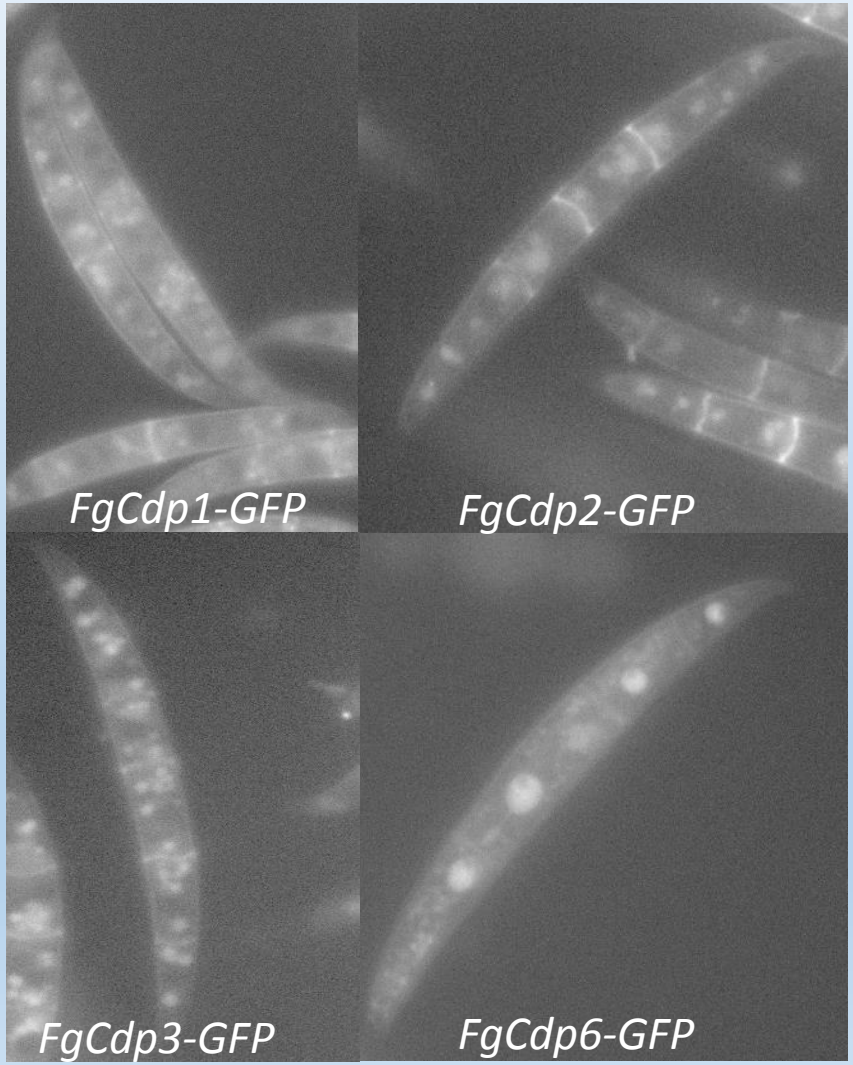


# *FgCdp6* Transformant Strain Phenotypes

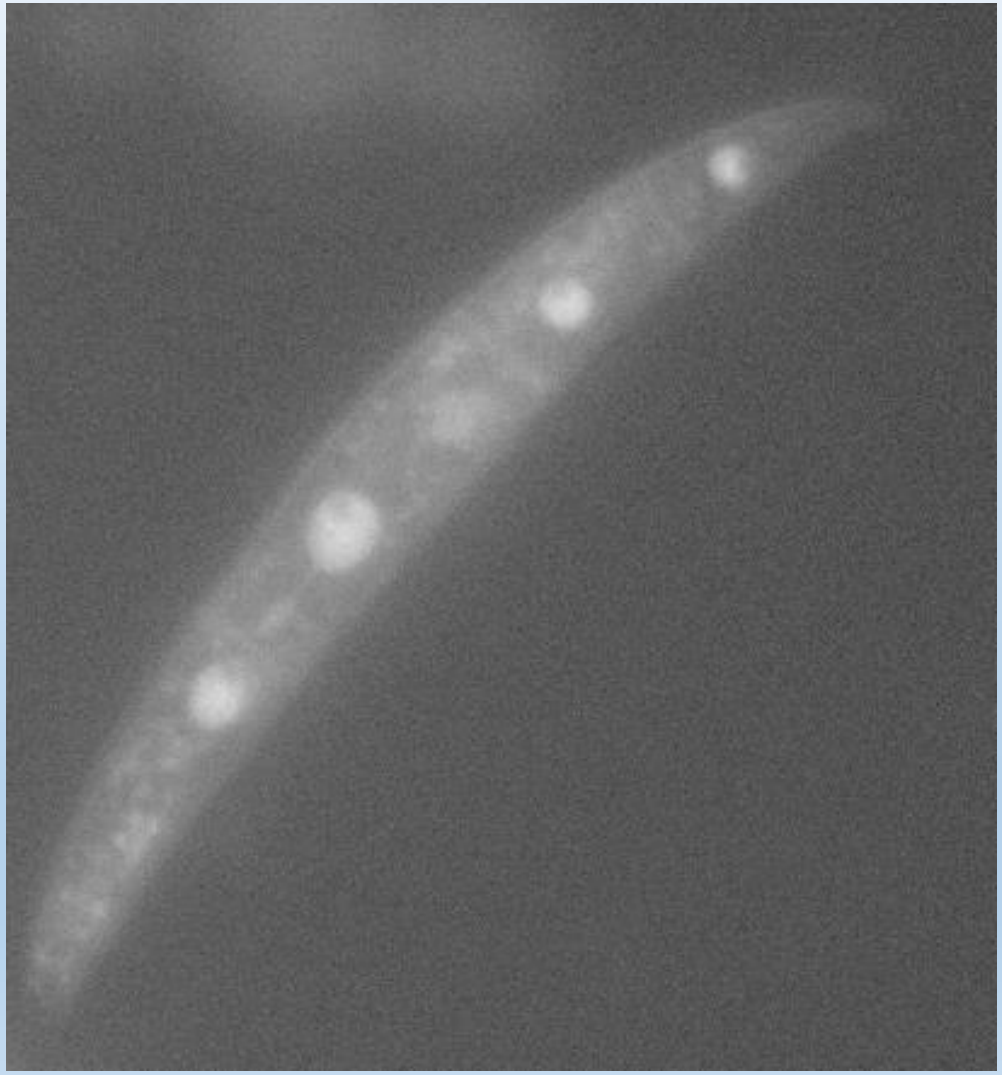
YPD, 3 days of growth



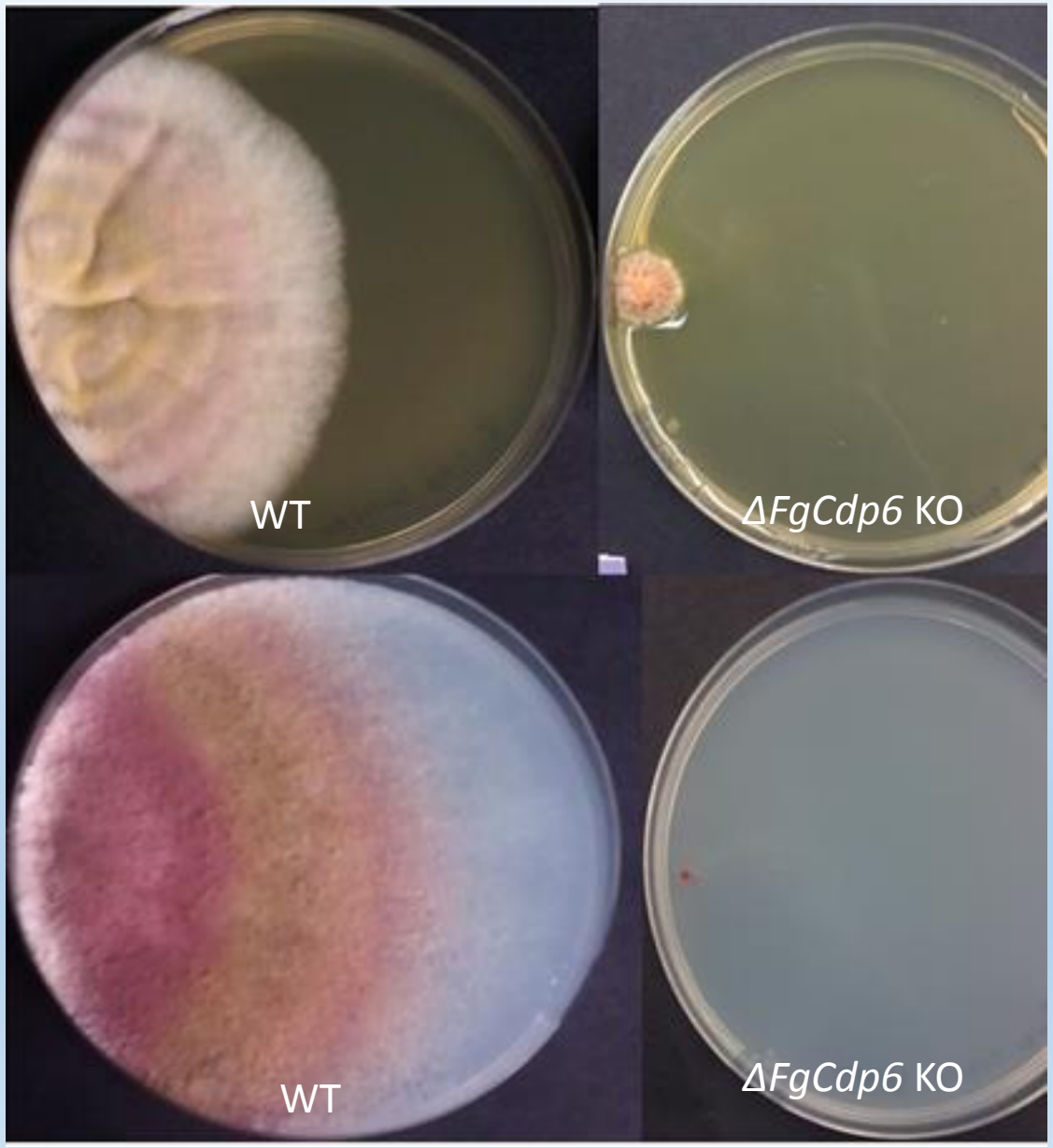

GFP tagged strains, conidia



# *FgCdp6* GFP



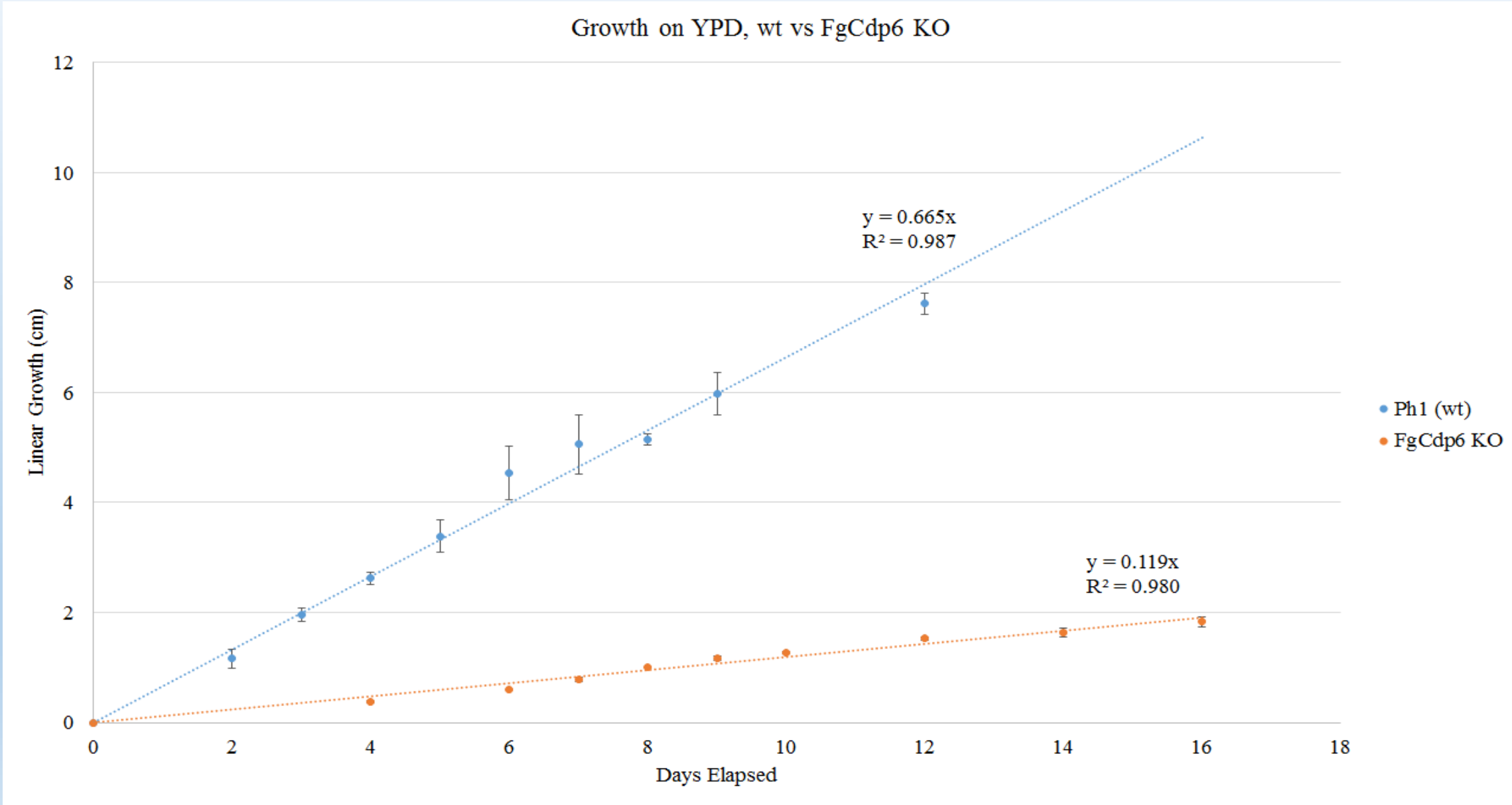
# Growth Assay



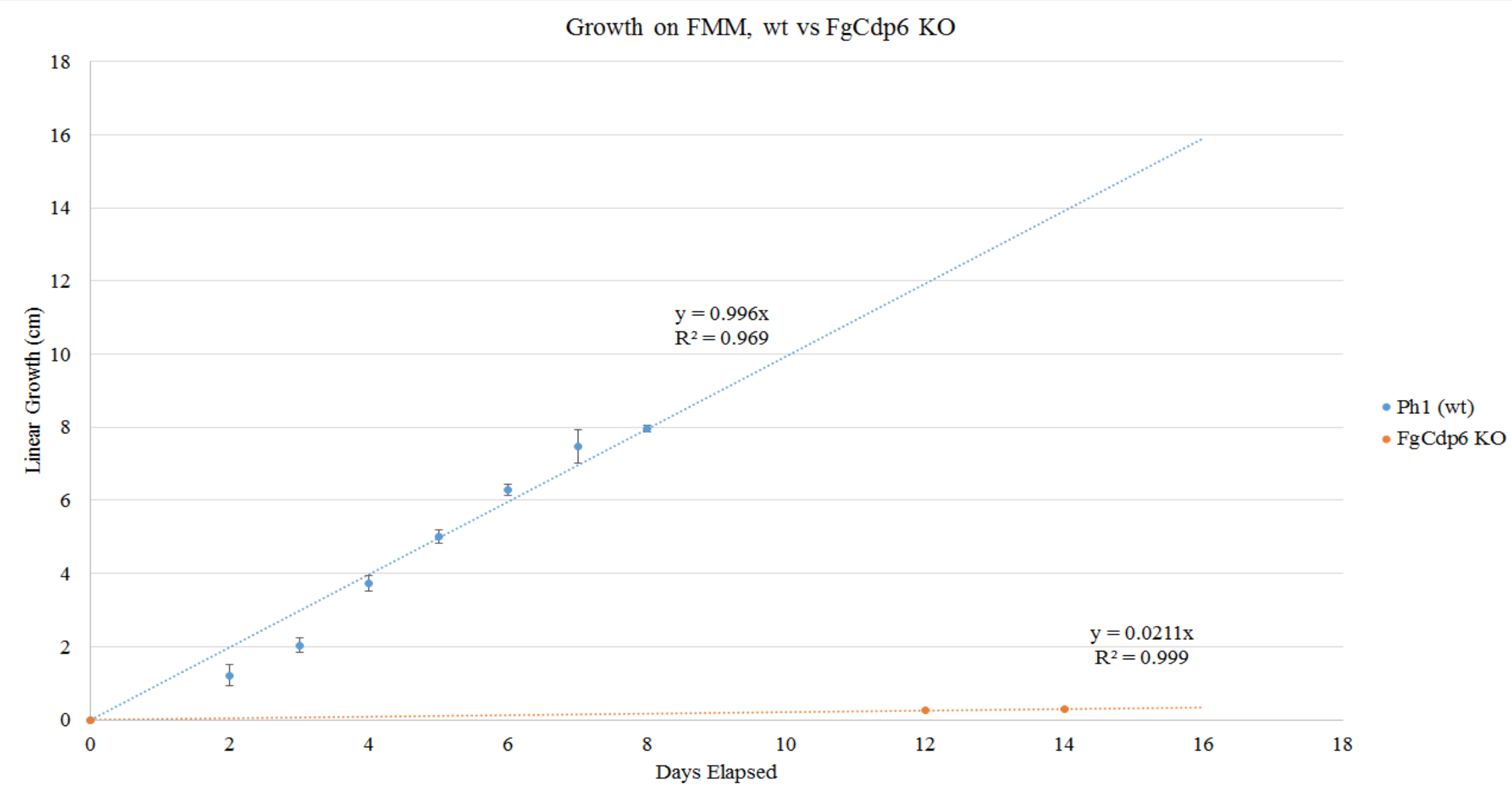

YPD, 7 days of growth

FMM, 7 days of growth

# Growth Assay Quantification

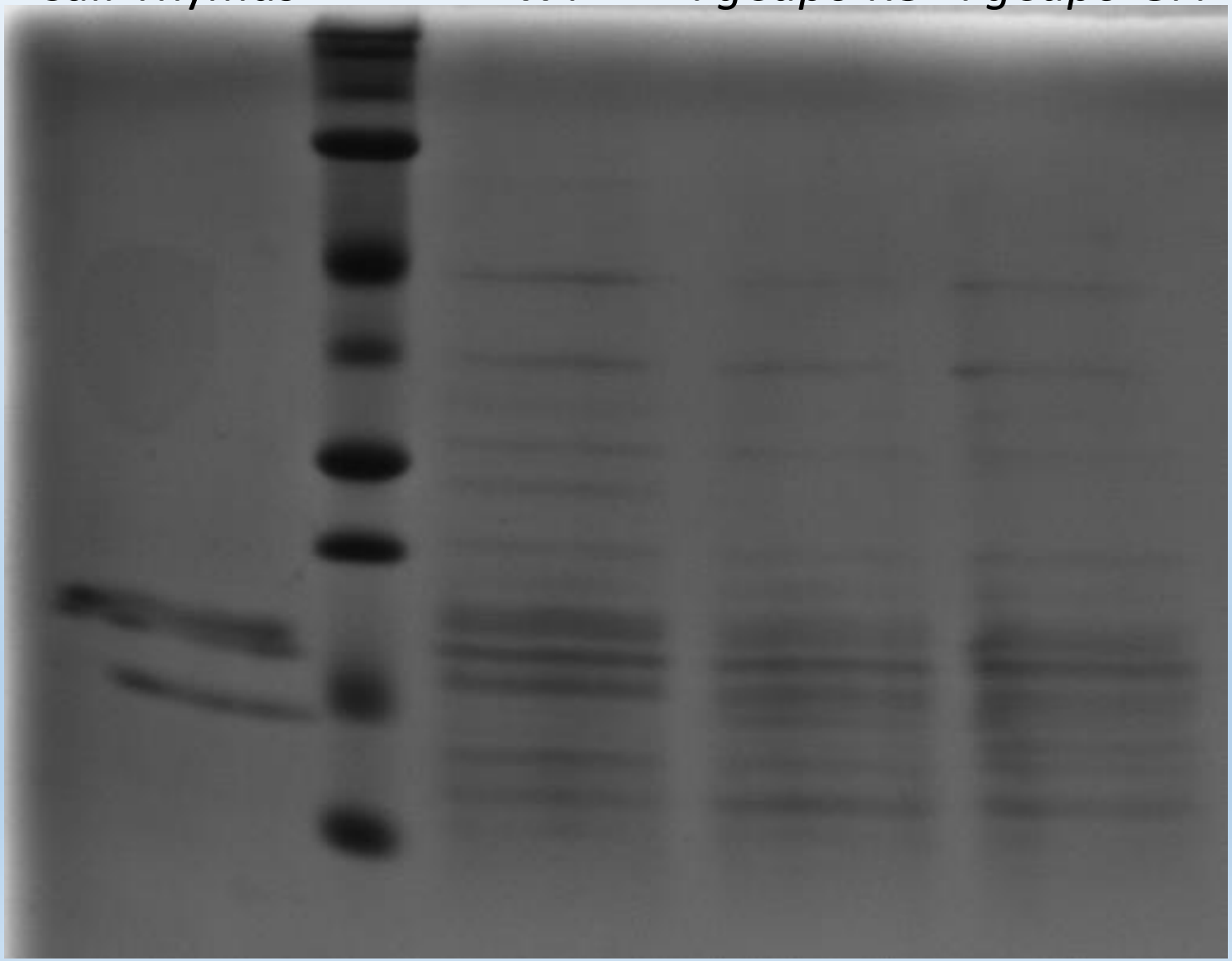


# Growth Assay Quantification

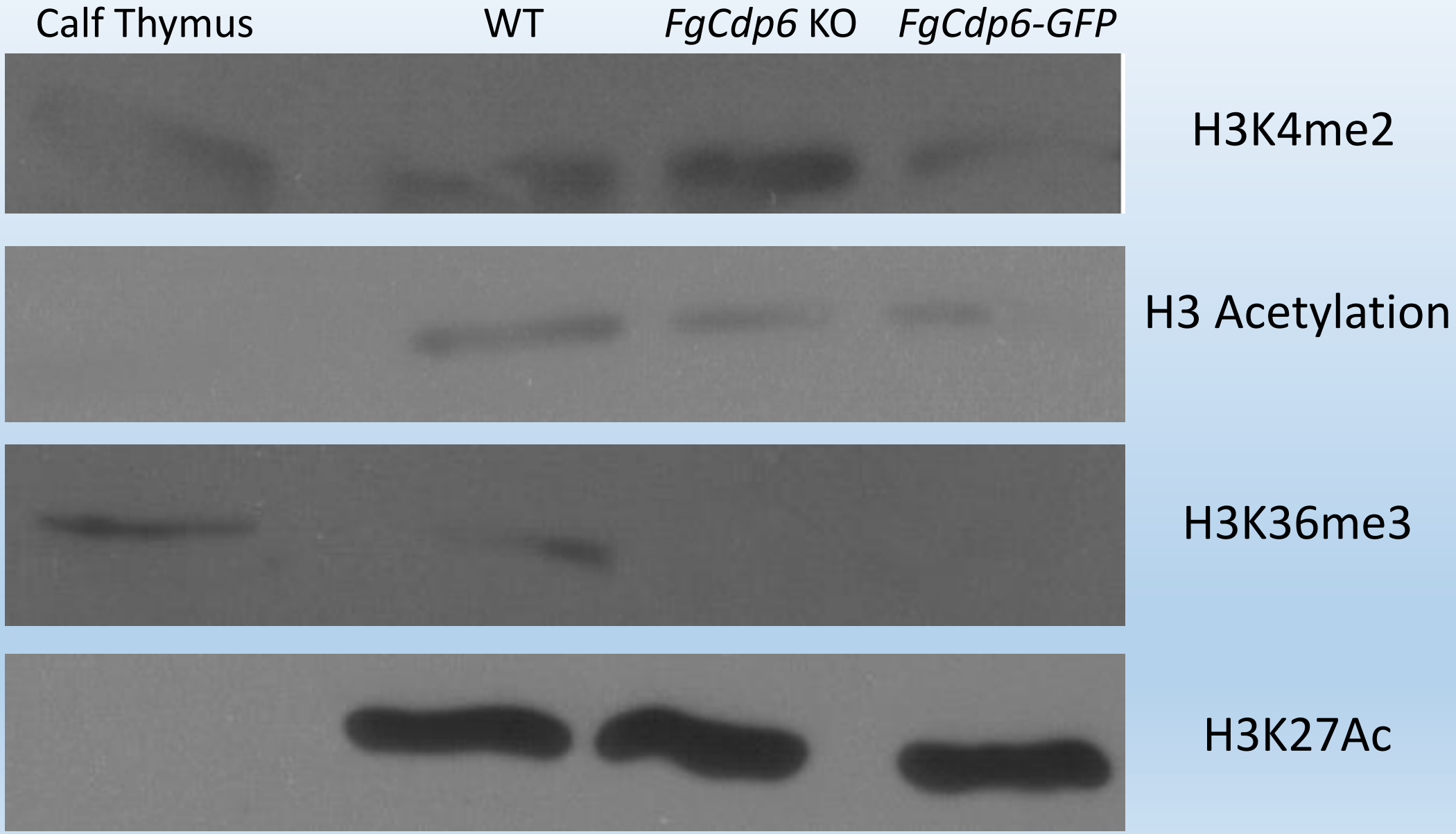


# Coomassie Stain

Calf Thymus WT *FgCdp6* KO *FgCdp6-GFP*



# Western Blot



# Gene Candidates With WT Phenotype

- Different Possibilities
  - Pseudogenes
  - Redundant Function
  - Further Studies
    - Multi-KO and gene reintroduction
- Transformation with no colonies
  - Lethal phenotype
  - Unsuccessful transformation

| Transformation | Obtained Verified Strains |
|---|---|
| *ΔFgCdp1::neo* | 1 |
| *FgCdp1_GFP-S tag-hph* | 0 |
| *ΔFgCdp2::neo* | 2 |
| *FgCdp2_GFP-S tag-hph* | 1 |
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| *ΔFgCdp8::neo* | 0 |
| *ΔFgCdp9::neo* | 1 |
| *ΔFgChd3::neo* | 1 |

# *FgCdp6*

- Functional CDP gene
  - Necessary for normal growth
  - Nuclear localization (GFP)

- *Eaf3* homolog
  - Sequence homology
  - Western Results
    - H3K36me3
  - NuA4 complex, Rpd3S recruitment
    - Histone Acetylation, Transcriptional Elongation (H3K36me3)

# Further Studies – *FgCdp6*

- Re-introduce gene
  - Reversion to wildtype
    - More evidence for gene existence
- Western blots
  - H3K36me3 quantification
- Complex pull-down/MS analysis
  - Identify other protein in complex with tagged protein (GFP)
- Chromatin Immunoprecipitation
  - DNA segments influenced by gene product

# Acknowledgements


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