

# Cloning of MreB from *Caulobacter crescentus* into *E. coli*

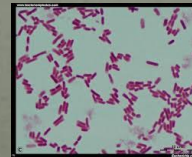
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Presented by:  
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# Project Goals

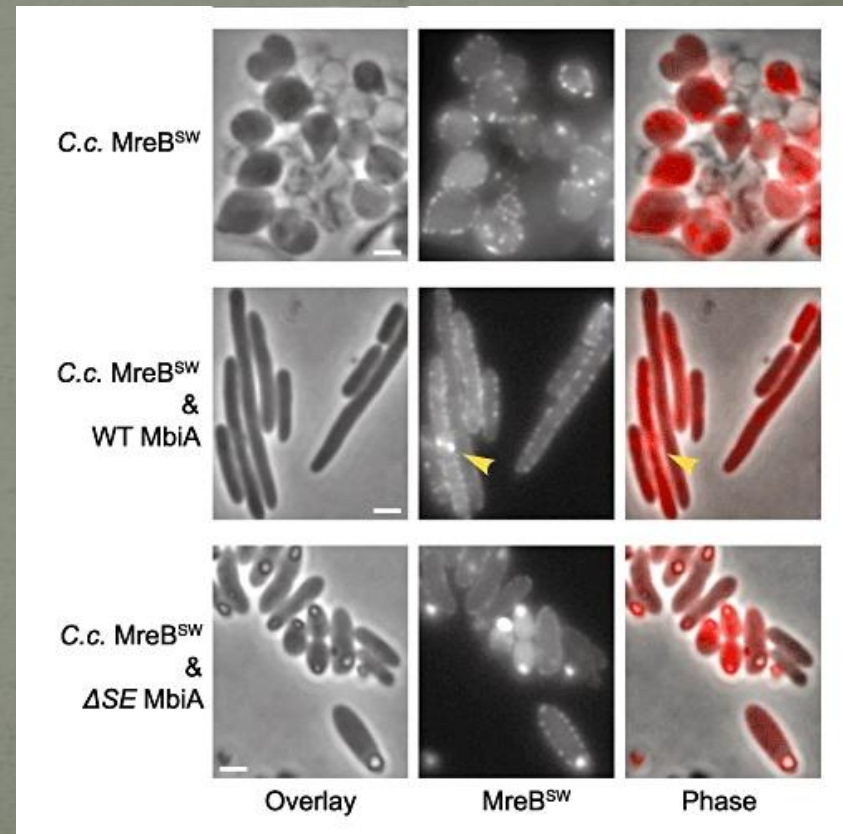
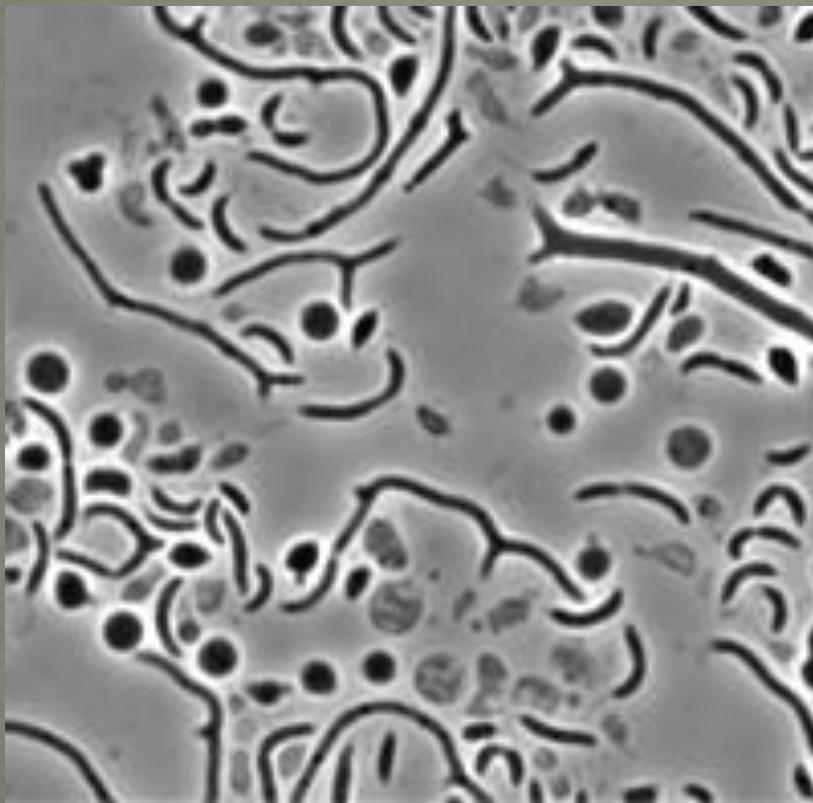
1. Clone the *mreB* gene from *Caulobacter crescentus* (stalked shaped cell) into *E. coli* (rod shaped cell)
2. Will the gene affect *E. coli*'s cell shape in some way since each bacteria are a different shape?

- *C. crescentus* – wildtype *CB15N*
- *E. coli* – strain *DH5 $\alpha$*



# After cloning of mreB...possible outcomes

## Cell shape change



# Gene of Interest: mreB from *C. crescentus*

- Rod-shape determining gene
  - Accession # NC\_011916
  - 1,044 base pairs
  - No introns – prokaryote
- Appears as bands or spirals encircling the cell
- Associates with mreC and penicillin-binding proteins (PBPs) to catalyze precursors for peptidoglycan cell wall
- Correctly positions polar bacterial proteins

# Experiment 1: DNA Extraction

## Purpose

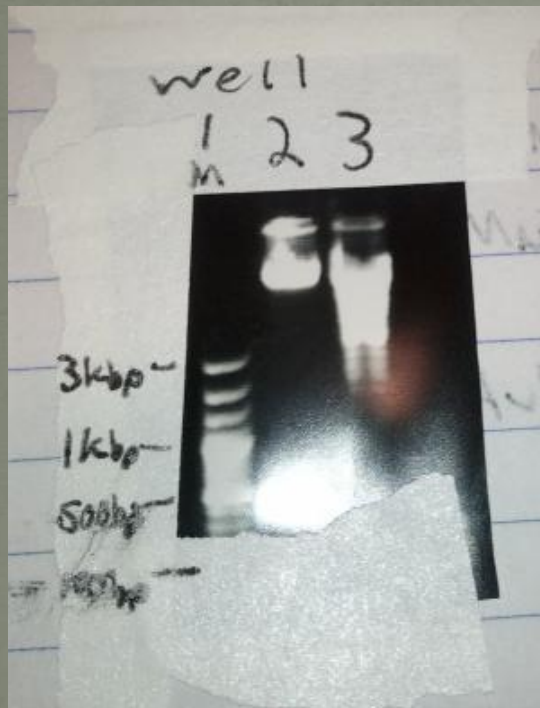
- Extract DNA from *Caulobacter crescentus*

## Methods

- Bacterial Genomic DNA Extraction

# Experiment 1: DNA Extraction

## Results



- Well #1: 100bp DNA Ladder Plus
- Well #2: CC genome, undigested
- Well #3: CC genome, digested w/EcoR1

## Conclusions

- Shows digestible DNA

# Experiment 2: Initial PCR

## Purpose

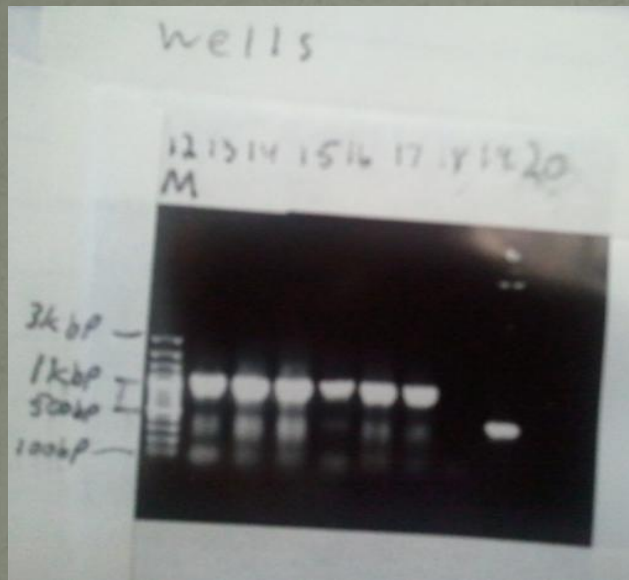
- Amplify our gene, mreB, out of *Caulobacter crescentus* genome

## Methods

- Followed PCR protocol
    - 95°C – 4min
    - 95°C – 30sec
    - 50-65°C – 30sec
    - 72°C – 1:30min
    - 72°C – 30min
    - 8°C – hold
- } Repeat 35x
- Positive & negative controls
  - Gel Electrophoresis

# Experiment 2: Initial PCR

## Results



- Well 12: 100bp DNA Ladder Plus
- Wells 13-18: PCR products
- Well 19: Negative Control
- Well 20: Positive Control

## Conclusions

- Amplified at correct length
- Clean controls

# Experiment 3: Large Quantity PCR

## Purpose

- Amplify a larger amount of DNA

## Methods

- Followed PCR protocol
    - 95°C – 4min
    - 95°C – 30sec
    - 65°C – 30sec
    - 72°C – 1:30min
    - 72°C – 30min
    - 8°C – hold
  - Positive & negative controls
- } Repeat 35x

# Experiment 4: Purification & Digestion of PCR

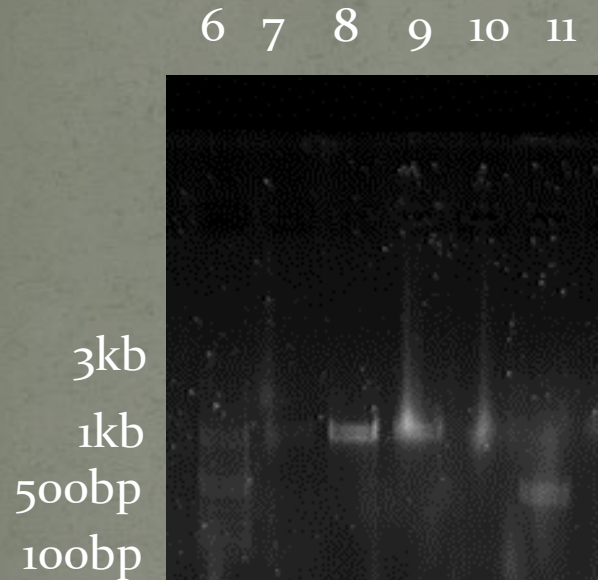
## Purpose

- Purify PCR
- Preparatory digest of purified PCR

## Methods

- GeneJET PCR Purification Kit
- DNA digest
  - Xba1
  - Pst1

# Experiment 3/4: RESULTS



- Well 6: 100bp DNA Ladder Plus
- Well 7: Raw PCR (exp 3)
- Well 8: Pure PCR (exp 4)
- Well 9: Pure Digested PCR (exp 4)
- Well 10: Negative Control
- Well 11: Positive Control

## Conclusions

- Purification worked
- Digestion worked as well

# Experiment 5: Prepping Plasmid for Ligation

## Purpose

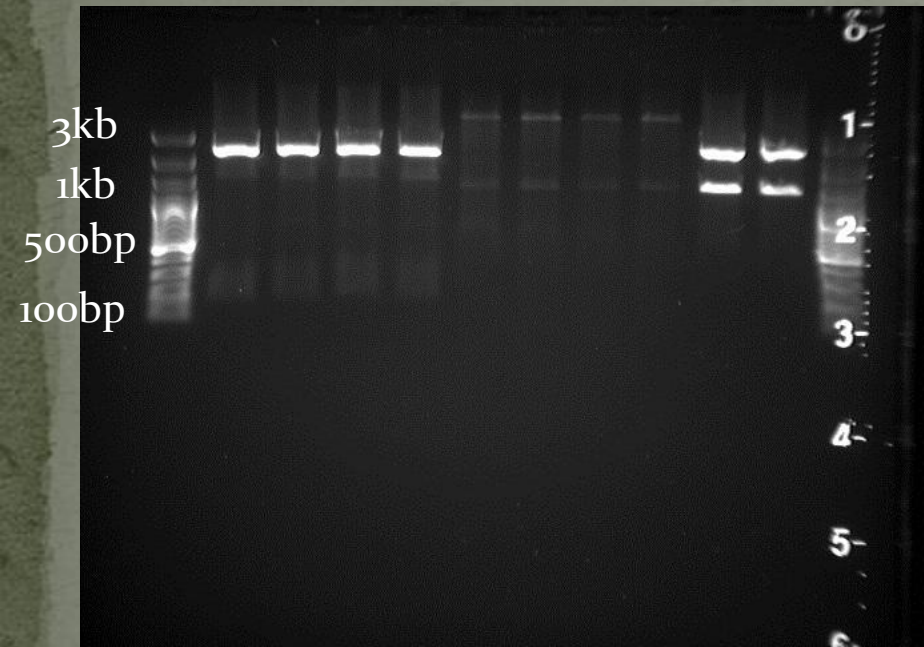
- Grow multiple copies of Bba\_K20600
- Miniprep for plasmid DNA
- Digestion of plasmid DNA

## Methods

- Plasmid extraction from distribution kit
- Transformation w/controls
- Tube cultures
- GeneJET Plasmid Miniprep Kit
- DNA digest
  - EcoR<sub>1</sub>
  - Pst<sub>1</sub>

# Experiment 5: Prepping Plasmid for Ligation

M 1 2 3 4 5 6 7 8 9 10 M



## Results

- Wells M: 100bp DNA Ladder Plus
- Wells 1-4: Bba\_K20600
- Wells 5-8: Bba\_I10500
- Well 9/10: Positive Controls

## Conclusions

- Promoter is present in both parts
- Plasmid backbone present
- Controls as expected

# Experiment 6: Preparatory Digest & Purification of Bba\_K20600

## Purpose

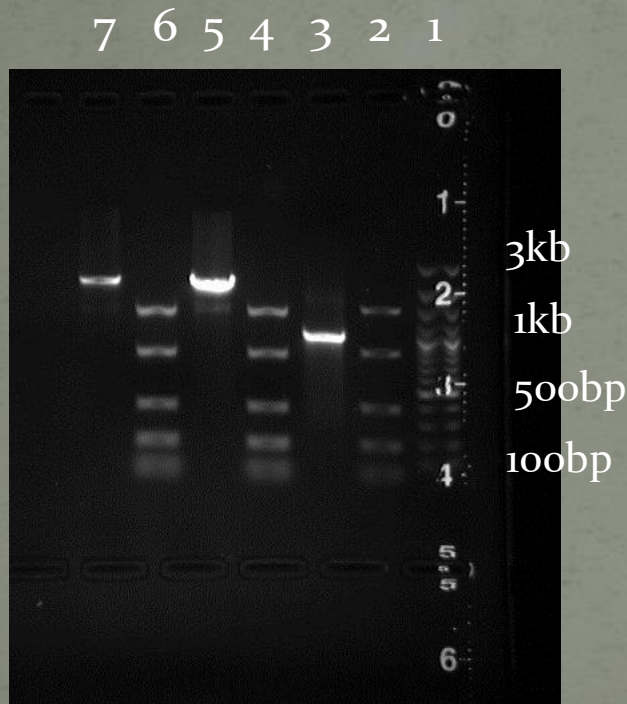
- Preparing the plasmid to be ligated with insert
- Compare mass/intensity of insert & vector
  - 2:1 ratio is ideal

## Methods

- DNA digest
  - SpeI
  - PstI
- GeneJET PCR Purification Kit
- FastRuler Low Range DNA Ladder

# Experiment 6: Preparatory Digest & Purification of Bba\_K20600

## Results



- Well 1: 100bp DNA Ladder Plus
- Well 2/4/6: FastRuler
  - 1500, 850, 400, 200, & 50bp
- Well 3: gene fragment
- Well 5: Part 1 Vector
- Well 7: Part 2 Vector

## Conclusions

- Vector & insert were as expected
- Intensities of vector & insert were comparable

# Experiment 7: Ligation & Transformation of mreB and Bba\_K20600

## Purpose

- Insert mreB into Bba\_K20600
- Insert recombination into *E. coli*
- Grow recombinant *E. coli*

## Methods

- Three ligation tests
  - 1:1, 1:5, 5:1 insert:vector ratios
- Ligation & transformation controls
- Plate selection
- Tube cultures

# Experiment 7: Ligation & Transformation of mreB and Bba\_K20600

## Results

- Plates
  - L1 = 130 colonies
  - L2 = TMTC
  - L3 = 160 colonies
  - L4 (no insert) = 5 colonies
  - L5 (no insert/ligase) = none
  - L6 (no vector) = none
  - Plasmid Control = 210 colonies
  - Pre-T Control w/Amp = none
  - Pre-T Control w/o Amp = confluent
  - Post-T Control w/Amp = none
  - Post-T Control w/o Amp = confluent

Ligation controls {

## Conclusions

- Vector was growing with or without the insert (L4)
- Controls as expected
- Hoping for recombinant *E. coli*

# Experiment 8: Verification

## Purpose

- Verify that mreB was inserted into Bba\_K20600

## Methods

- GeneJET Plasmid Miniprep Kit
- Gel Electrophoresis
- Gene Sequencing

# Experiment 8: Verification

## Results



Wells 5 & 10 = 100bp DNA Ladder Plus

Wells 4, 3, 2, 1 = L3A- L3D

Wells 9, 8, 7, 6 = L2A-L2D

Wells 14, 13, 12, 11 = L1A-L1D

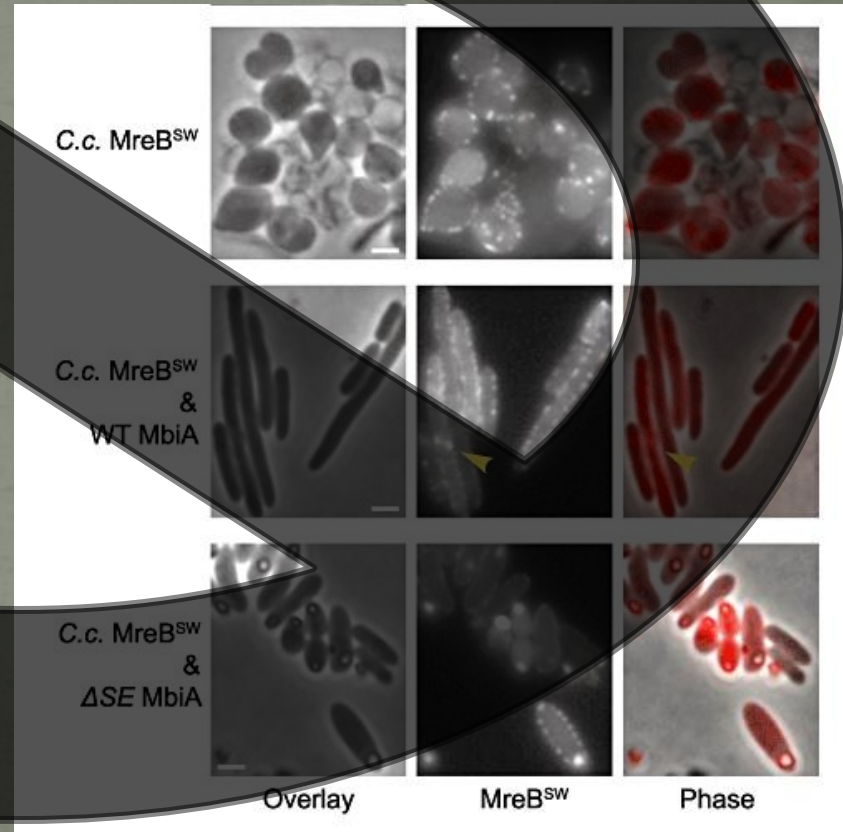
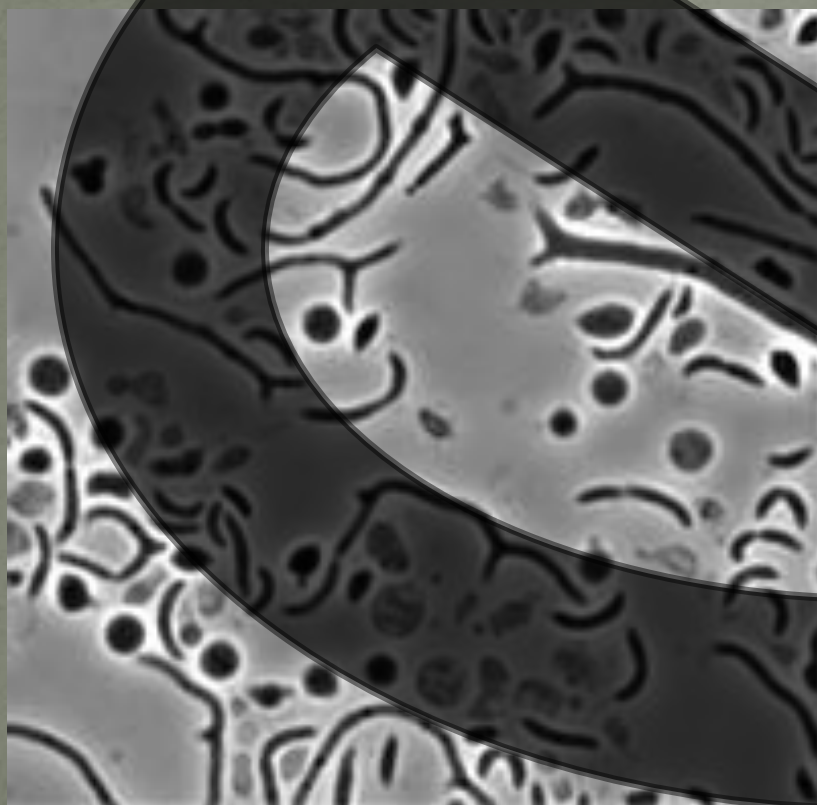
# FINAL CONCLUSIONS



- Unfortunately, none of our plasmids appeared to contain our insert, mreB.
- In light of this conclusion, we still sent 2 plasmids to be sequenced to verify the gel from exp. 8
- 10 more colonies were selected, grew and glycerol stocked for future testing
  - Hopefully, one or more of these DOES contain our insert

# After cloning of mreB...possible outcomes

## Cell shape change



# References

Gitai, Z., & Yakhnina, A.A. (2012). The small protein mbiA interacts with mreB and modulates cell shape in *caulobacter crescentus*. *Molecular Microbiology*. doi: 10.1111/j.1365-2958.2012.08159.x

Jacobs-Wagner, C., et al. (2012). Osmolality dependent relocation of penicillin-binding protein PBP<sub>2</sub> to the division site in *Caulobacter crescentus*. *Journal of Bacteriology*. doi: 10.1128