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

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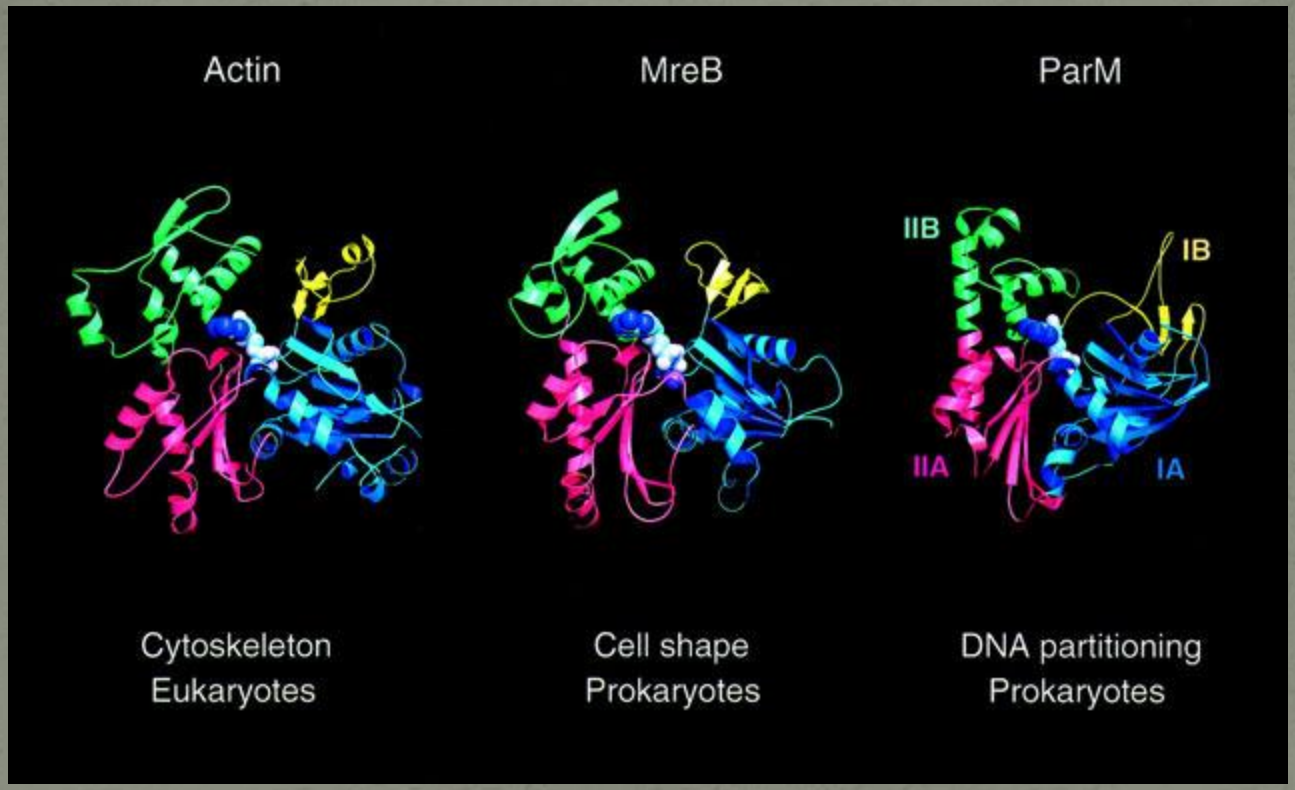
Gene of Interest: mreB from *C. crescentus*

- 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

Structure of mreB



Caulobacter crescentus vs. *Escherichia coli*

Goals

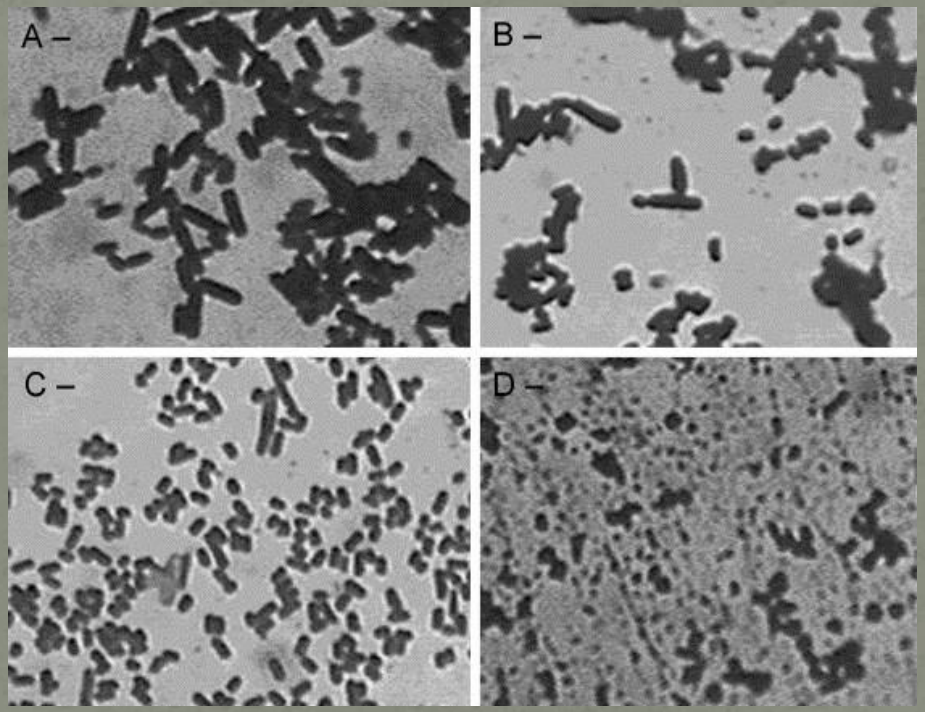
1. Clone the mreB gene from *Caulobacter crescentus* (stalked shaped cell) into *E. coli* (rod shaped cell)

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 DH5α

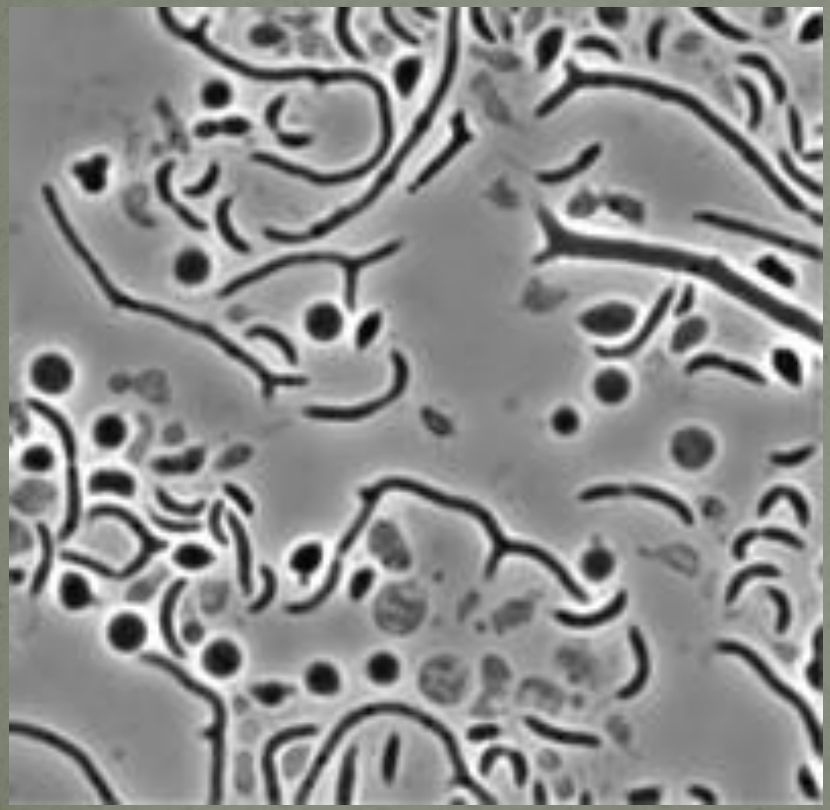
After cloning of mreB…possible outcomes

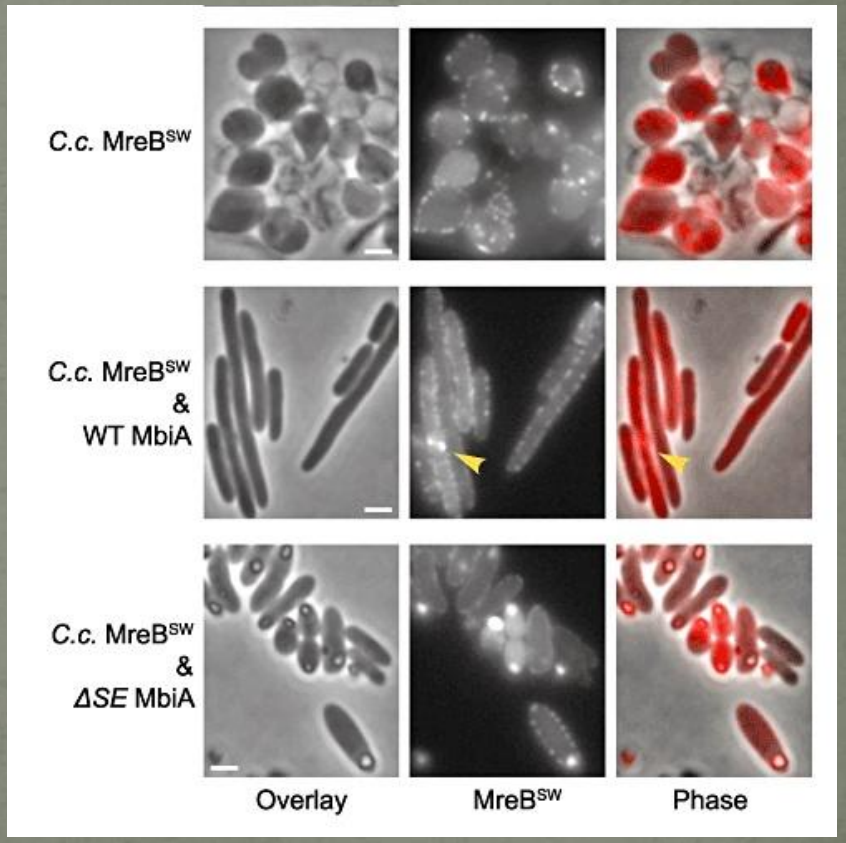
Cell Lysis



After cloning of mreB…possible outcomes

Cell shape change





Alternate (back-up) Plan

- Antisense (negative sense) RNA
  - Make a ssRNA that is complementary to a part in the mRNA of the mreB gene of the *E. coli*

- GOAL:
  - To inhibit translation of *E. coli's* mreB gene and allow the translation of *C. crescentus* cloned mreB gene

Procedure

1. Grow C. *crescentus* & *E. coli* on …. media
2. PCR to extract mreB gene + Biobrick (BB) enzymes

Primers

Extended Forward Primer

5’ {GAATTCTCT AGAATGTTCTCTTCCCTTTTCGGCGTGATCTCGAACG} 3’

Blue = EcoR1
Yellow = Xba1
Purple = beginning sequence

Extended Reverse Primer

5’ {CTGCAGACTAGTCTAGGCCAGCGTGGATTCCA} 3’

Green = Pst1
Red = Spe1
Purple = ending complementary sequence

Forward Primer

(same sequence)

gaattctctagaatgttctcttccctttcggcgtgatctcgaacg

5’atgttctcttccctttcggcgtgatctcgaacgacatcg
ccatcgacct cggaacggcc aacaccctga tctatcagaa gggtaagggc atcgtgctga acgagccgtc
ggtggtggcg ctacgcaatg tgggcgggcg caaggtcgtc cacgccgtgg gcatcgaggc caagcagatg
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cgacgatccg ctctcgtgcg tggccctggg ctgcggcaag gtgctggaac atcccaagtg gatgaagggc
gtcctggaatccacgctggcctag3’

accttaggtgcgaccggatctgatcagacgtc

(compliment sequence)

ctgcagactagtctaggccagcgtggattcca

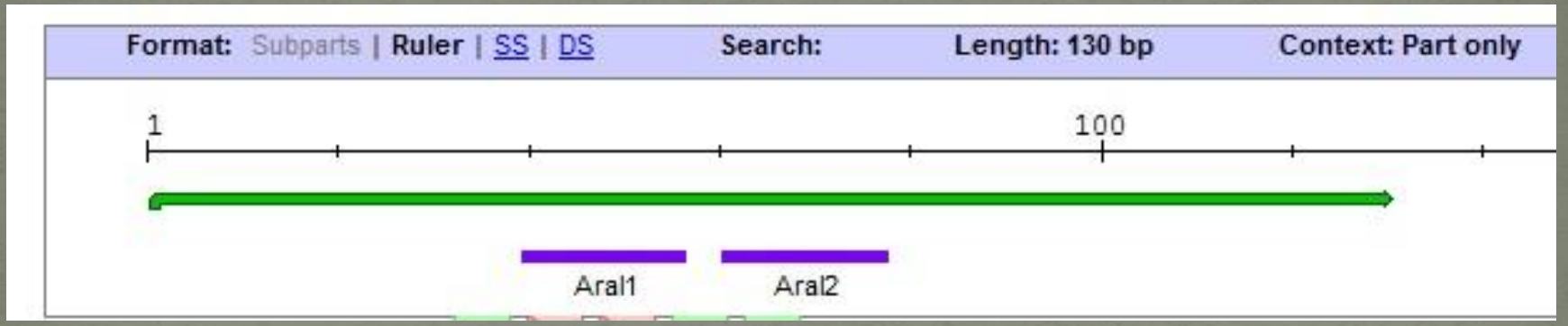
Reverse Primer

Procedure

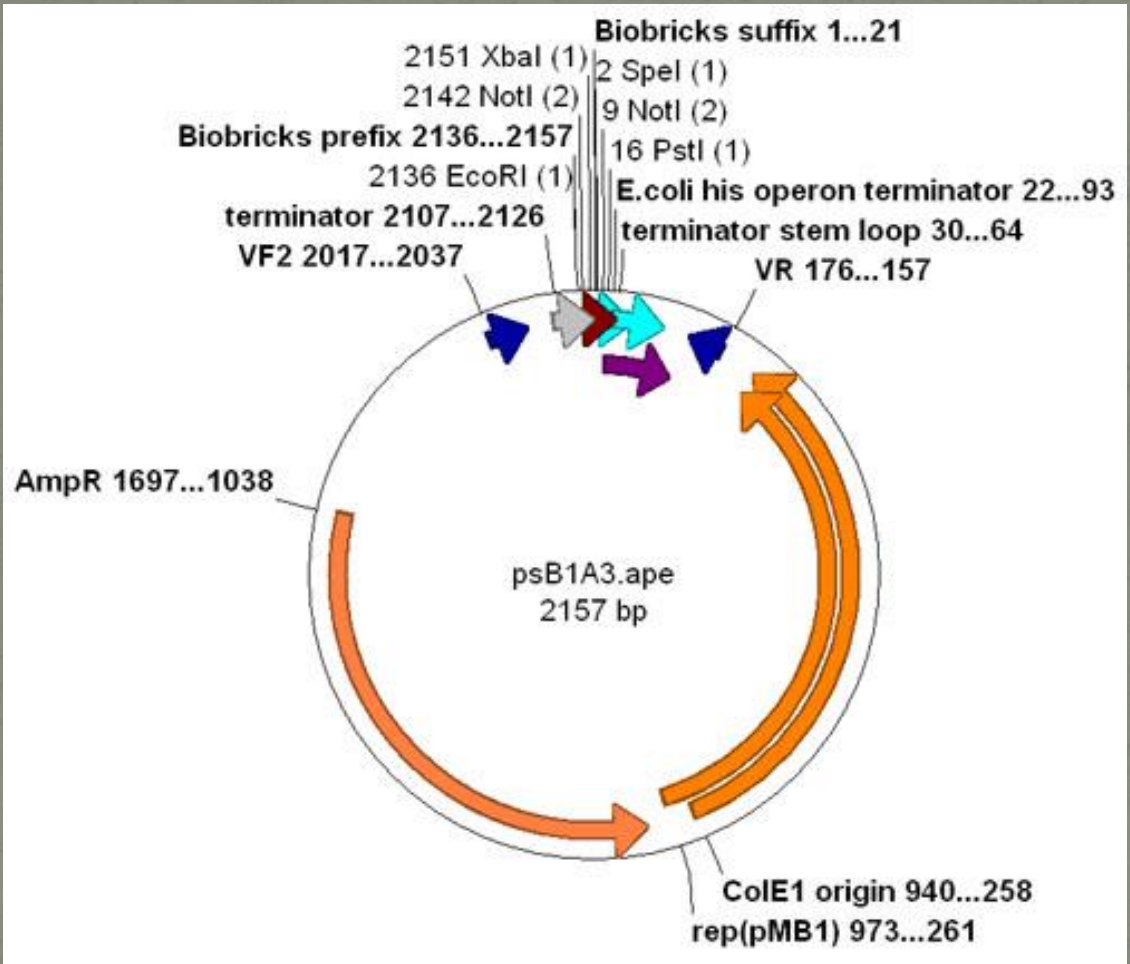
1. Grow C. *crescentus* & *E. coli* on …. media
2. PCR to extract mreB gene + Biobrick (BB) enzymes
3. Isolate and purify mreB + BB enzymes
4. Cut mreB + BB enzymes and ligate with pSB1A3

Promoter: BBa_K206000

- pBad Strong Promoter
  - Inducible by L-arabinose
    - When induced, AraC binds and changes conformation interacting with Arai1 and Arai2 operator sites, permitting transcription



pSB1A3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2 SpeI (1)
2142 NotI (2)
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Biobricks prefix 2136...2157
16 PstI (1)
2136 EcoRI (1)
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terminator 2107...2126
terminator stem loop 30...64
VF2 2017...2037
VR 176...157
AmpR 1697...1038
psB1A3.ape
2157 bp
ColE1 origin 940...258
rep(pMB1) 973...261

Procedure

1. Grow C. *crescentus* & *E. coli* on …. media
2. PCR to extract mreB gene + Biobrick (BB) enzymes
3. Isolate and purify mreB + BB enzymes
4. Cut mreB + BB enzymes and ligate with pSB1A3
5. Perform bacterial transformation to import the plasmid into *E. coli*
6. Grow *E.* coli bacteria on experimental plates
   - No ampicillin
   - Ampicillin
   - Ampicilin + L-arabinose
7. Verification test

Verification Test

- Light Microscope
  - Take samples of living *E.* coli with cloned mreB gene and view under microscope to see any physical cell shape changes
- Sequence Verification
  - Send sample of cloned DNA to Iowa State to verify that mreB was successfully inserted into *E. coli*
- Other
  - Plasmid sequence

Parts

- Tissue Source
  - Yale – wildtype *C. crescentus* CB15N
- Primers
  - Ordering through IDT
  - First 34 & Last 20 bases of our gene sequence
- Promoter
  - Part: BBa_K206000
  - Part: BBa_I10500 (alternate)
- Plasmid vector
  - pSB1A3
  - pSB2K3 (alternate)
- Restriction Enzyme Sites
  - EcoR1 & Xba1 sites added in front of forward primer
  - Spe1 & Pst1 sites added in front of the reverse primer
- Intron Removal
  - Not necessary because our organism is a prokaryote

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 PBP2 to the division site in *Caulobacter crescentus*. *Journal of Bacteriology*. doi: 10.1128