Newcastle University

iGEM

L-forms

Bacteria without cell walls

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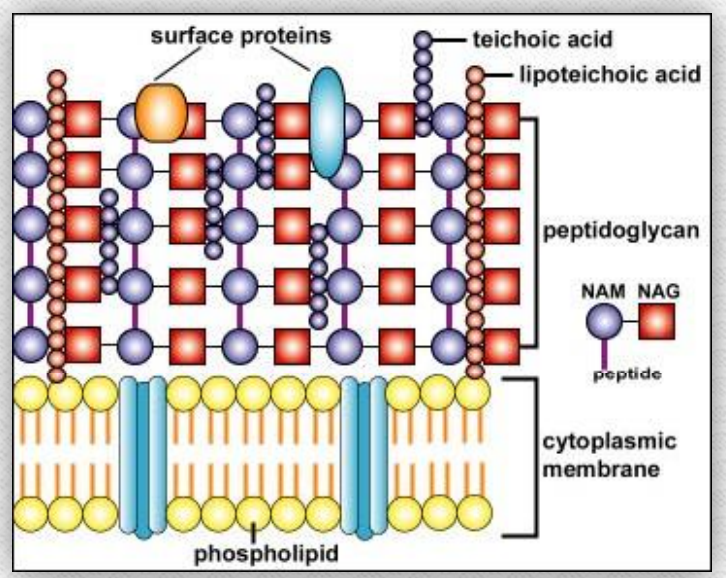
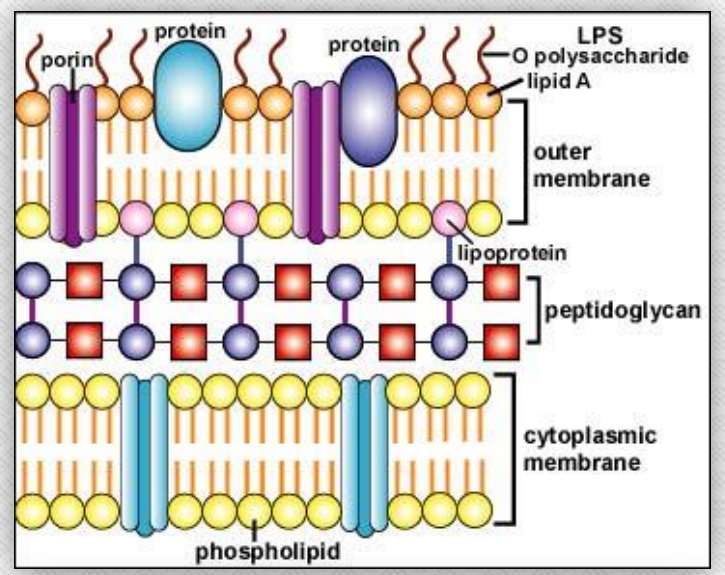
TEAM Newcastle

iGEM 2013

Gram positive vs. Gram negative bacteria





Gram ***positive***:

- Thick peptidoglycan layer (15-80 nm)
- Stain crystal violet when Gram staining
- E.g. *Bacillus subtilis*

Gram ***negative***:

- Thin peptidoglycan layer (10nm)
- Do not stain with crystal violet when Gram staining
- E.g. *Escherichia coli*

Images courtesy of Dr Gary Kaiser, Community Collage of Baltimore County

The bacterial cell wall is important because it…

- Protects the cell from mechanical damage
- Prevents the cell from lysing due to osmotic pressure
- Acts as a target for antibiotics
- Allows the cell to grow and divide

Losing the cell wall

L-forms and protoplasts both do not have a cell wall however there are key difference between them…

Protoplasts

- Formed in the presence of lysozyme which removes the cell wall.
- Cannot grow and divide.
- Fragile – osmotically unstable.

L-forms

- Formed by a mutation in the *murE* and *ispA*-like genes that causes peptidoglycan to stop being made.
- They can grow and divide using a specific media (NA/MSM).
- Can regrow their cell wall
- Fragile – osmotically unstable.

Leaver, M., et al. (2009). "Life without a wall or division machine in Bacillus subtilis." Nature **457**(7231): 849-853.

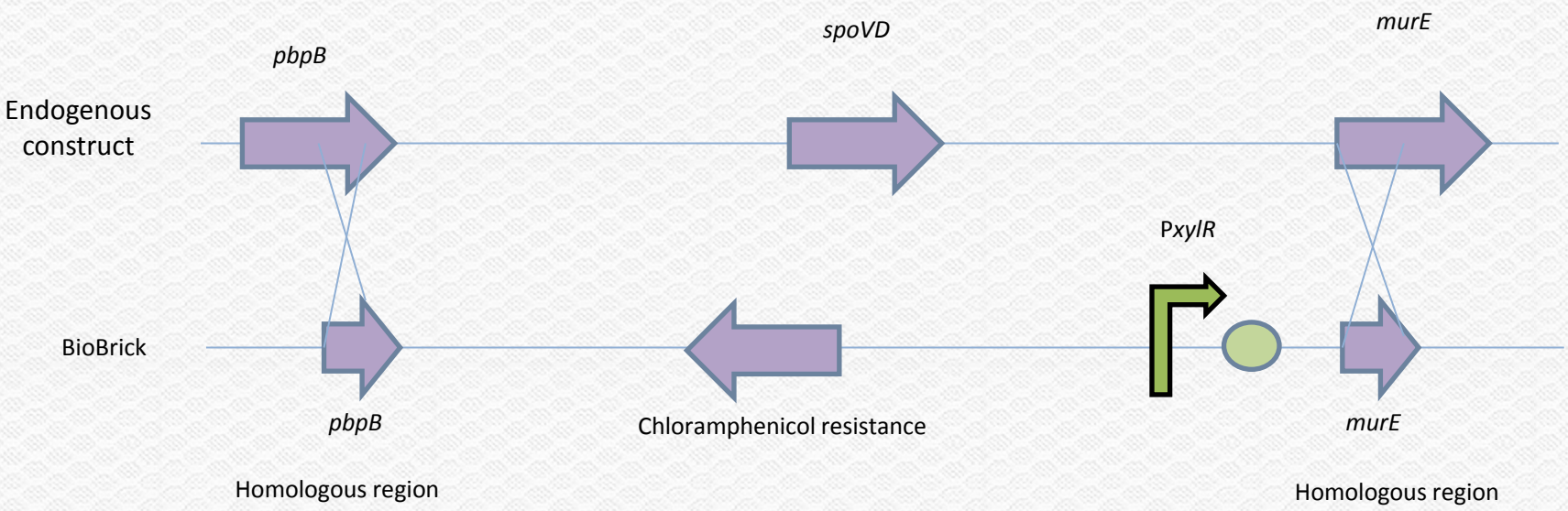
Applications

L-form switch BioBrick

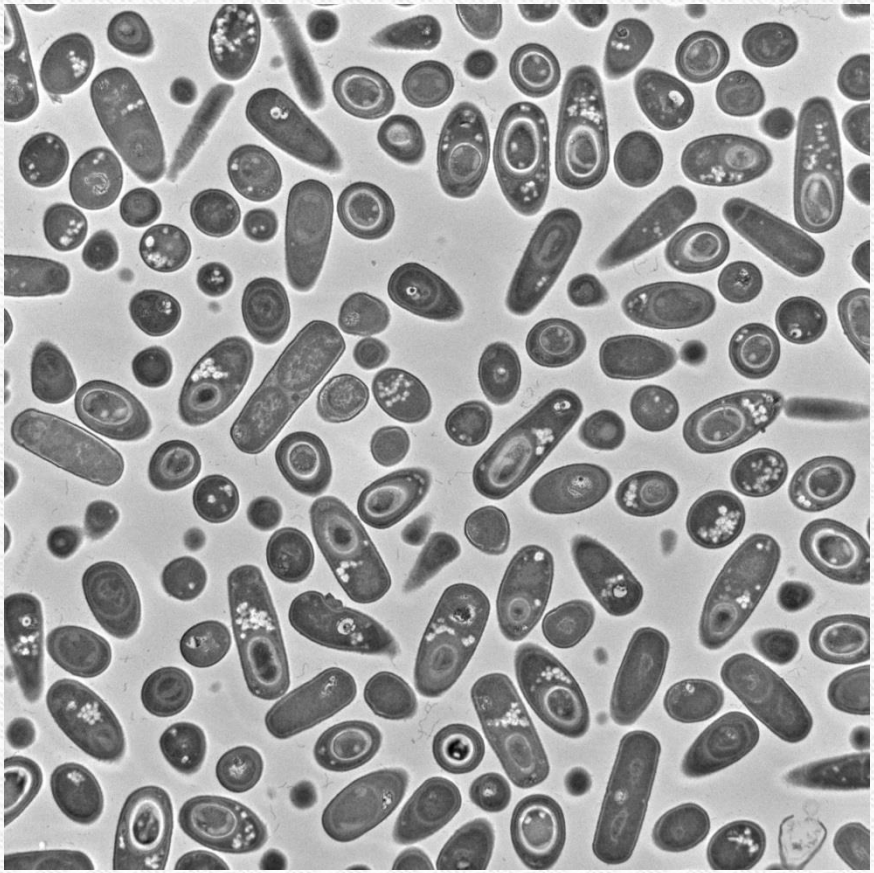
Purpose:

To produce *Bacillus subtilis* that can be switched back and forth from L-form to rod shape

This requires:
- The controllable expression of *murE* under P*xlyR*
- Also mutation in *ispA* (naturally occurring) in order to produce excess phospholipids

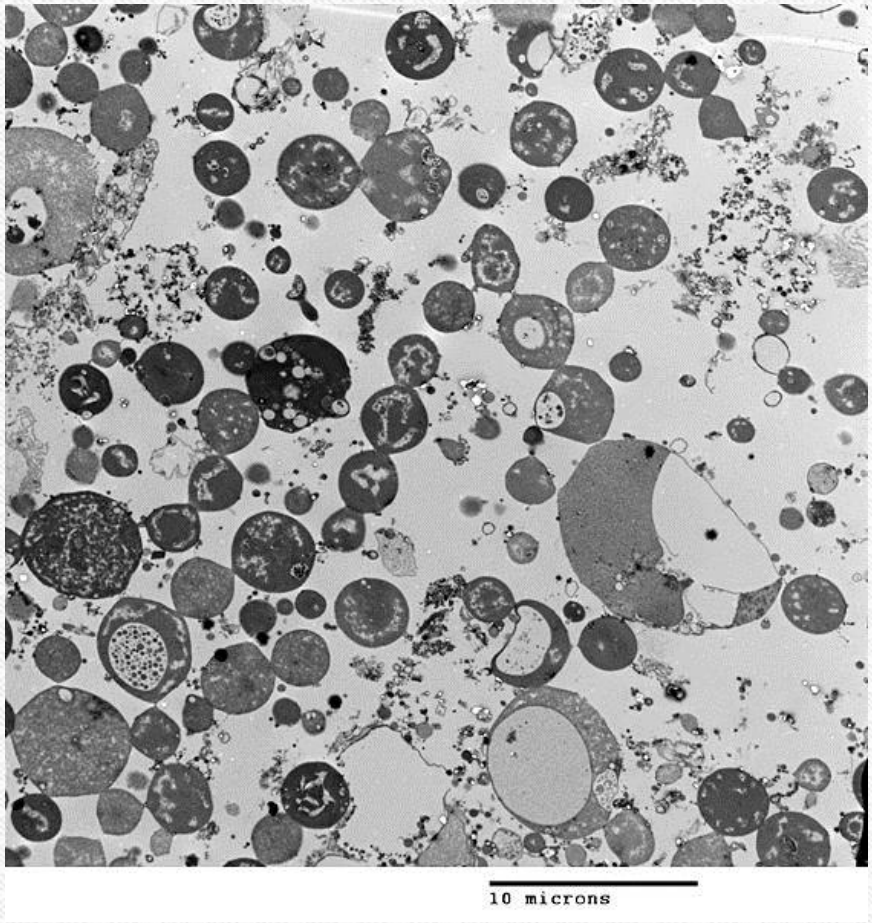


Rod cells



10 microns

L-form



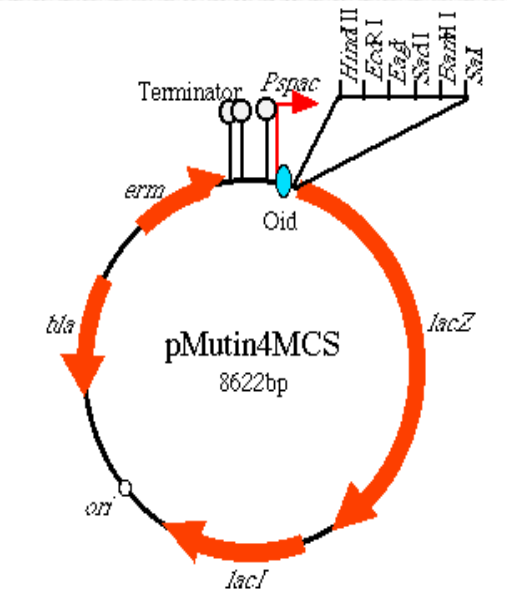
Images courtesy of: Gilpin, R. W., Young, F. E. & Chatterjee, A. N., 1973. Characterization of a Stable L-form of *Bacillus subtilis* 168. *Journal of Bacteriology,* 113(1), pp. 486-499.
&
Frederick National Laboratory for Cancer Research

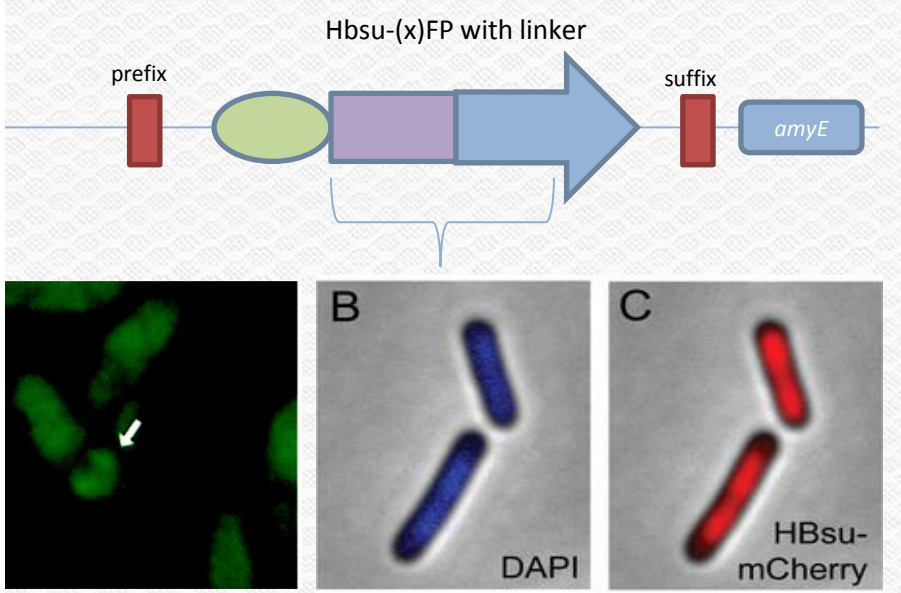
Hbsu-(x)fp BioBrick

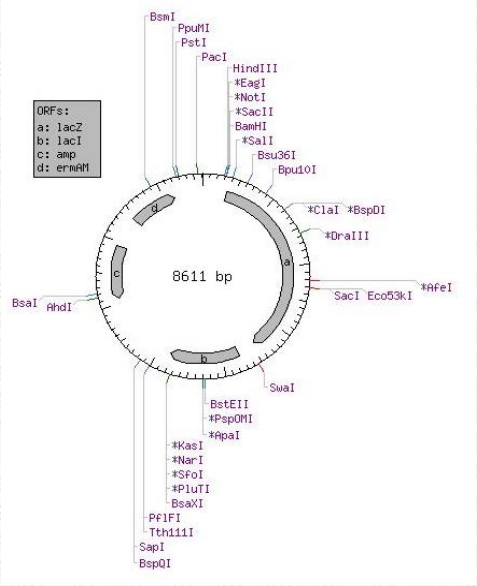
Purpose:
To tag the bacterial chromosome with Fluorescent protein, in order to observe recombination

Hbsu is a non-specific DNA binding protein

Interacts with DNA through the formation of homodimers

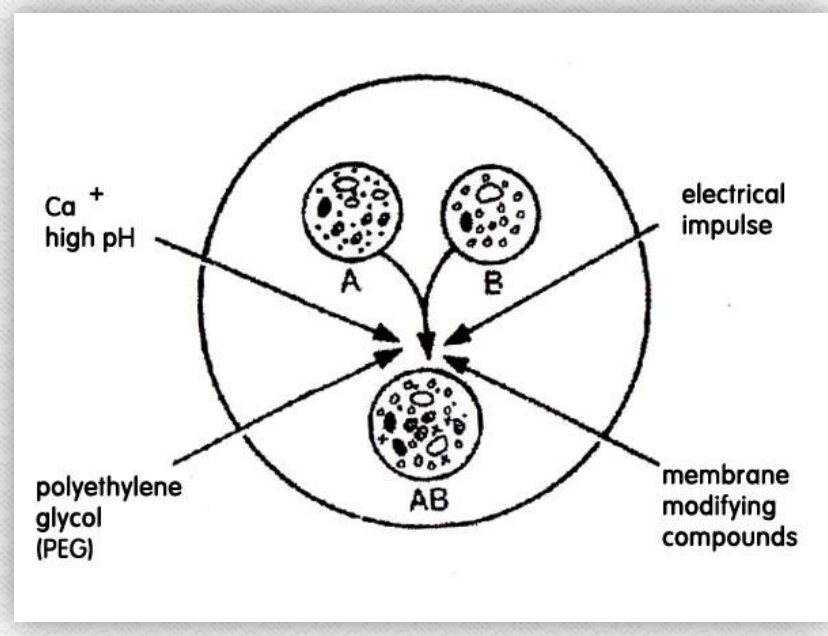






Images courtesy of: Smits WK, Grossman AD (2010) The Transcriptional Regulator Rok Binds A+T-Rich DNA and Is Involved in Repression of a Mobile Genetic Element in Bacillus subtilis. PLoS Genet 6(11): e1001207
Ross M A , and Setlow P (2000) Analysis of Nucleoid Morphology during Germination and Outgrowth of Spores of *Bacillus* Species. J. Bacteriol, 182:1942-1948

Genome Shuffling

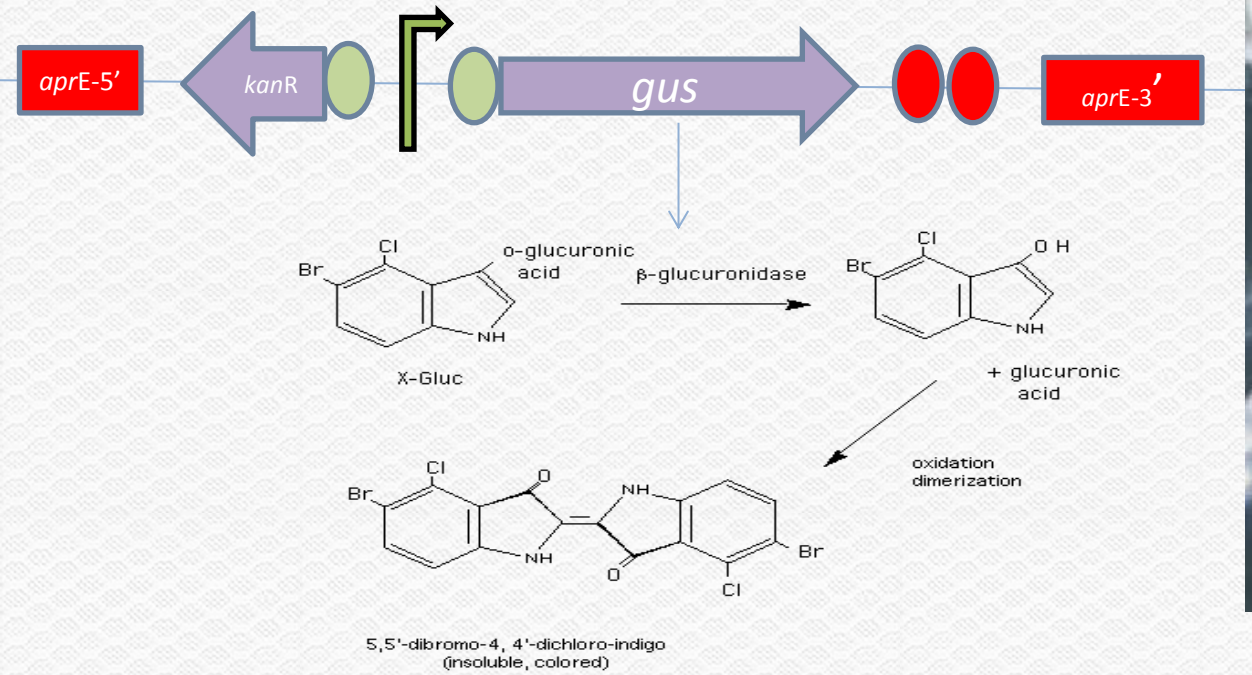


Images courtesy of: http://www.biotechnology4u.com/biotechnology_agriculture_methods_transfer_genes_plants.html

GUS reporter BioBrick

Purpose:
Act as a staining system to observe localisation of *Bacillus subtilis* in plants and to determine concentration of the colonies

gus encodes ß-glucuronidase which converts X-Gluc (a colourless, insoluble products) into blue insoluble products.



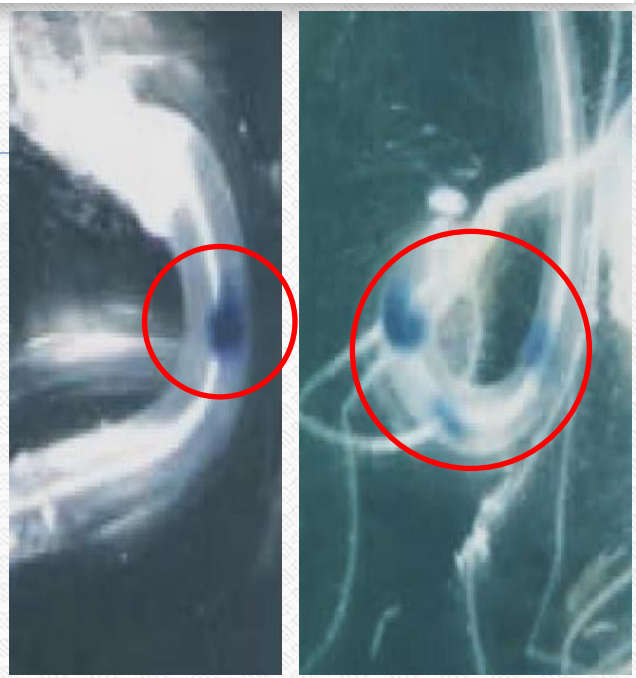


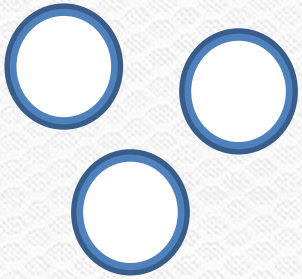
Fig. 1. *Bacillus subtilis* in Chinese cabbage seedling(3 days)

Images courtesy of: Tsomlexoglou, E., Daulagala, P.W.H.K.P., Gooday, G.W., Glover, L.A., Seddon, B. and Allan, E.J. (2003), Molecular detection and *β*-glucuronidase expression of *gus*-marked *Bacillus subtilis* L-form bacteria in developing Chinese cabbage seedlings. Journal of Applied Microbiology, 95: 218–224

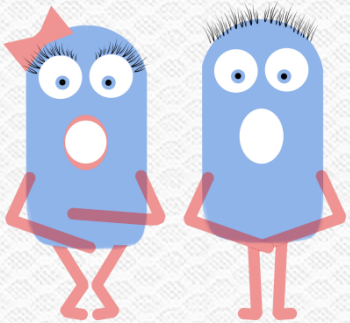
gus-model

```
# A simple gus Model
begin parameters
    c1      0.1
    c2      1e6
    cr2     1e2
    cdeg    1e-3
end parameters

begin molecule types

    beta(a)
    xgluc(a)
    blue(a)
    mRNA(a)
    promoter(a)

end molecule types

begin seed species
    beta(a)         1
    xgluc(a)        10
    blue(a)         0
    promoter(a)     1

end seed species

begin observables
    Molecules   blueproduct     blue(a)
    Molecules   xgluc           xgluc(a)
    Molecules   betaglucuron    beta(a!?)
end observables

# the actual reactions
begin reaction rules

    beta(a) + xgluc(a) <-> beta(a!0).xgluc(a!0) c2, cr2
    beta(a!0).xgluc(a!0) -> blue(a) + beta(a) + xgluc(a) c1
    blue(a) -> 0                                cdeg*blueproduct
    promoter(a) -> promoter(a) + mRNA(a)        0.1
    mRNA(a) -> beta(a)                          0.1
end reaction rules



## actions ##
generate_network({overwrite=>1})
writeSBML()
simulate_ode({t_end=>150,n_steps=>2000})
```

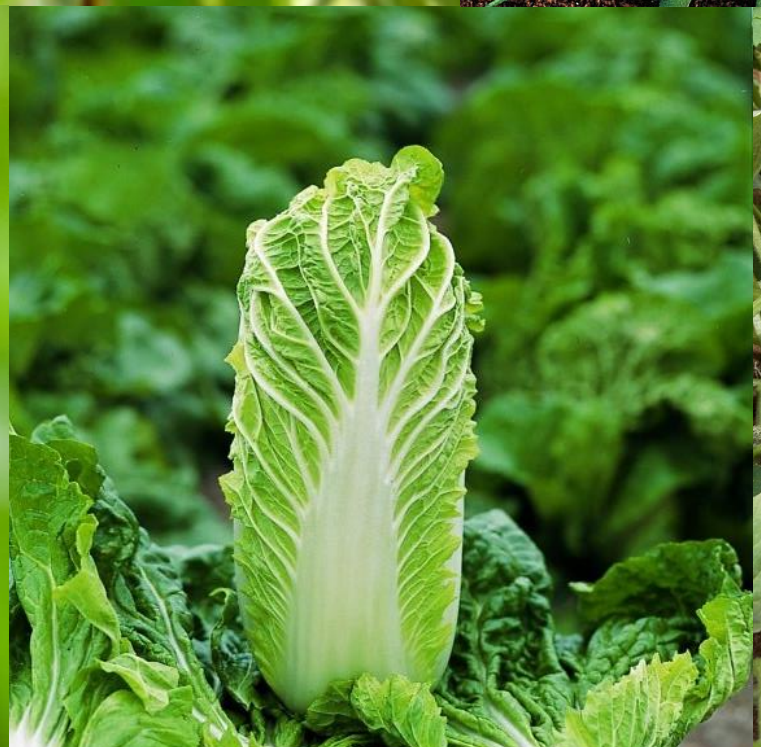
L-forms in Plants



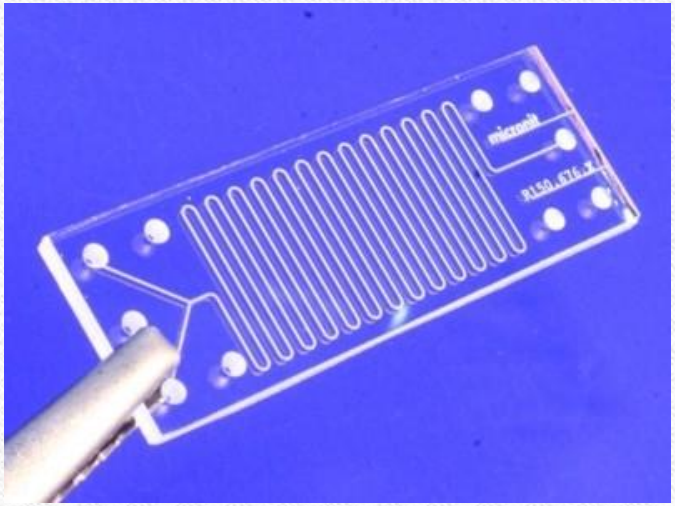
gus Reporter Gene

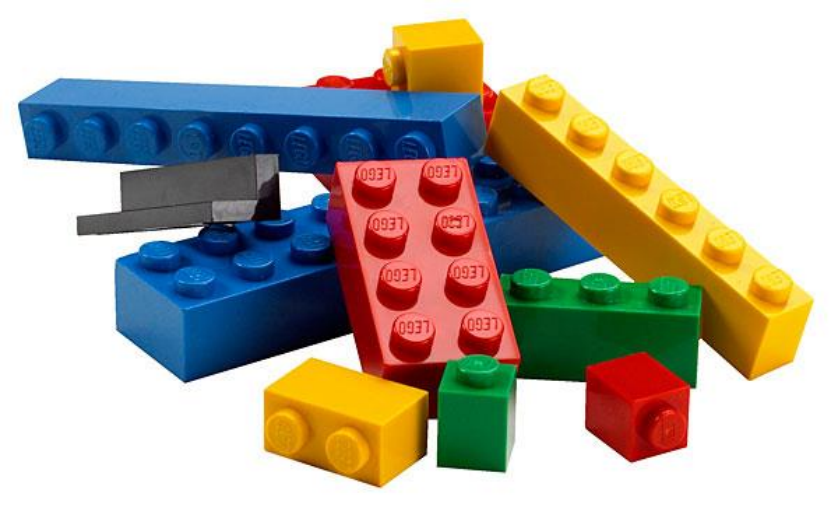





Shape Shifting





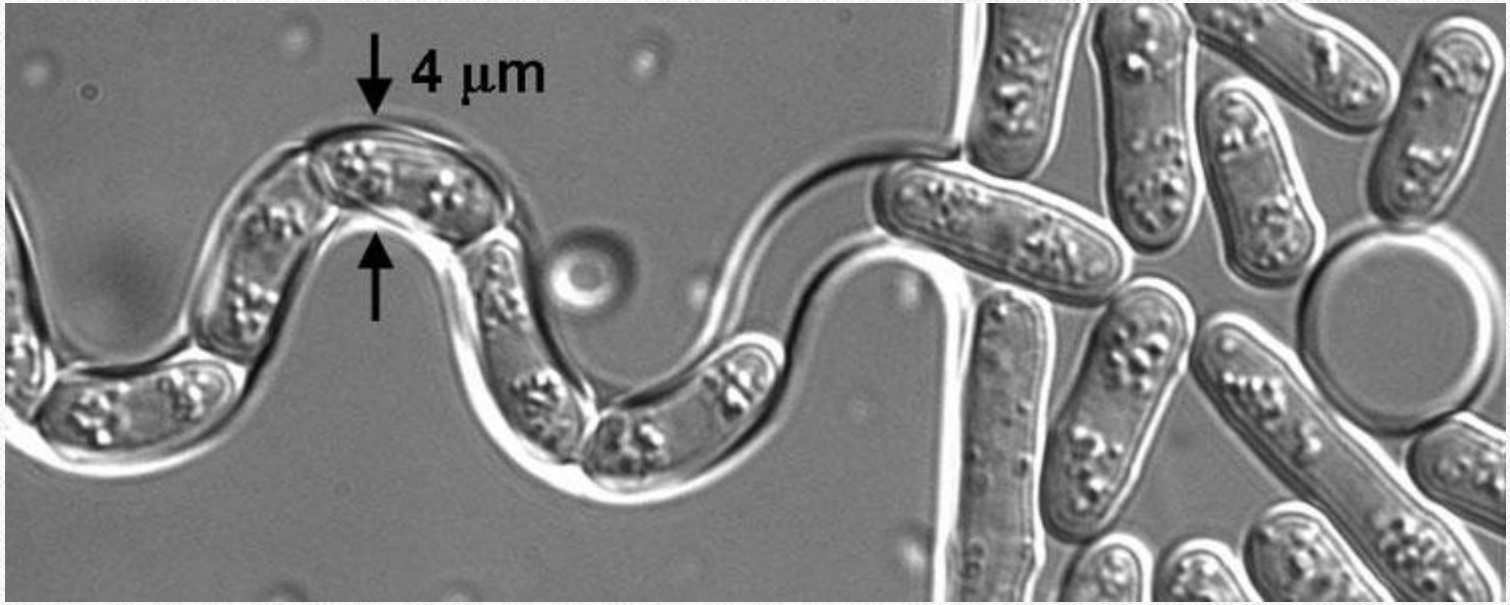
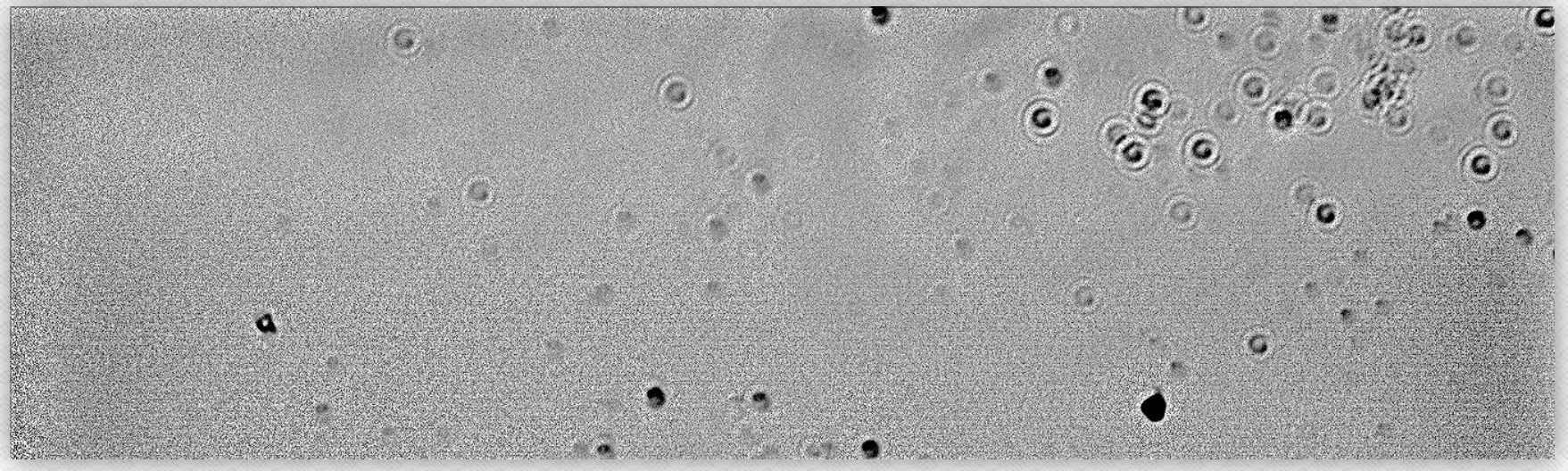


Image courtesy: Sarah Webb, Web of Science

Phong Tran, PhD, University of Pennsylvania School of Medicine

Summary

- Using L-forms we aim to research:
  - Biophysical properties of bacteria
  - Genomic shuffling
  - Symbiotic relationship between plants and L-forms



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QUESTIONS?

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