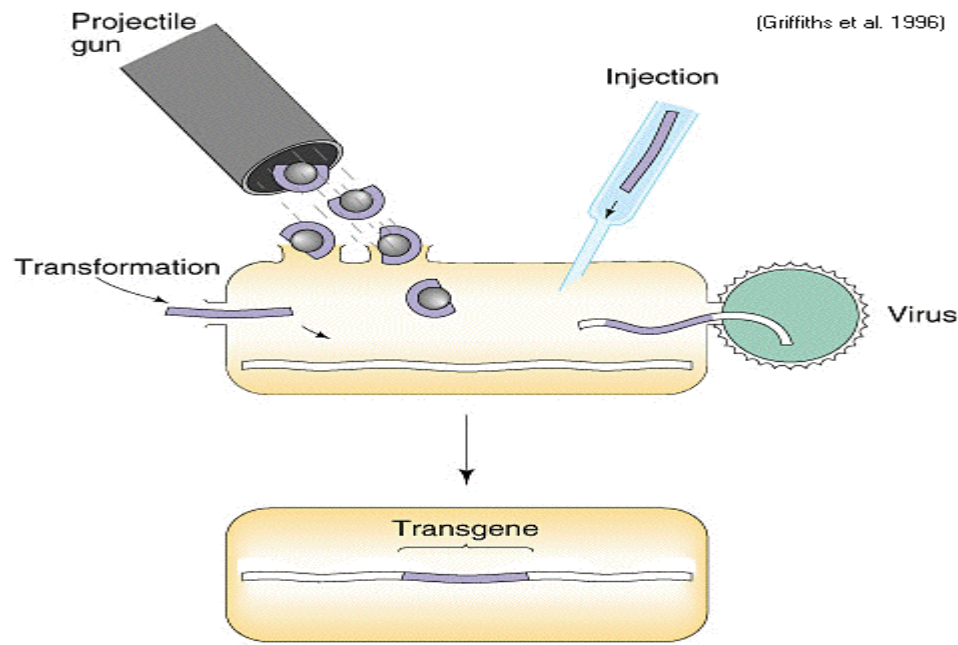




# Transformation/Transfection

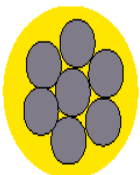
## Getting DNA into your host.



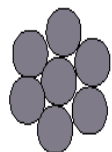


**Frederick Griffith**  
*transforming principle*  
1929

*Streptococcus pneumoniae*



Smooth colonies secrete a capsule and kill mice.

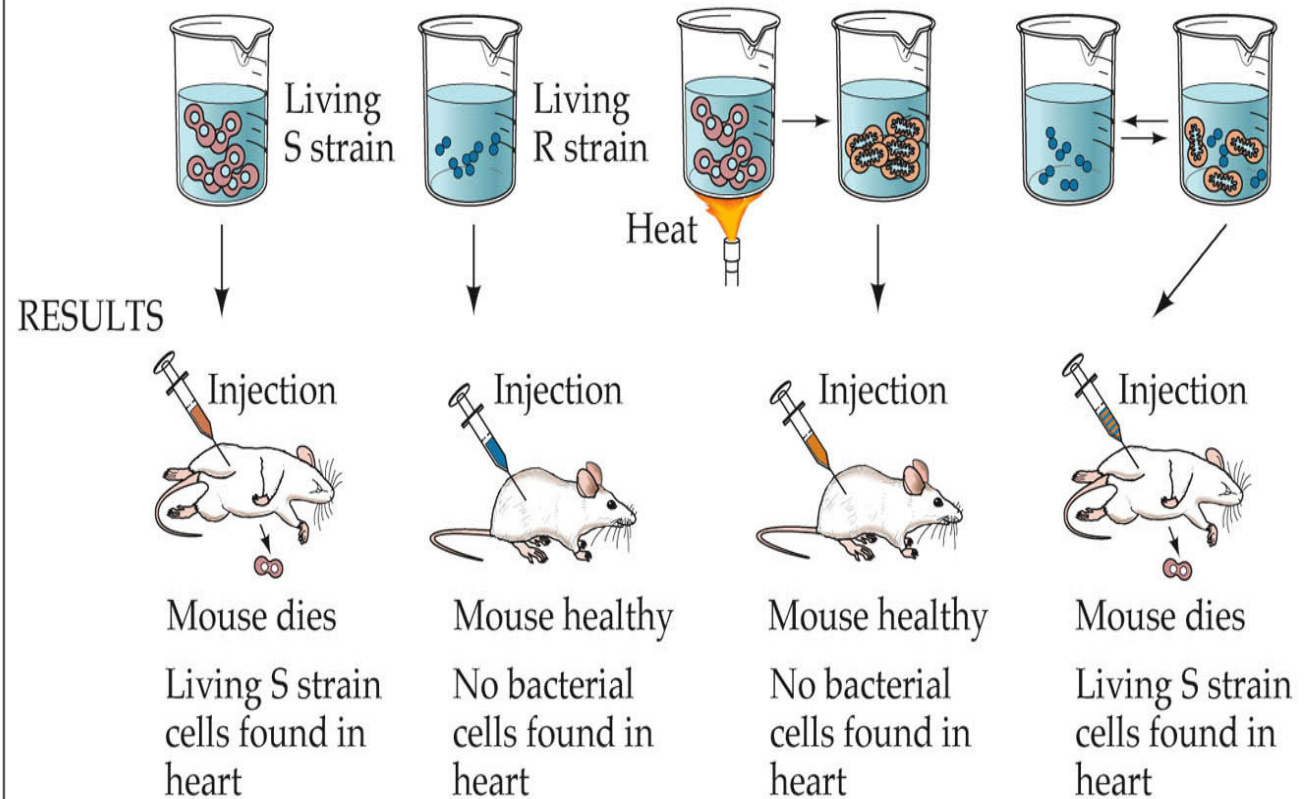


Rough colonies do not secrete a capsule and do not kill mice

## EXPERIMENT

**Question:** Can the presence of dead bacterial cells genetically transform living bacterial cells?

### METHOD



**Conclusion:** A chemical component from one cell is capable of genetically transforming another cell.

Transformation is getting DNA into a prokaryote

Transduction is the use of infection (i.e. viruses) to get DNA inside

Transfection is DNA into a eukaryote

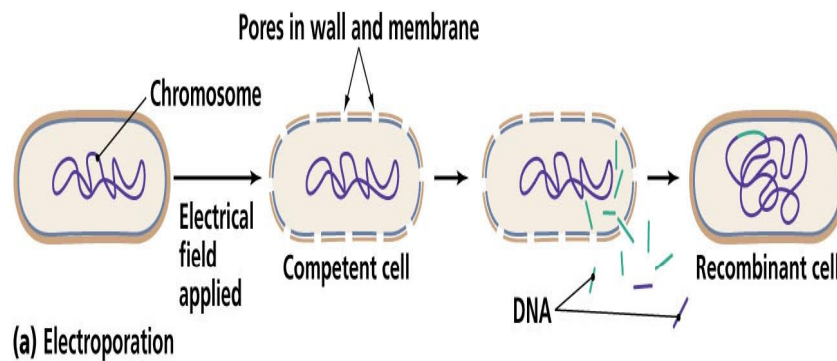
It happens in real live



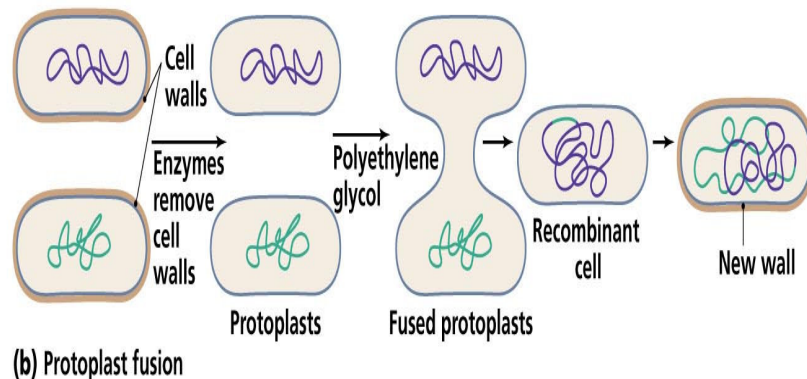
Joshua Lederberg  
Nobel Prize 1958  
33 years old

It happens in the lab  
competent (able to transform)

# Getting DNA into cells--Transformation of bacteria



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- Cells are made competent via electroporation or via pre-treatment with  $\text{CaCl}_2$ /cold
- DNA (plasmid) is added and cells that have taken up the plasmid are identified by plating on selective media

# Electroporation vs heat shock

- Plasmid size
- Efficiency





# Setting up your transformation reaction



- Ice, Ice, Ice, Ice
- Mix your ligation reaction with competent cells (never exceed 10%)
- No vortex of competent cells
- Incubate on Ice for few minutes
- Heat shock at 42 °C for 30 seconds
- Back on Ice for 2 minutes
- Add SOC Media
- Incubate with shaking 1 h then plate on selective media

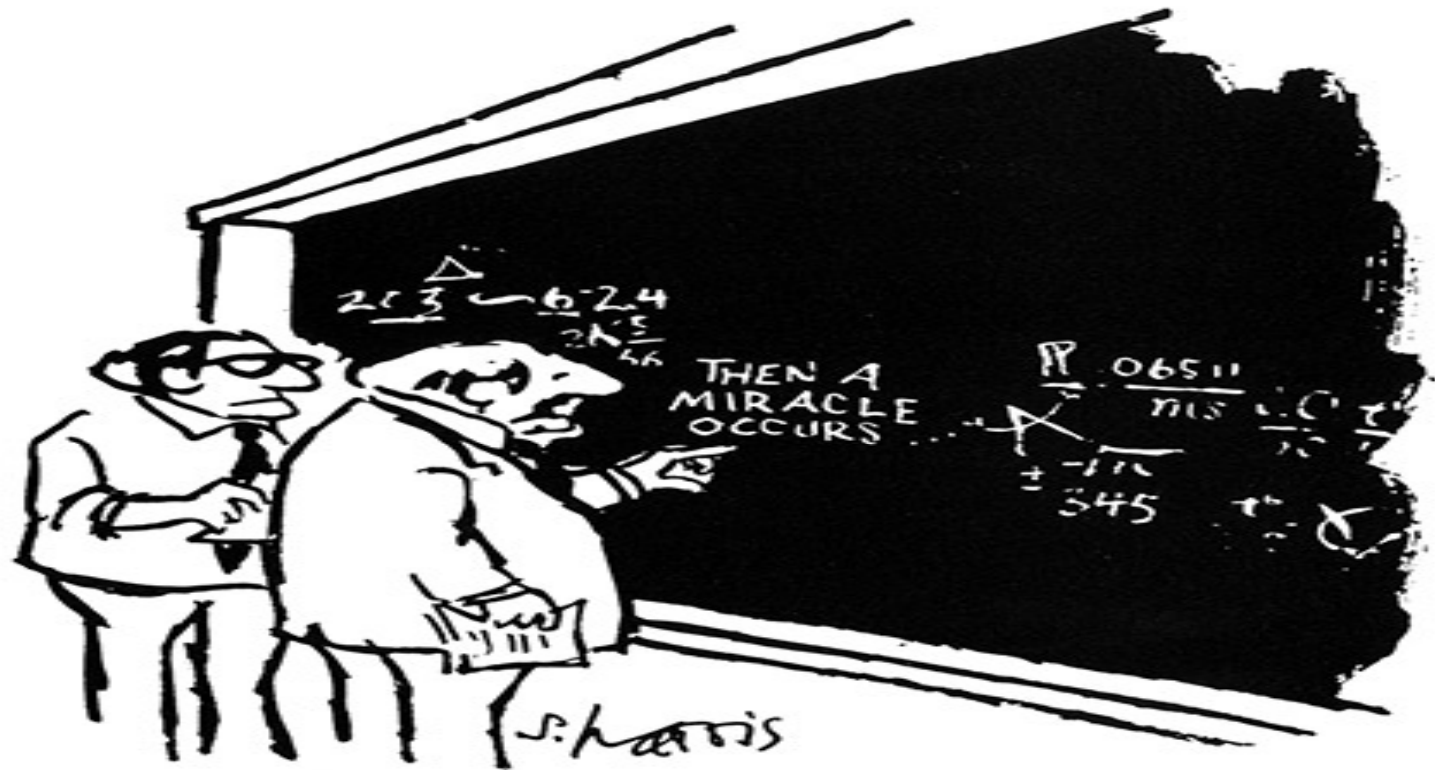
# Making your own competent cells

- Not all bacteria can be competent
- Easier with electroporation
- Very easy technique for *E. coli* in the lab





## Questions



"I think you should be more explicit here in step two."

If we knew what it was we were doing, it would not be called research, would it?" - Albert Einstein