Recombinant DNA technology

Lecture by:
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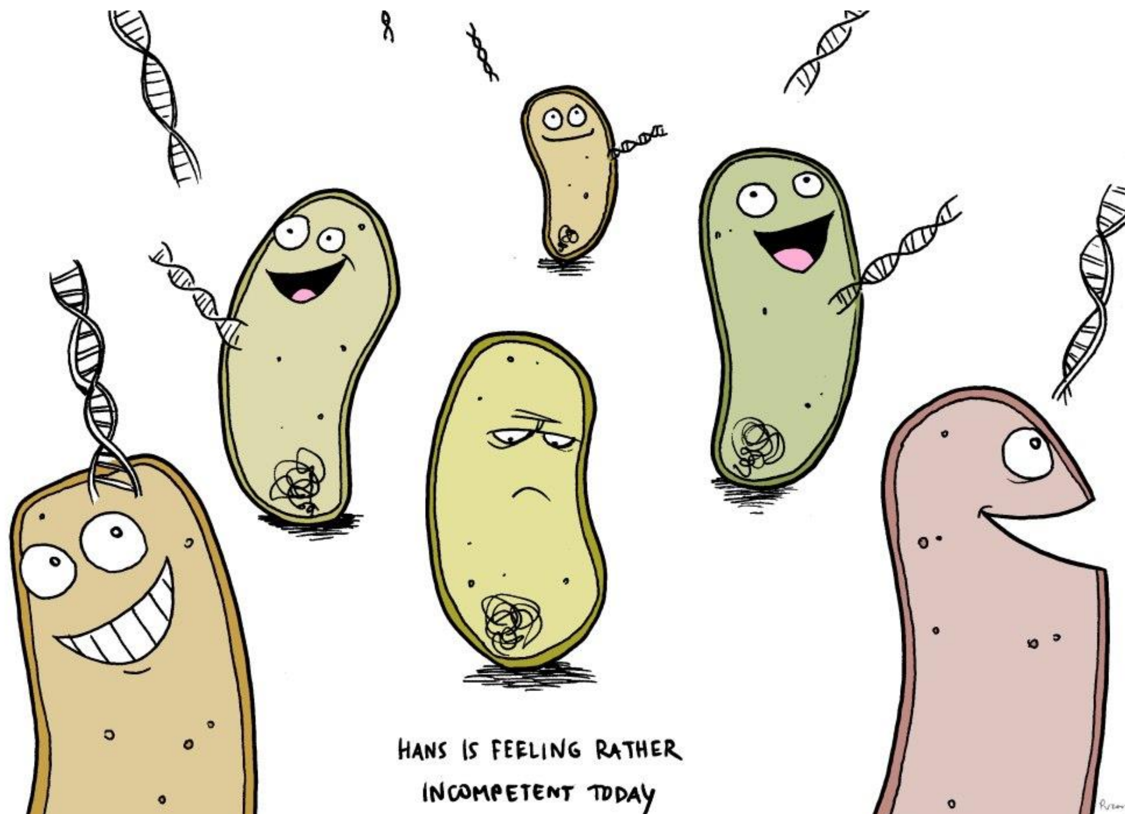


Recombinant DNA technology

Molecular cloning | **Transformation** | **Selection and Replication**

piece of DNA

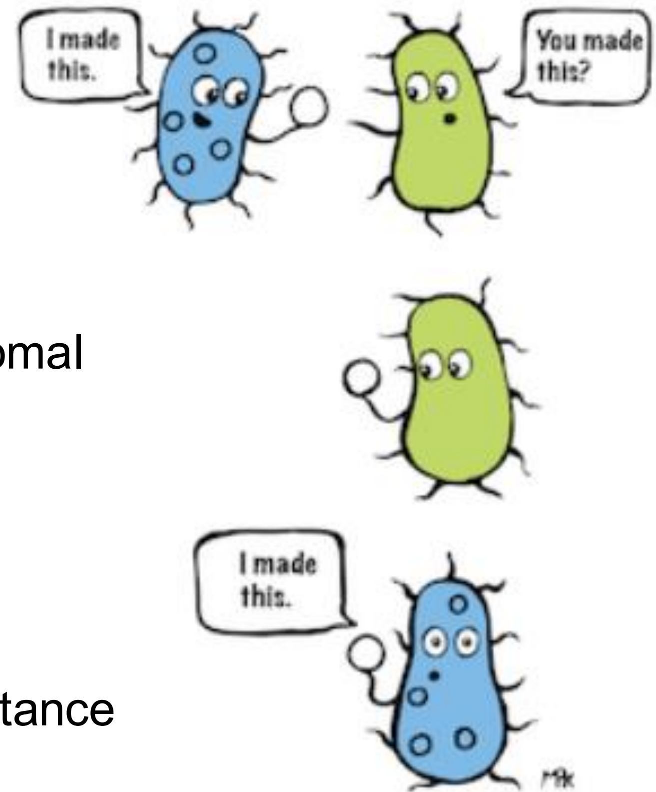
plasmid: holder for our piece of DNA

host cells

culturing

Performed during this lab

Plasmids



By: Maya Kostman

- Commonly found in bacteria as extra-chromosomal circular dsDNA molecules
- Able to self-replicate during cell division
- Often carry beneficial genes like antibiotic resistance
- Bacteria can share genetic information through plasmid transfer

How could this be useful for us?

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Applications of recombinant DNA technologies

Biopharmaceuticals:
Vaccines eg hepatitis B vaccine
recombinant proteins eg Insulin (Diabetes), Factor VIII (hemophilia)

Genetically modified organisms:
Organisms that have been genetically modified to exhibit specific traits eg herbicide-resistant crop plants

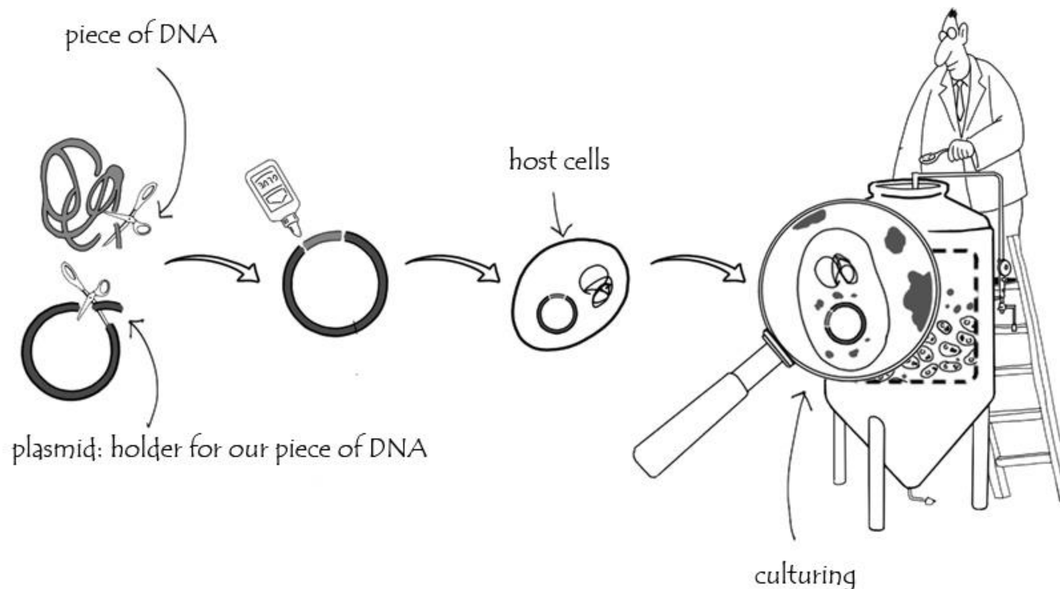
Gene Therapy:
In some genetic disorders, patients lack the functional form of a gene. Gene therapy attempts to provide a normal copy of the gene to the cells of the patient.

Gene Analysis:
build artificial, recombinant versions of genes that help understand how genes in an organism function

Khan S., 2016

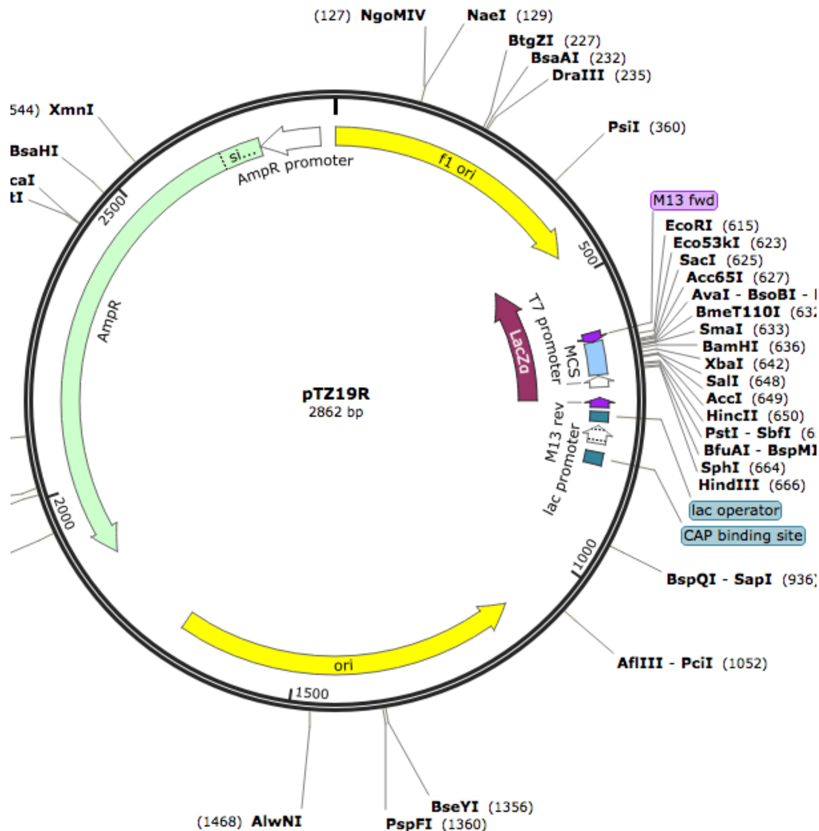
Toolbox for molecular cloning

- Scissors: DNA restriction enzymes (DNA digestion)
- Cutting sites: multiple cloning sites (MCS)
- Glue: DNA ligase (DNA ligation)
- Host: bacterial cells (Transformation)
- Environment: LB medium or LB agar plates (Culturing)
- Goal: making more identical copies, or expression (making proteins)



Vectors

- ✓ DNA molecule that acts as a vehicle to carry foreign genetic materials into another cell, where it can be replicated or expressed.



Ori (origin of replication):
Replication is initiated here, enabling the plasmid to reproduce itself.

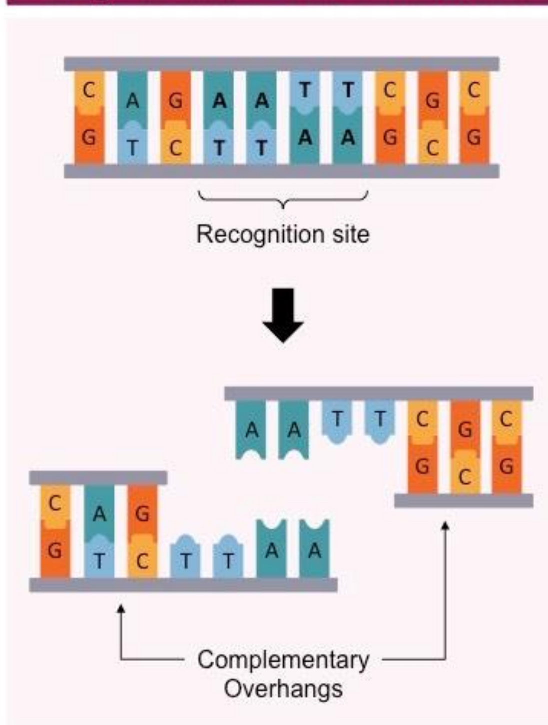
MCS (multiple cloning site):
Short segment of DNA which contains many restriction sites. This allows a piece of DNA to be inserted into that region. The used plasmid contains a BamHI cleavage site in its MCS.

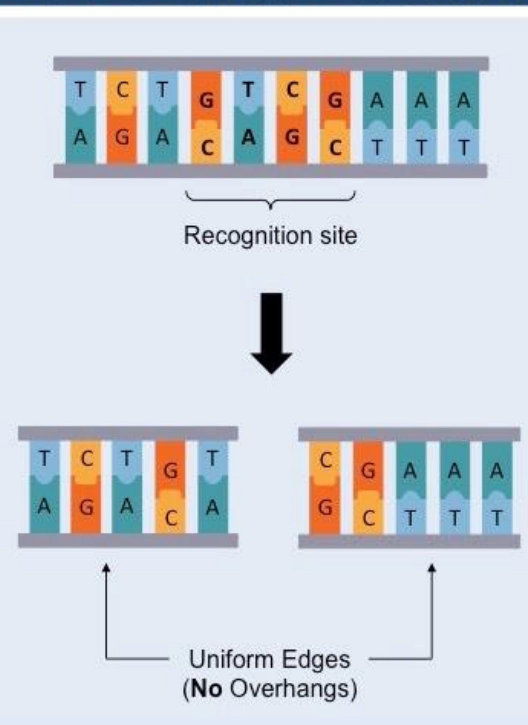
AmpR gene:
encodes the enzyme beta-Lactamase, which inactivates ampicillin. Cells containing a plasmid vector which expresses AmpR can be selected from those that do not by growth in an ampicillin-containing medium.

Lac promoter:
binding site of RNA polymerase to initiate expression. IPTG binds and inactivates the LacI repressor protein and thereby enables expression of genes downstream of the promoter.

LacZα gene:
encodes the alpha-peptide of the enzyme beta-galactosidase. Functional beta-galactosidase consits of the alpha- and omega-peptide. The used E-coli strain carries the lacZ deletion mutant which contains the omega-peptide but lacks the alpha-peptide. The activity of mutant beta-galactosidase is rescued by the presence of the alpha-peptide present in the plasmid (alpha-complementation).

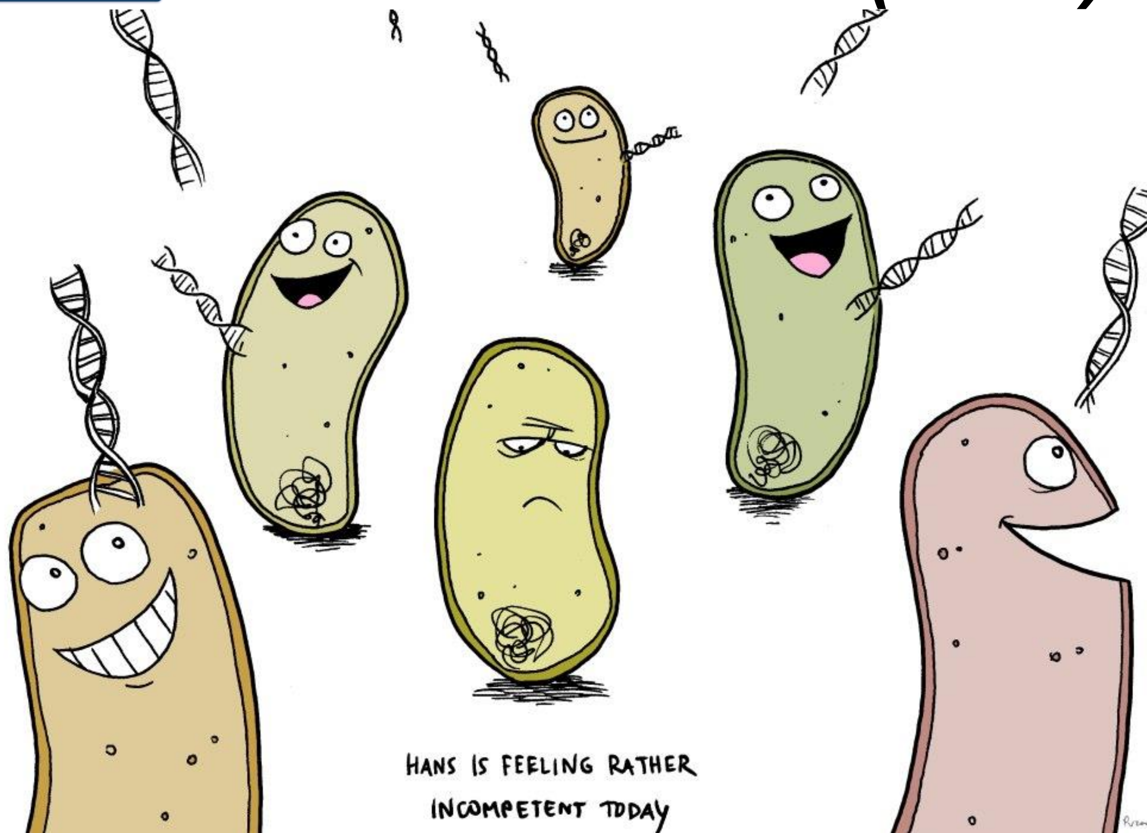
Restriction enzymes (Scissors)





- ✓ Sequence-specific DNA endonucleases
- ✓ Recognise and cleave DNA sequences at specific restriction sites
- ✓ Generate "sticky end" or "blunt end"

Escherichia coli (Host)



- ✓ Model organism in molecular biology
- ✓ Gram negative, rod shaped bacteria
- ✓ Located in lower intestine

The History of Insulin Production

1921: Discovery of insulin

1922: Leonard Thompson became the first person with diabetes ever treated through administration of insulin

1923: Insulin is commercialized

14 cows or 70 pigs to sustain a diabetic patient for 1 year

Insulin sales kit, Eli Lilly and Company, 1940s

1970: Recombinant DNA technology is developed

1982: Recombinant insulin is commercialized

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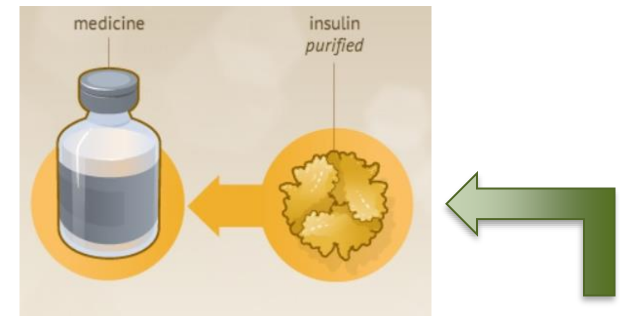
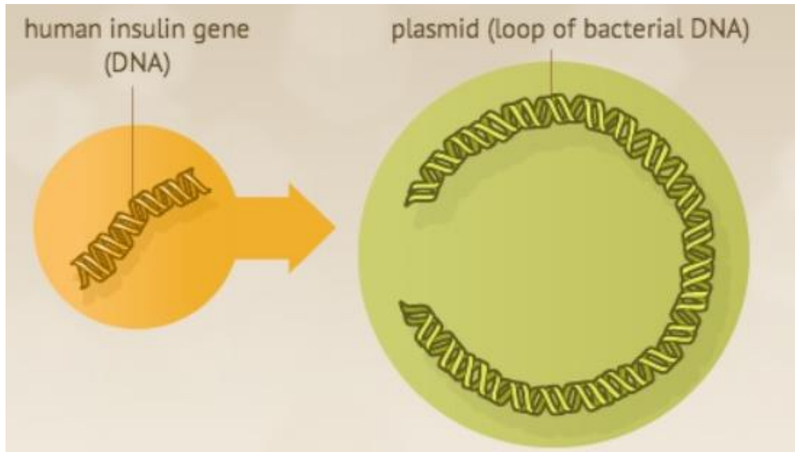
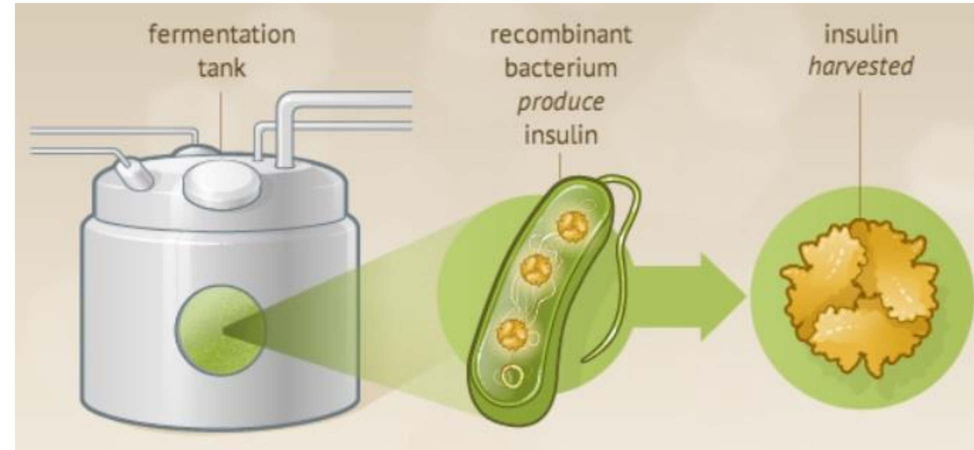
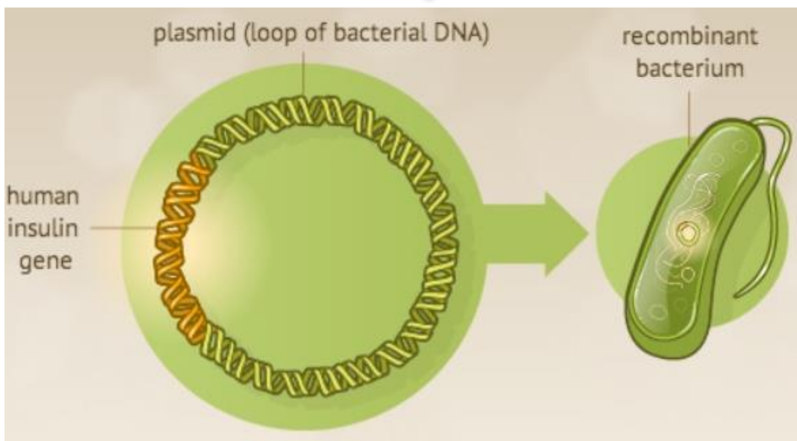
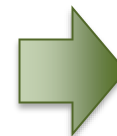
Production of Insulin

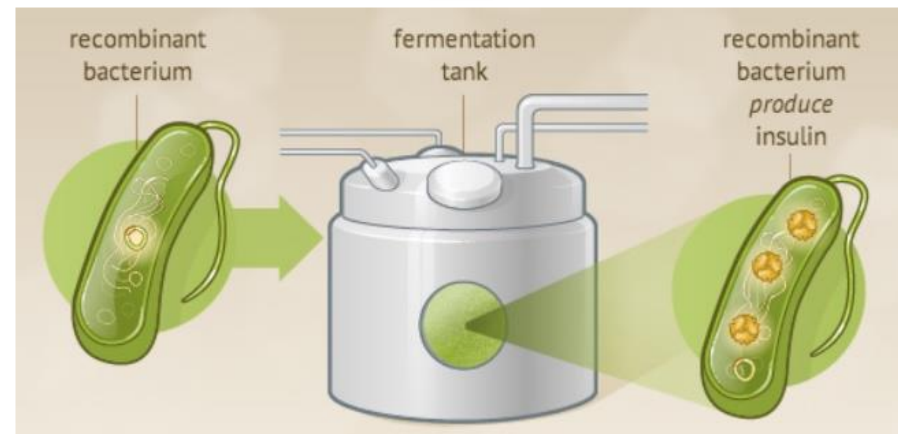












Adapted from “From DNA to Beer: Harnessing Nature in Medicine & Industry”

Purpose of this lab:

Determine whether a gene of interest has been successfully cloned into a vector.

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Transformation

recombinant Plasmid

competent E.coli Host cell

transformed cell

Transformation

During the incubation on ice, DNA binds to the surface of the bacterium as a calcium-phosphate-DNA complex

Permeabilized membrane

Following a sudden increase in temperature, one or more DNA molecules bound to the surface of the cell is taken up by the competent cell

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How can we selectively grow bacteria that have taken up the plasmid?

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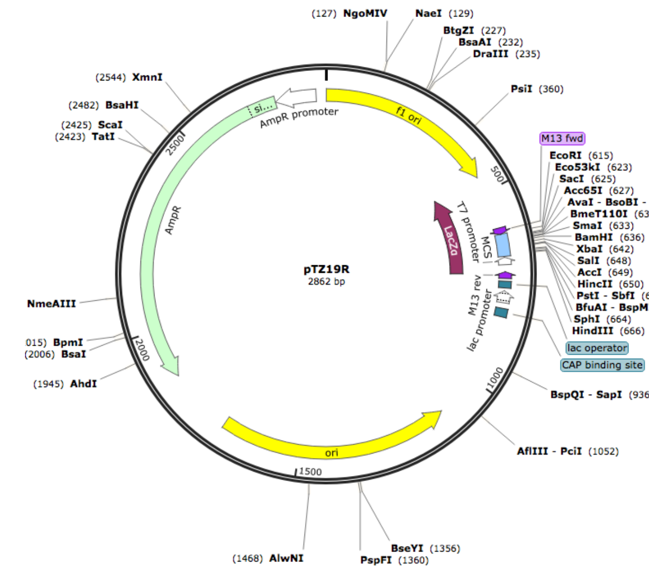
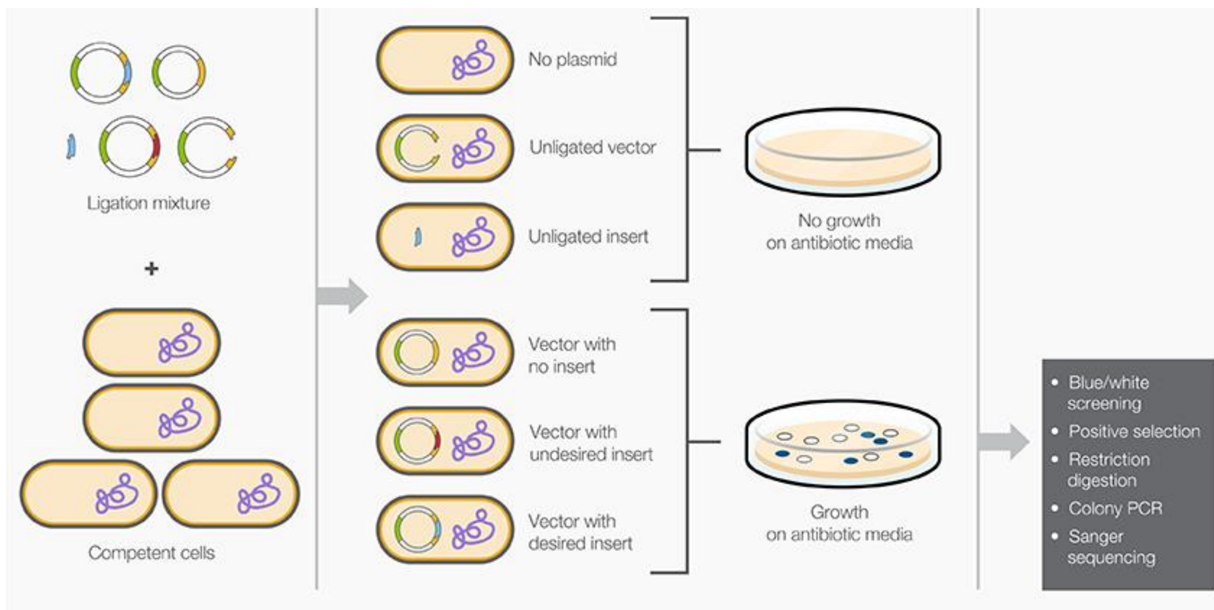
Selection pressure

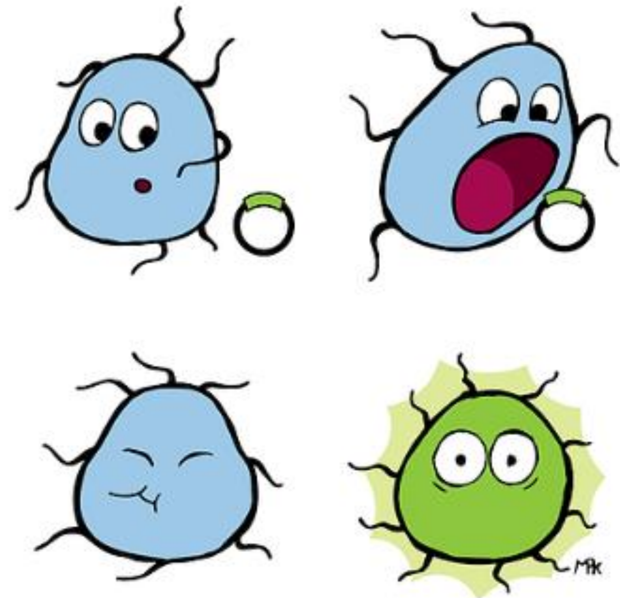


- ✓ *ampR* gene encodes for beta-lactamase
- ✓ Inactivates ampicilin antibiotics
- ✓ Only cells containing vector DNA will grow in the presence of ampicilin



Selection based on antibiotic resistance

How do we select for bacteria with the plasmids carrying the inserts?



By: Maya Kostman

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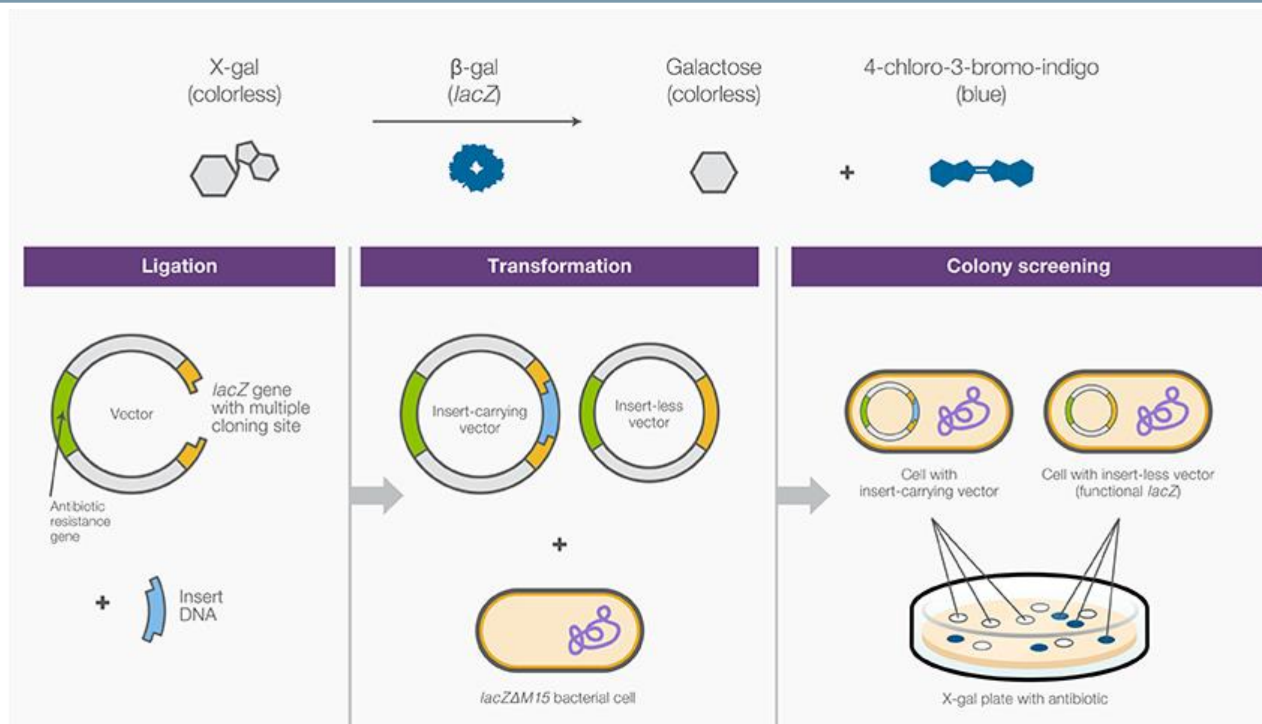
Blue-white screening

LacZ gene naturally found in *E. coli*, encodes β-galactosidase.

We use an E.coli strain that carries the LacZ deletion mutant which contains the omega-peptide but lacks the alpha-peptide and is therefore non-functional. The plasmid we use carries the alpha-peptide, rescuing the function of mutant beta-galactosidase.



More detailed info: thermofisher.com

Lab Schedule

- Monday (11/24)
  Introductory lecture 12:15 – 13:00
  Lab 13:15 – 14:00 Groups 1-19
  Lab 14:15 – 15:00 Groups 20-38

- Tuesday (11/25)
  Lab 11:15 – 11:45 Groups 1-19
  Lab 12:00 – 12:30 Groups 20-38

- Wednesday (11/26) Groups 1-19
  Introductory lecture 8:15 – 9:00
  Lab 09:15 – 16:00

- Thursday (11/27) Groups 20-38
  Introductory lecture 8:15 – 9:00
  Lab 9:15 – 16:00

Deadline for submitting lab reports: 07/12/2025

Working in the lab

- ✓ Work in groups of **2 people, stick to your assigned partner**
- ✓ Always wear **gloves** and **lab coat** to protect you and your samples, wash your hands thoroughly before leaving the lab
- ✓ When using the pipette, check the **volume limits** (0.1-10μl, 10-200μl, and 100-1000μl)
- ✓ Pipette **into the bottom** of the tube, do not ”shoot” it *(especially when working with very low volumes)*

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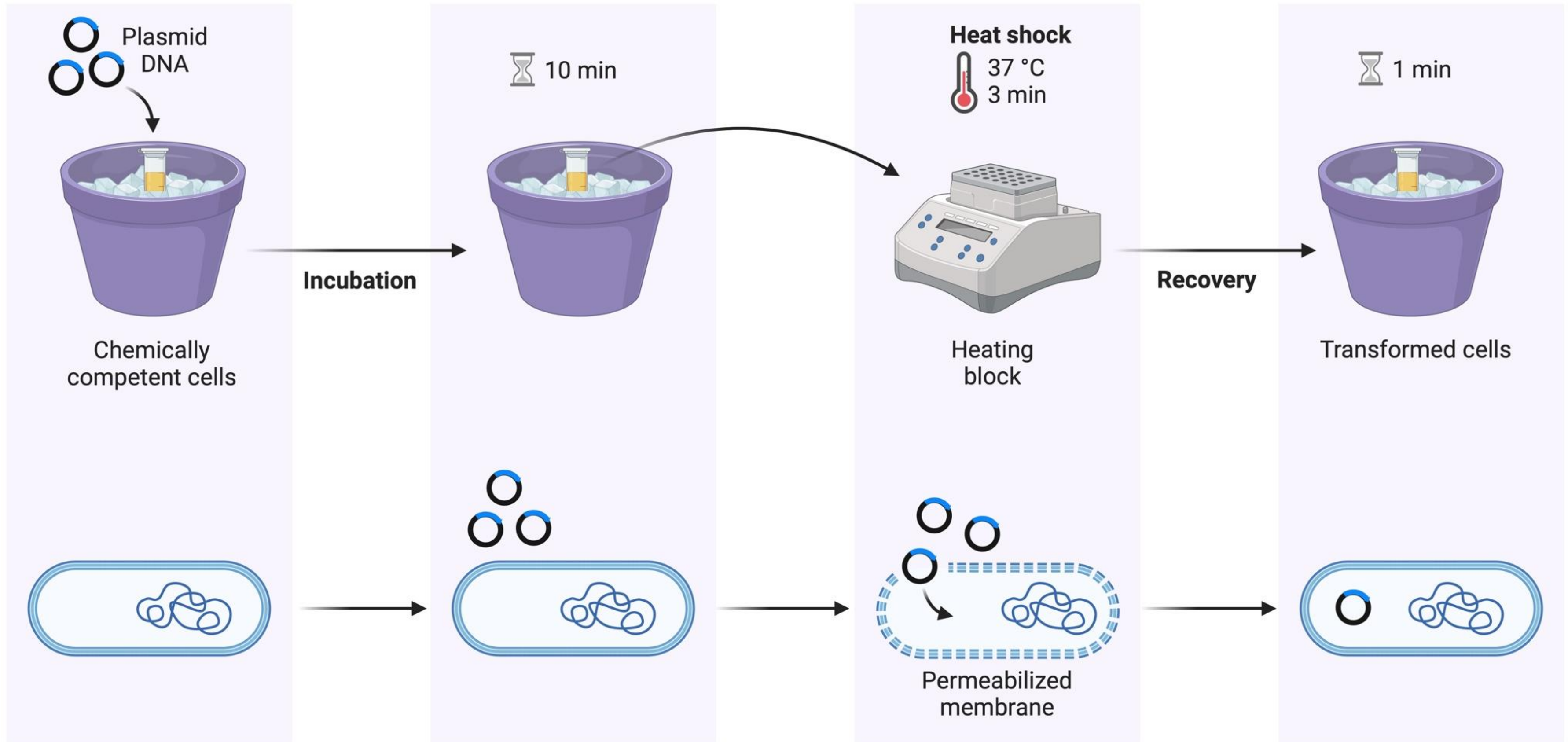
Transformation of competent cells

→ performed during this lab (day 1)



- ✓ 1 tube of plasmids (P) (with/without insert)
- ✓ 1 tube of competent bacteria (C)

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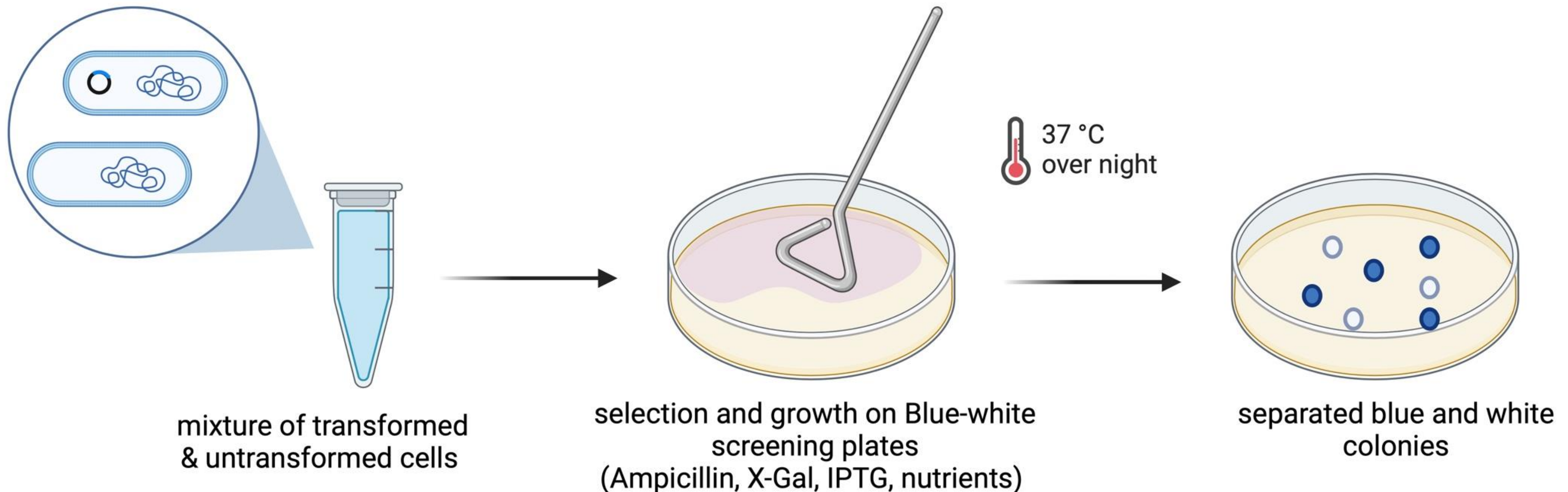
Day 1: Selection of transformed cells

→ performed during this lab (day 1)



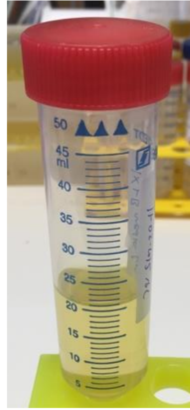
Day 2: Picking & expansion of blue and white colonies

→ performed during this lab (day 2)

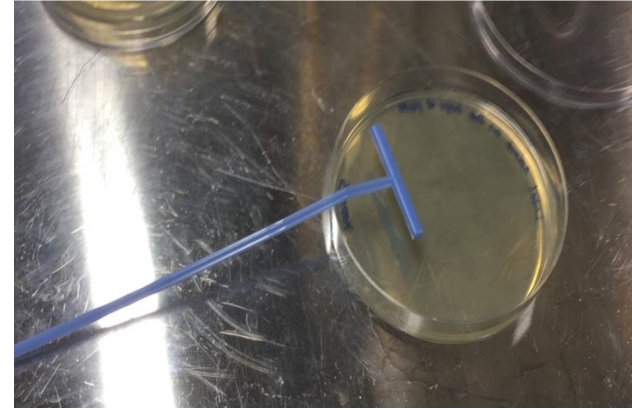
- ✓ Pick up bacterial colonies (**2 white, 2 blue**) from plate
- ✓ Grow in LB medium with antibiotics (expand the colony and replicate plasmids)

Day 1: Materials for Transformation

Eppendorf tube

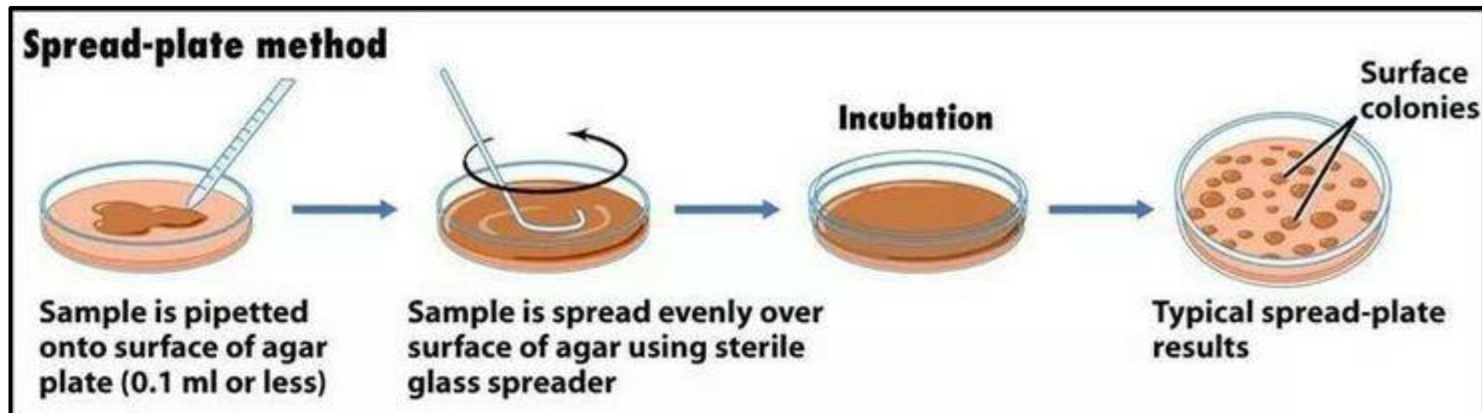


Falcon tube (50ml)



LB-agar plate

Day 1: Spread plate method



- Apply light pressure to not tear or stab the agar

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Day 3 - work overview

- ✓ Purify plasmids
- ✓ Restriction enzyme digestion
- ✓ Run on agarose gel
- ✓ Interpret results

Lab reports

- ✓ Write according to the guidelines on the handout on Canvas
- ✓ **One lab report per group** (your names and project group number on the cover page)
- ✓ **In English**
- ✓ Upload your lab reports on CANVAS deadline on 07/12/2025

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Lecture Questions

1. Name three applications of molecular cloning.
2. What is a plasmid?
3. Name the three main steps involved in recombinant DNA technology?
4. What is a restriction enzyme?
5. What are the bacteria used in your protocol?
6. Explain the calcium/phosphate (heat-shock) method and what it is used for.
7. How can we selectively grow bacteria that have taken up the plasmid?
8. Explain the principle behind blue and white screening and its purpose in this lab.

References

Kehoe A (1989). "The story of biosynthetic human insulin". In Sikdar SK, Bier M, Todd PW (eds.). Frontiers in Bioprocesssing. Boca Raton, FL: CRC Press. ISBN 978-0-8493-5839-5.

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https://www.thermofisher.com/se/en/home/life-science/cloning/cloning-learning-center/invitrogen-school-of-molecular-biology/molecular-cloning/cloning/traditional-cloning-basics.html