

BME

Molecular Biology
Experiment

Colony selection

SKKU BME

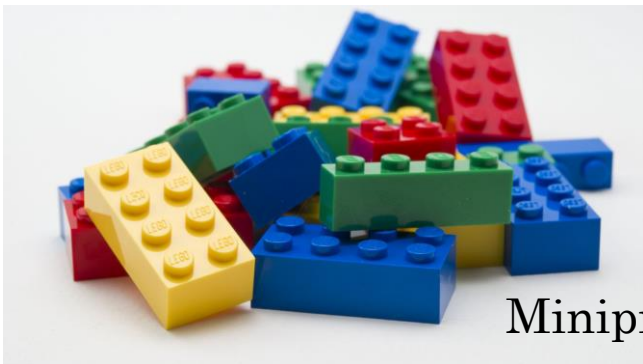
3rd grade, 2nd semester

Today

- Select colonies
- Colony PCR
- DNA electrophoresis
- Seeding for plasmid mini-prep.



Subcloning...SCIENCE!



Miniprep, PCR, seeding...

How to find right one?

- LacZ method
- Colony PCR
- Miniprep and enzyme cutting
- GFP leakage



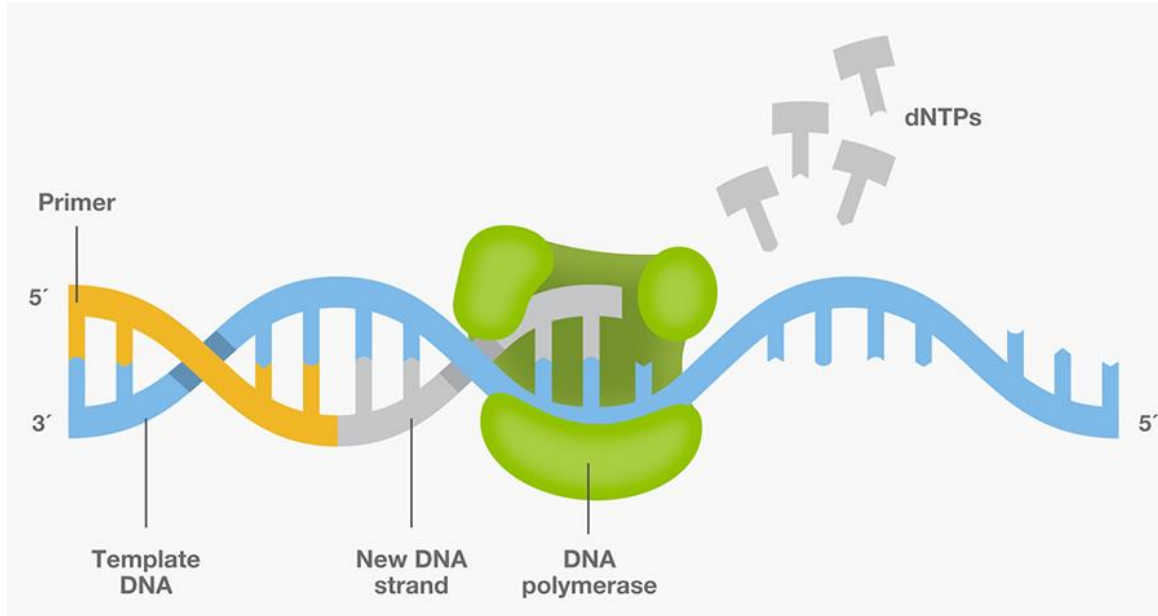
X-gal identifies cells with beta-galactosidase

How to find right one?

- Colony PCR

Colony PCR is a convenient high-throughput method for determining the presence or absence of insert DNA in plasmid constructs.



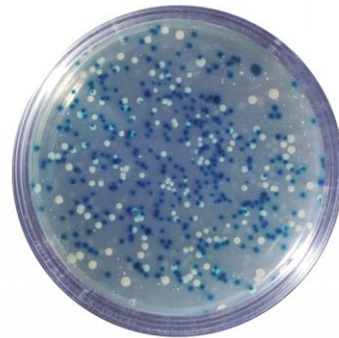


Colony PCR

- Question:
 - How to confirm the insert by PCR?
 - Make your own plan!!



X-gal identifies cells with beta-galactosidase





Colony PCR

- Picking colonies
- Suspending each colony in D.W. (~10 ul)
- Use 1 ul of suspended colony for PCR template



Colony PCR

- Marking colonies to be picked
- Picking each colony
- Suspending in 10 ul D.W. by up and down



Colony PCR mix!

- How many colonies will you pick?
- If 10 colonies,
- Making PCR mix for 10 reactions
- Calculate the PCR mix



Colony PCR mix!

- Total volume of the PCR mix?
 - 10 ul each
- What should be in the PCR mix?
 - Template: E. coli containing plasmid
 - Primers
 - Taq polymerase
 - dNTP
 - Buffer



Colony PCR mix!

- Total volume of the PCR mix?
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Making total PCR mix (11X for 10X)
Template: 1 ul in each tube
Rest of it: 9 ul
 $9 \text{ ul} \times 11 = 99 \text{ ul total}$

Colony PCR mix!

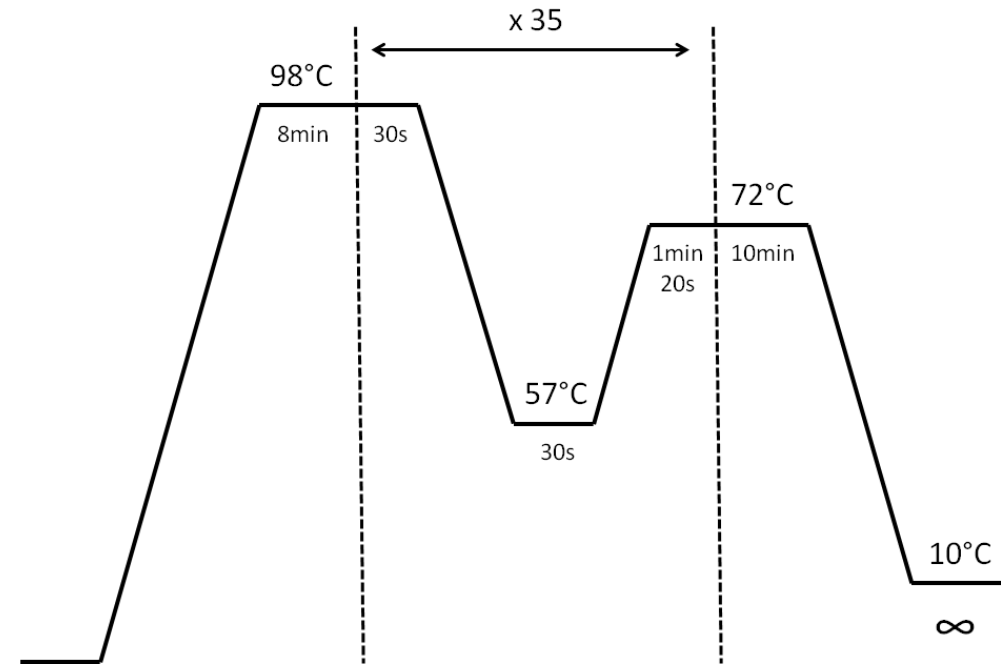
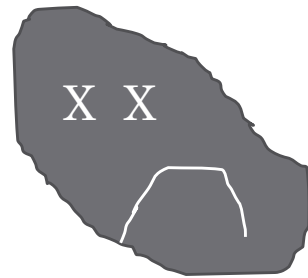
	1X	11X
Template	1ul	
Primer 1 Primer 2		
dNTP		
Buffer		
Taq polymerase		



Making total PCR mix (11X for 10X)
Template: 1 ul in each tube
Rest of it: 9 ul
 $9 \text{ ul} \times 11 = 99 \text{ ul total}$

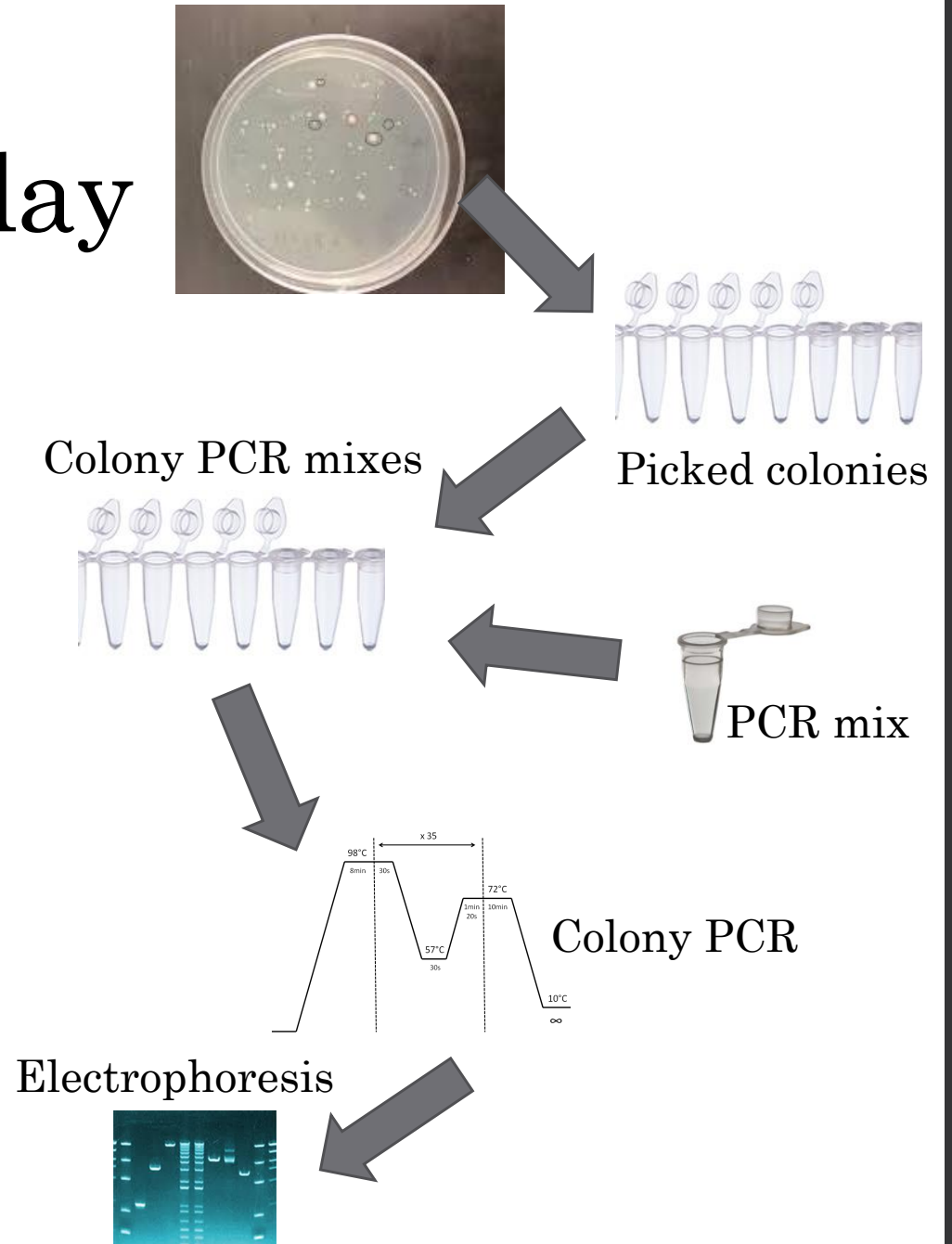
Colony PCR Cycle?

- Think what's different from usual PCR?
- Then, what should you change the cycle?

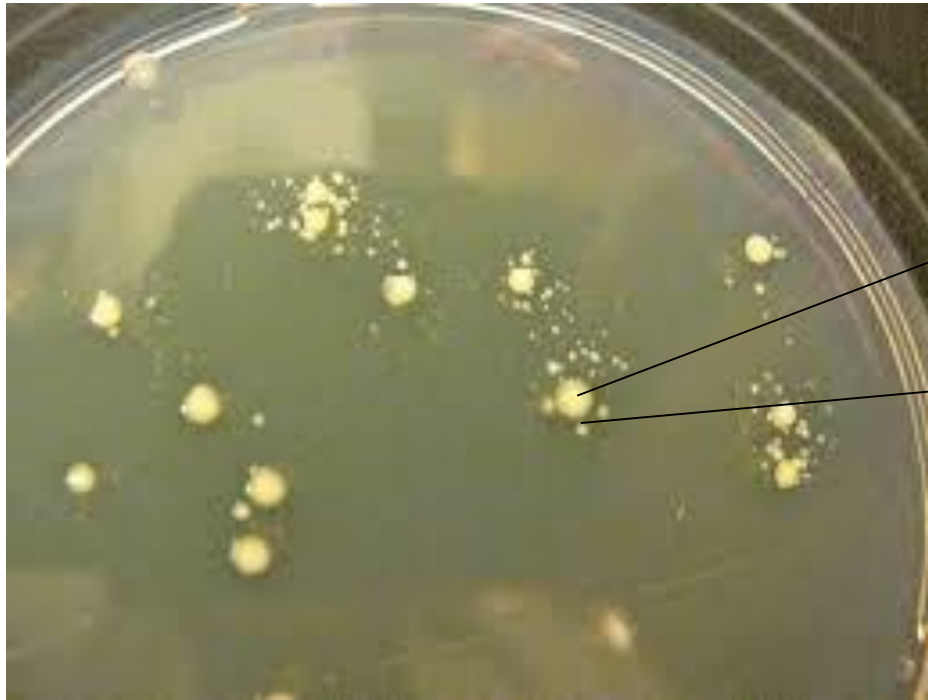


Overall schedule today

- Colony selection and marking
- Colony picking
- Colony PCR (mix => aliquot => RX)
- Agarose gel making
- Gel electrophoresis
- Miniprep seeding (4 ul suspension to 5 ml in LB/Amp media)
- E.coli streaking (1ul in each section)



Subcolony! Do NOT pick it up!



Colony

Subcolony

Homework



- Please find streaking method and explain the right figure.
- Explain what the subcolony is, how it is generated, and discuss about how to reduce the subcolony. Then explain the left figure.
- **For protein induction, a specific *E. coli* line is necessary. Explain the reason and find BL21(DE3) cell line.**

