

BME

Molecular Biology  
Experiment

Subcloning confirmation  
& Spreading for protein induction

SKKU BME

3<sup>rd</sup> grade, 2<sup>nd</sup> semester

# What did you do last class?

- Colony PCR
- Miniprep seeding
  
- Then?

# Today

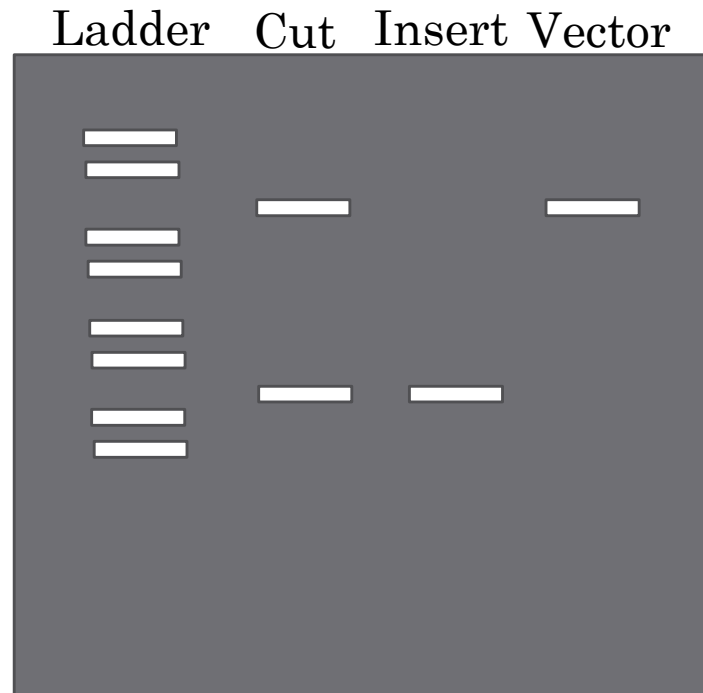
Plasmid  
miniprep

Enzyme cutting  
for insert and  
vector  
confirmation

Seeding for  
protein  
induction

# Enzyme cutting

- What is the point?
  - Right sizes of vector and insert



# Enzyme cutting

- Enzyme reaction!

	1X reaction	nX reaction
Miniprep product	How much ug?	
Enzyme 1 & 2	How much unit?	
10X buffer	Depending on final Rx volume	
DW		

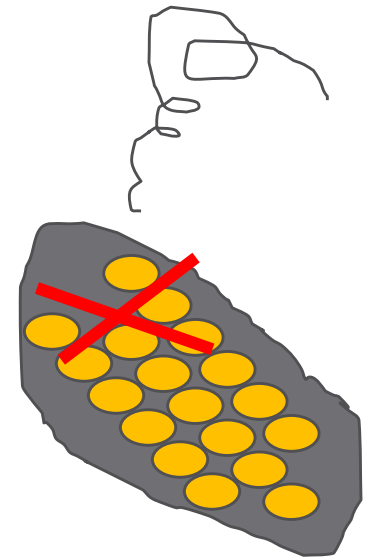
# Spreading for Protein induction

E. coli strain for miniprep...

What's necessary?

Replication

- For protein induction, use different strain...
  - *Why is it necessary?*
    - 1. Transcription
    - 2. Translation
    - One more!
      - 3. Translation regulation factor



# Homework

- Please explain E.coli growth curve with O.D. (right figure)
- Please explain the protein induction mechanism by IPTG

