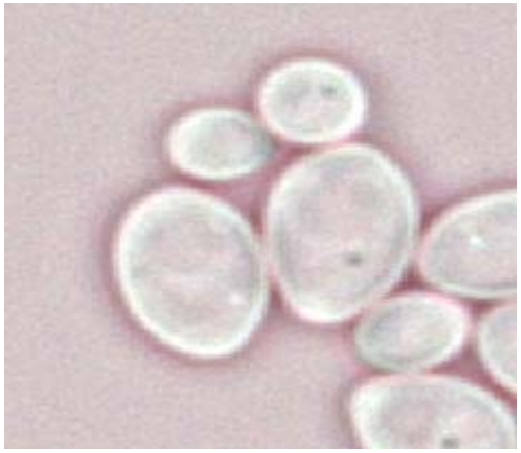
Welcome to Yeast Laboratory



Today's Experiments

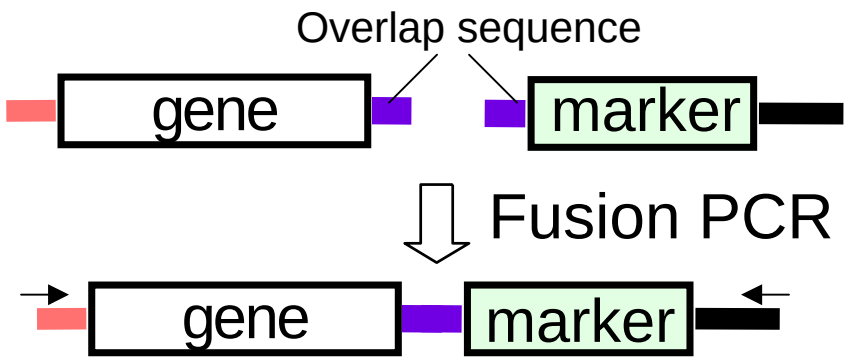
- Experiment I:
  Construction of *TDH3p-TAA-15G-URA3* by using fusion PCR

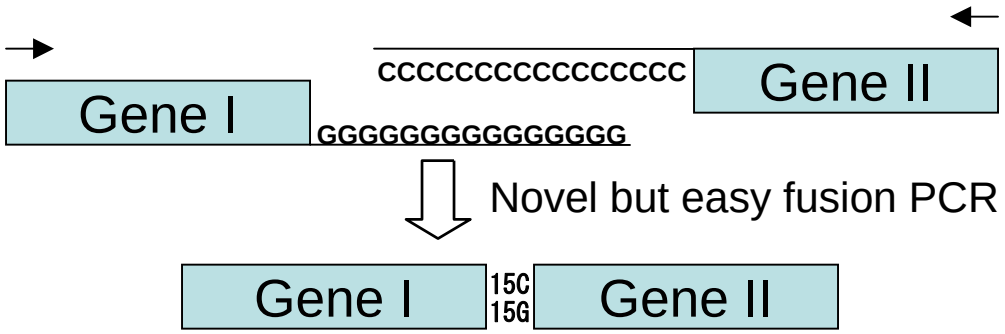
- Experiment II:
  Heterologous protein expression in *Kluyveromyces marxianus*

- Experiment III: Gene disruption in *K. marxianus*

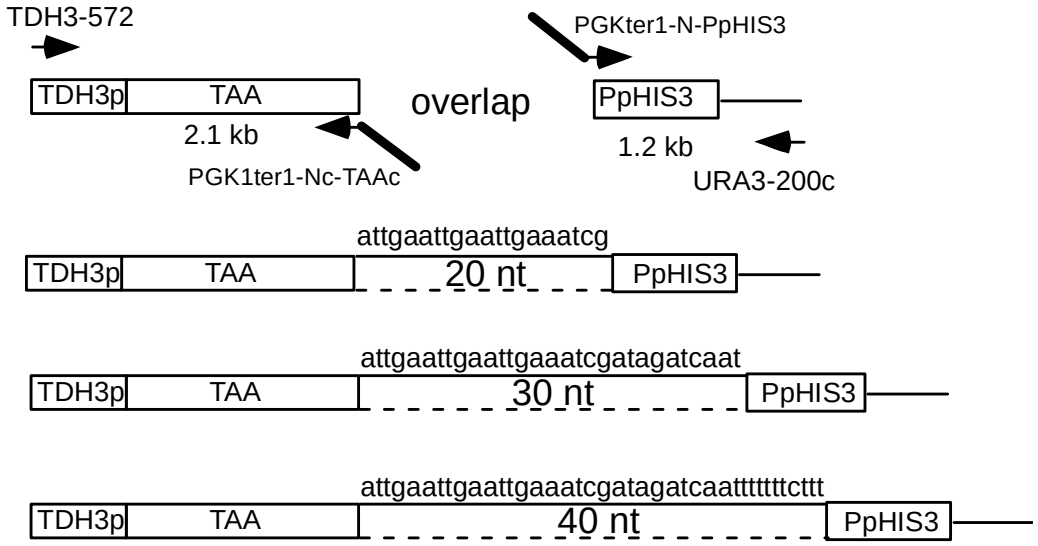
Fusion PCR

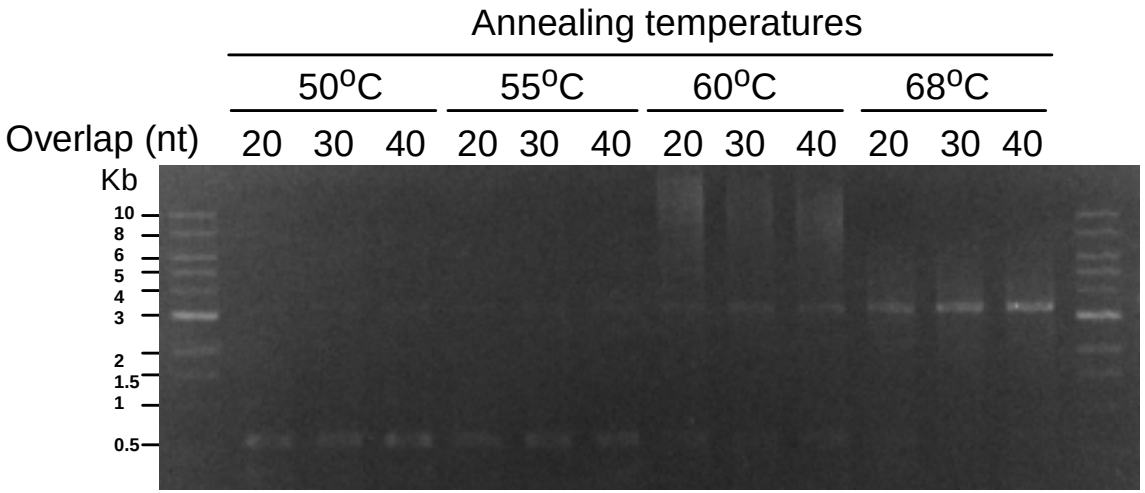
- Very useful PCR technique for construction of recombinant DNA.



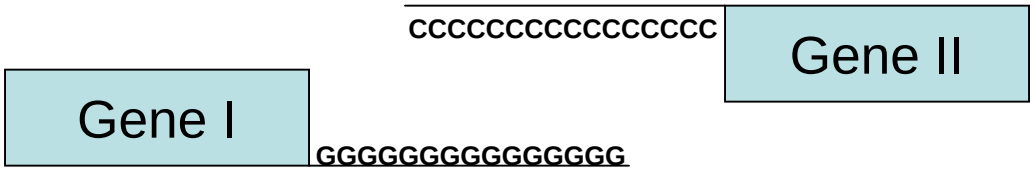


In some cases, fusion by using overlab sequences seems difficult





What sequences are good for overlap extension PCR?



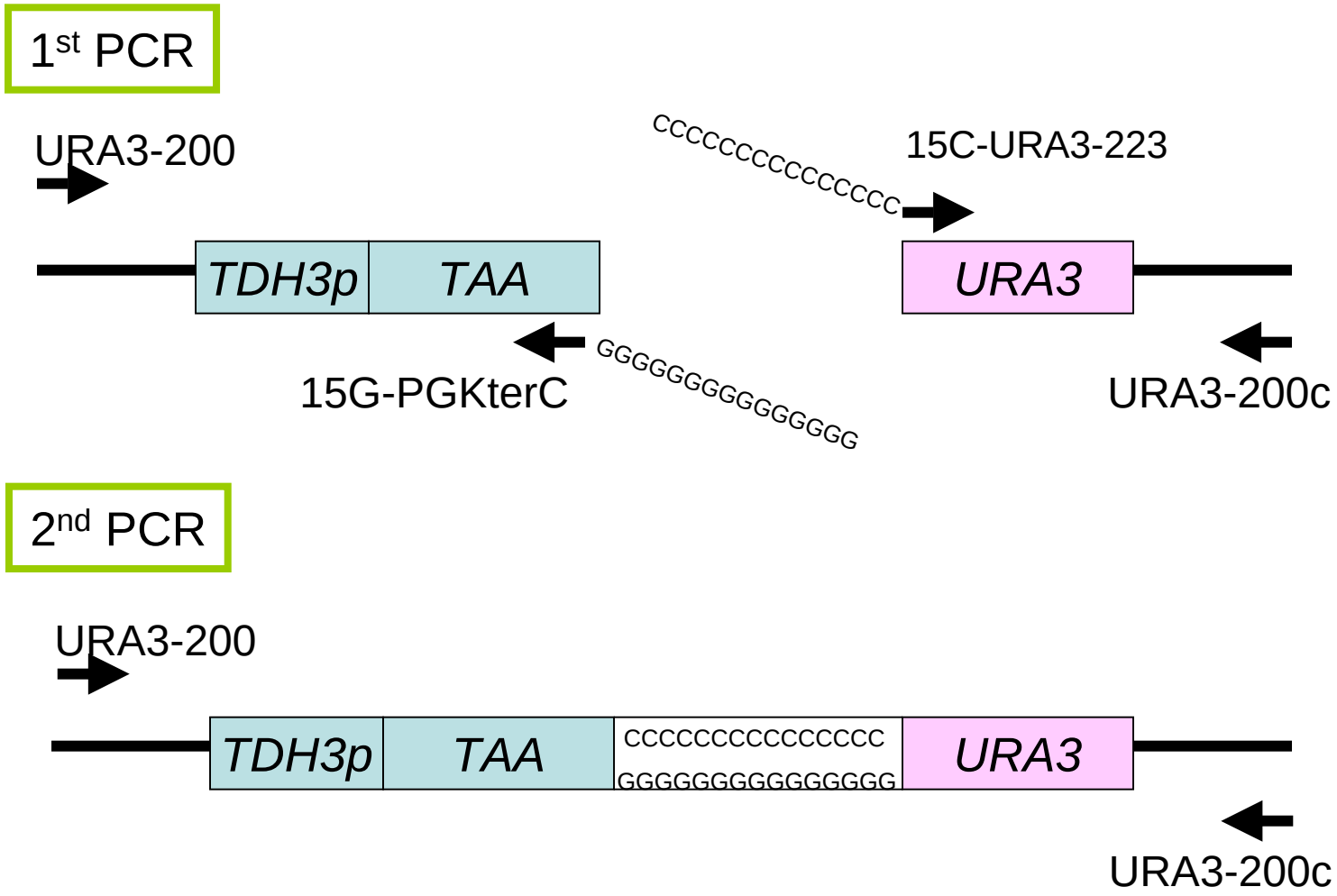
(15G) CCCCCCCCCCCCCCC

(3GCG) GGGCCCGGGCCCGGG

(5GCG) GGGGGCCCCCGGGGG

Kamonchai Cha-aim, Tomoaki Fukunaga, Hisashi Hoshida and Rinji Akada.2009. **Reliable fusion PCR mediated by GC-rich overlap sequences.** Gene. 434(1-2):43-9.

Construction of *TDH3p-TAA-15G-URA3* by using fusion PCR

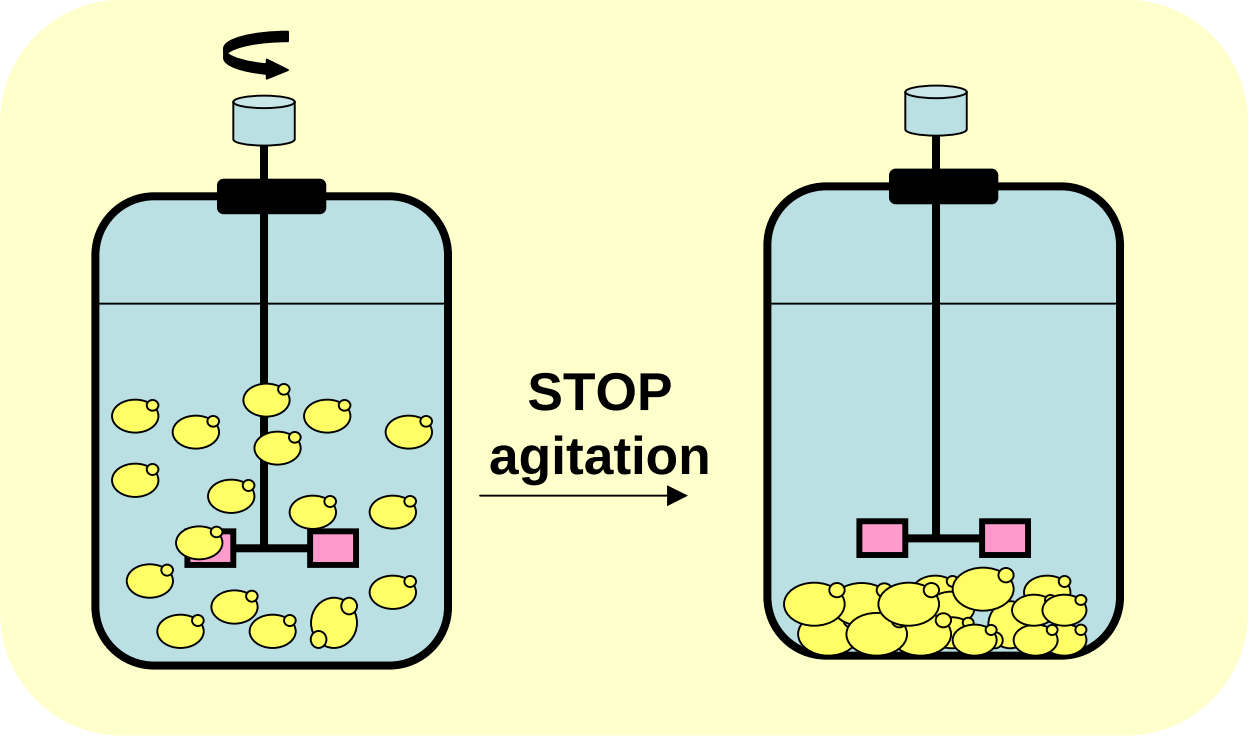


Experiment II:
Heterologous protein expression in *Kluyveromyces marxianus*

K. marxianus
- thermotolerant yeast
- can produce ethanol as well as *S. cerevisiae*
- utilize wide range of carbon sources
- can incorporate linear DNA into their chromosome easily
- can be a host for heterologous protein experssion

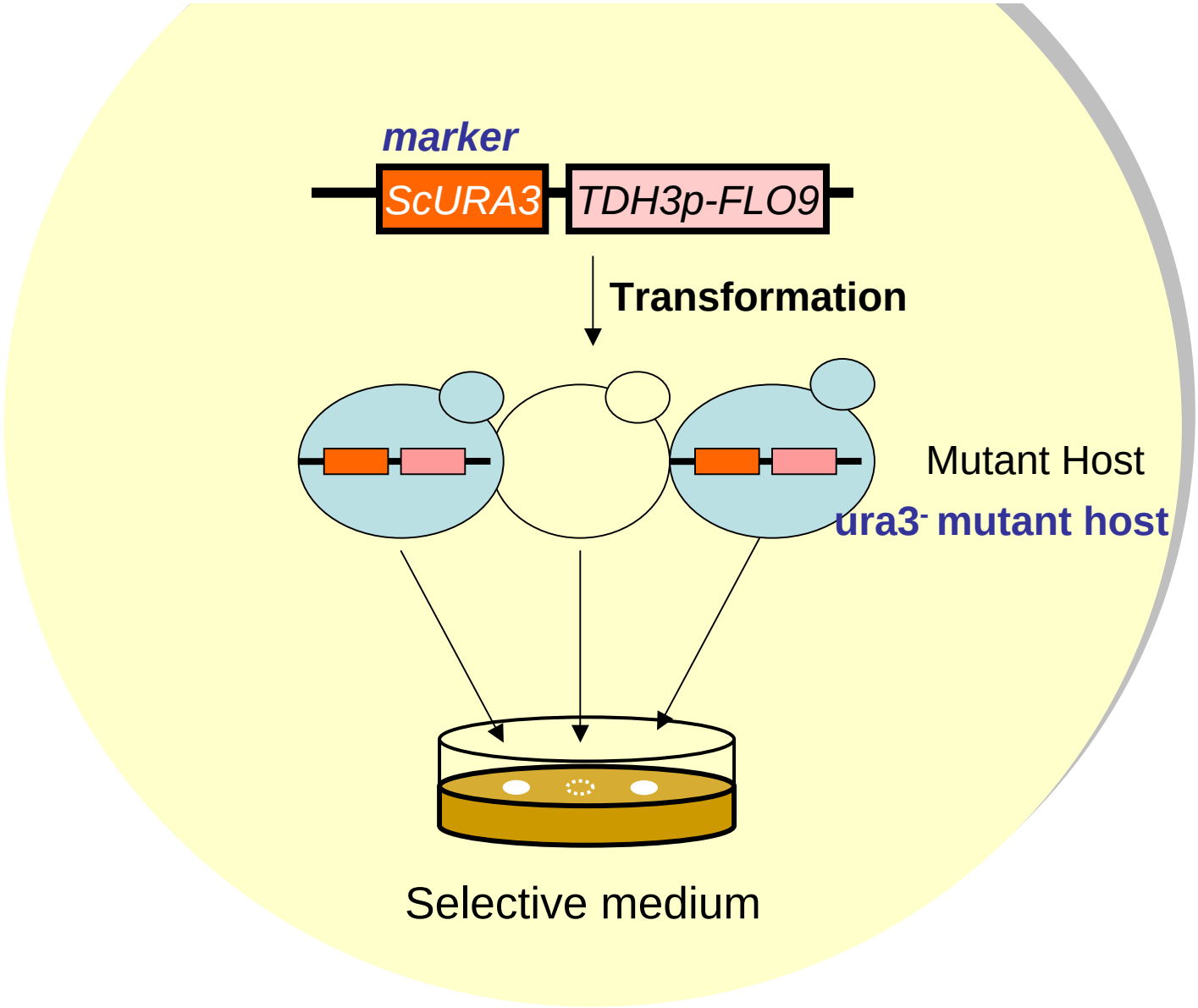
Heterologous protein expression
- Heterologous protein is a protein from other species
- In this experiment we are going to express *S. cerevisiae FLO9*
- The flo9 protein is a flocculin protein that confer flocculation phenotype in yeast

Advantages of flocculent yeast

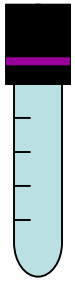


- **Easy and efficient separation process**
- **No energy consumption**
- **No centrifugation process**

How can we introduce gene of interest into yeast cells



Transformation procedure

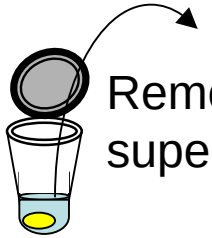


10 ml of cultured cells

Centrifuge 5,000 rpm, 3 min

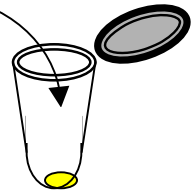
Discard supernatant and add 300 μl of One-step buffer

Transfer cell suspension to 1.5 ml tube and flash spin 5 sec

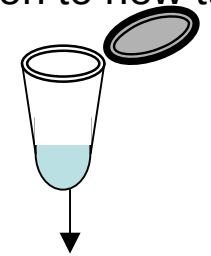


Remove all supernatant

Add 200 μl of One-step buffer



Transfer 100 μl of cell suspension to new tube

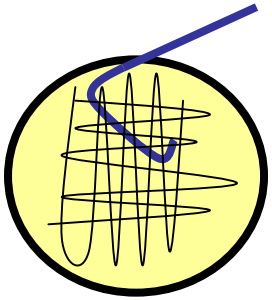


1-5 μl of DNA

Incubated in a 47°C water bath for 15 min

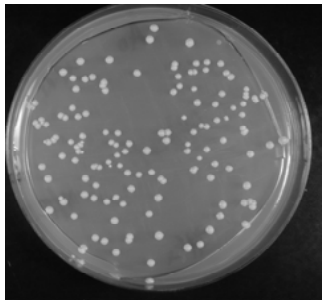
Add 100 μl of -U

Vortex 30s



-U plate

Incubate at 28C, 2-3 days



One-step buffer

• 60%PEG3350	667	333	67
• 4M LiAc	50	25	5
• 1M DTT	100	50	10
• DW	183	92	18
• Total (μl)	1000	500	100

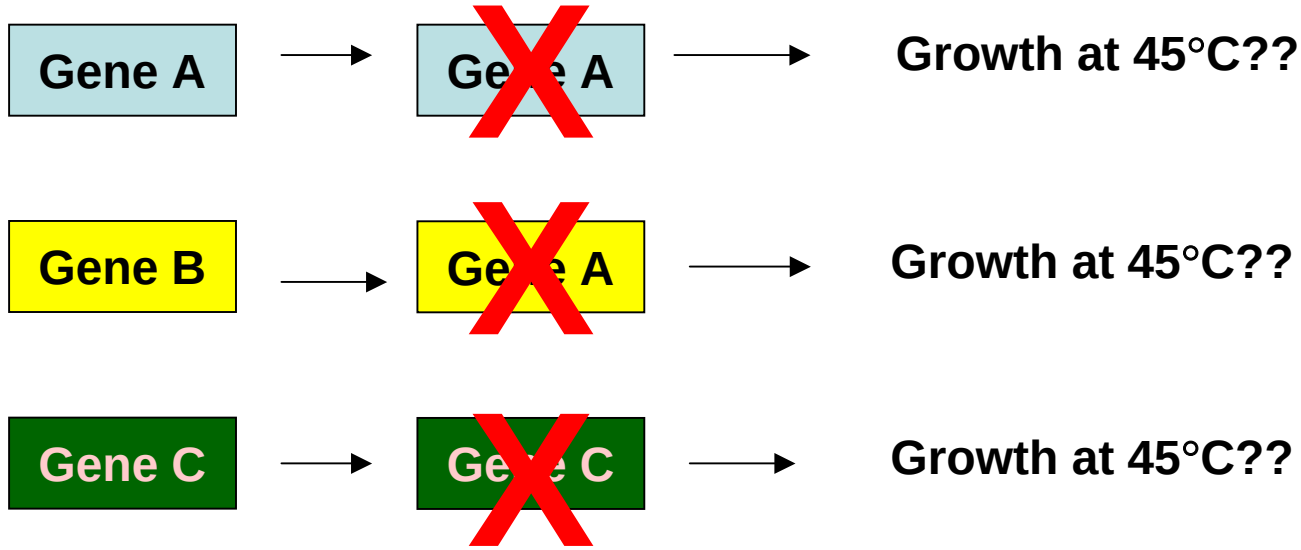
What happened after FLO9 was expressed??

Let’s see on Monday

Experiment III: Gene disruption in *K. marxianus*

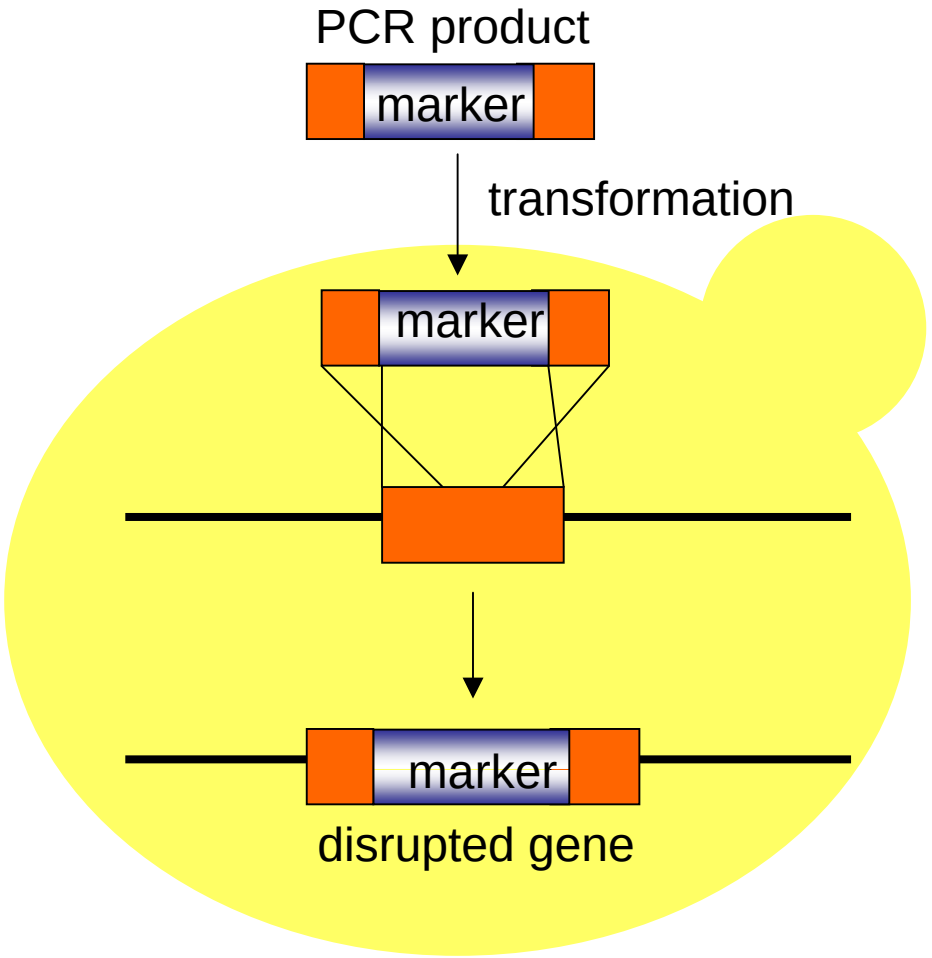
Gene disruption is a useful tool for gene function analysis

Example: study of the genes that involved in high-temperature growth



Gene disruption

How to disrupt gene



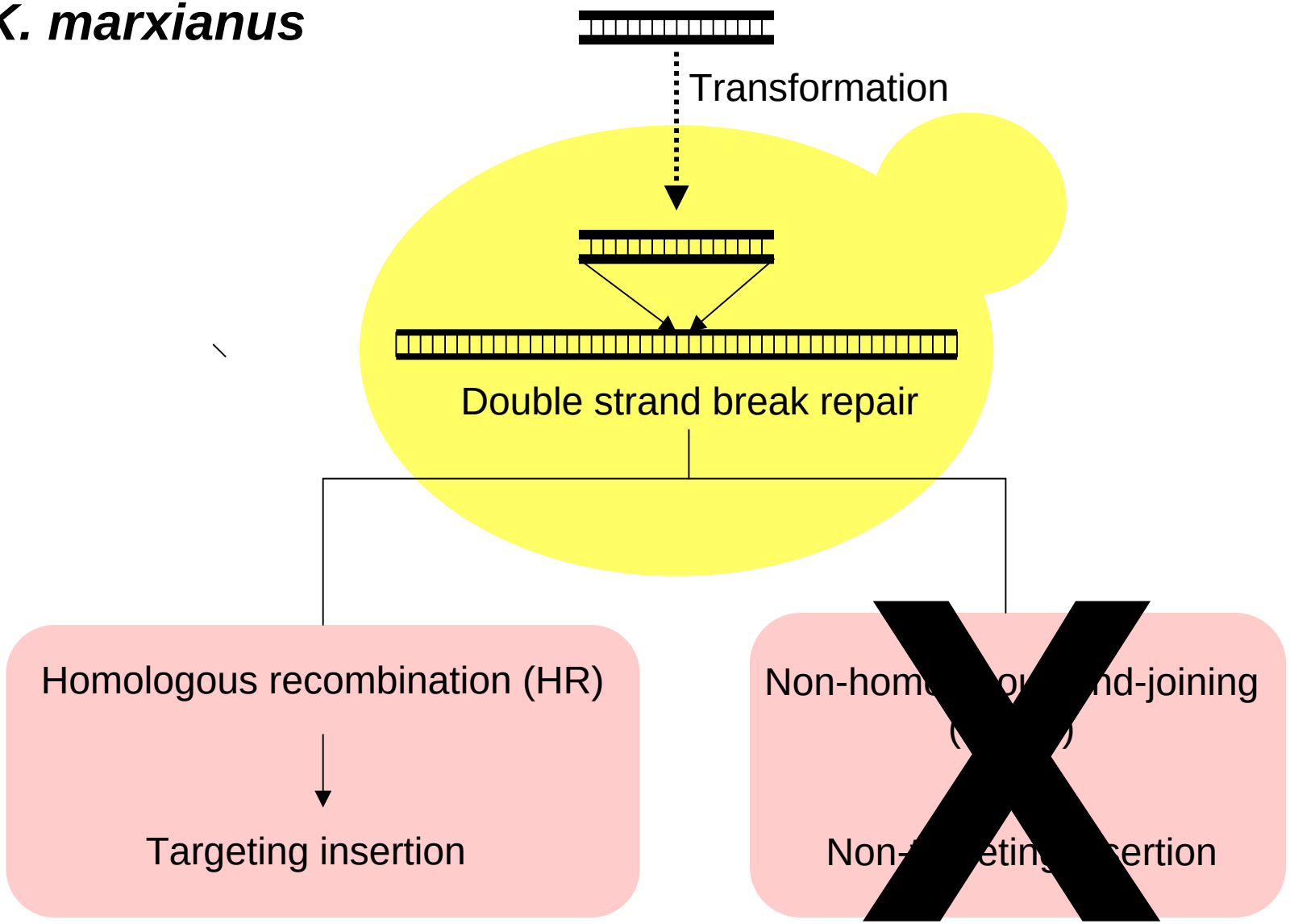
However, the efficiency of gene disruption is depend on the efficiency of homologous recombination in host cell

In our study

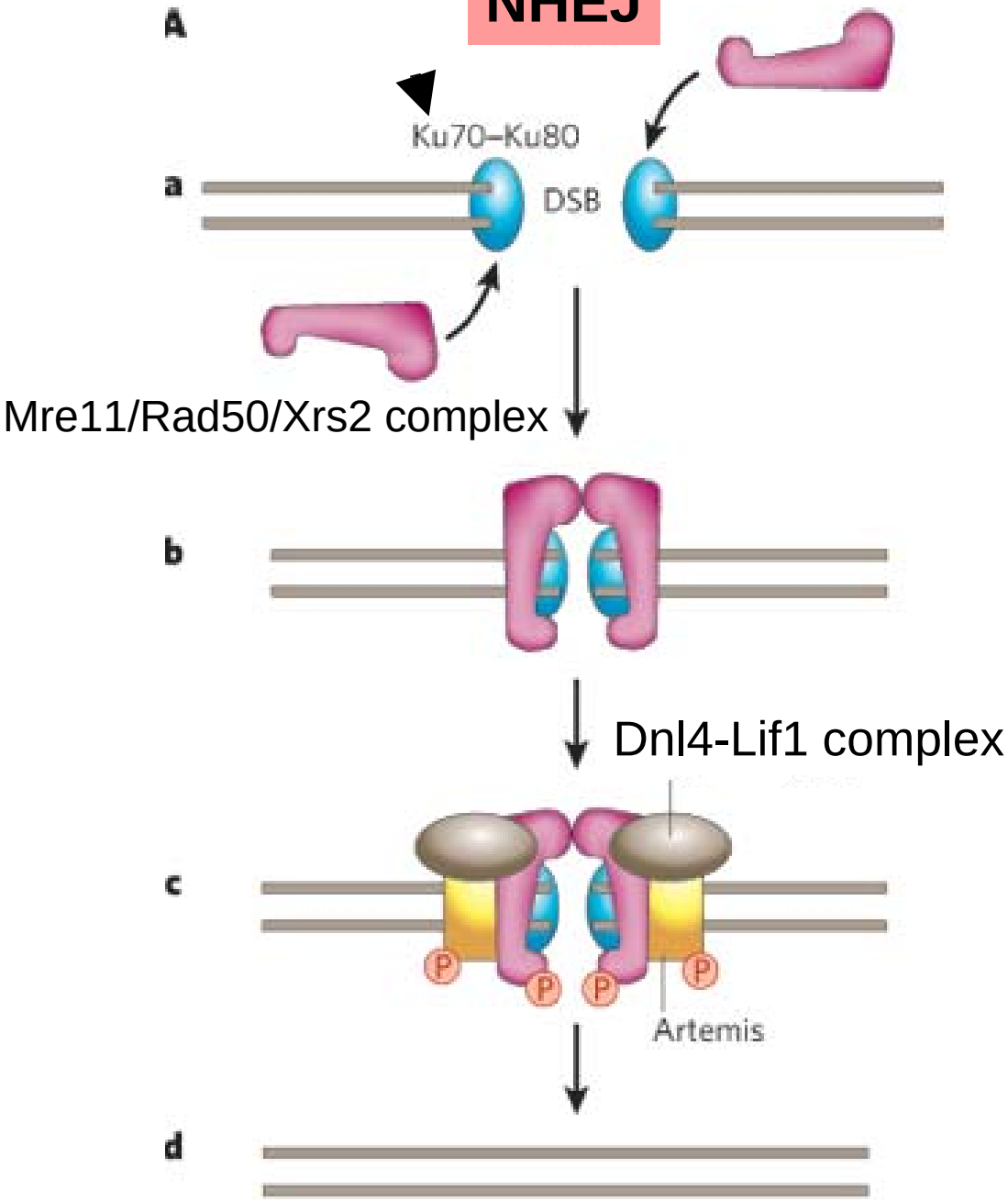
Transformation of linear DNA showed random insertion.

- *K. marxianus* has low efficiency of homologous recombination.
- Indicating that targeting of transformed fragment into desire locus would be difficult.
- How to increase gene targeting efficiency???

K. marxianus


Transformation
Double strand break repair
Homologous recombination (HR)
Targeting insertion

NHEJ



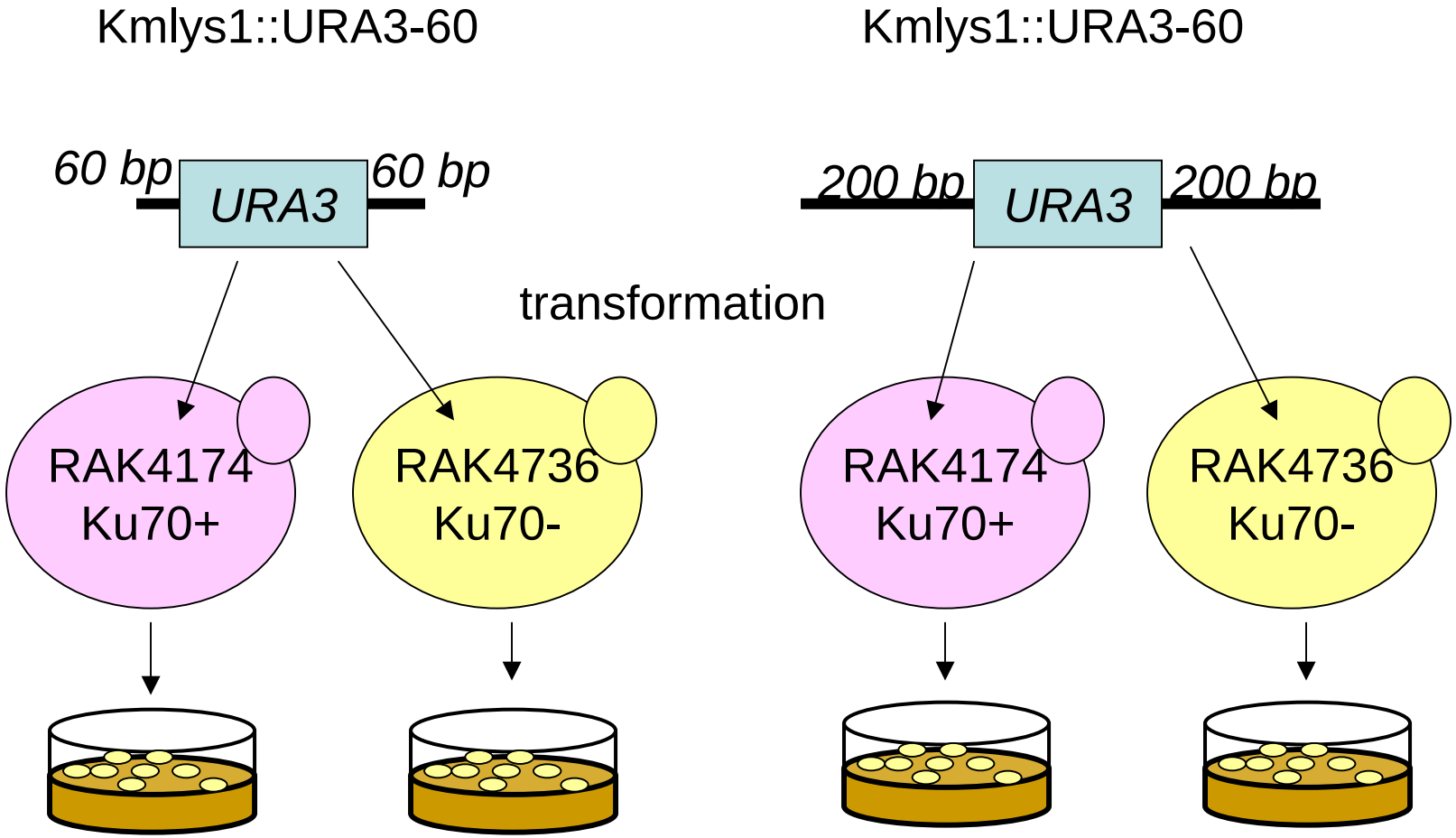
Ku70
Ku80
Mre11
Rad50
Xrs2
Dnl4
Lif1

Objectives of today experiment

- Disrupt Km*Lys1* gene by insertion Sc*URA3* marker into Km*Lys1* locus

- Compare gene targeting efficiency in Ku70+ and Ku70- strains
  - *K. marxianus* RAK4174 (Ku70+)
  - *K. marxianus* RAK4736 (Ku70-)

- Study the effect of homologous length on transformation efficiency
  - Kmlys1::URA3-60 (conc. 110 μg/mL)
  - Kmlys1::URA3-200 (conc. 60 μg/mL)



Count colonies and record and calculate transformation efficiency

$$\text{Transformation efficiency (CFU/}\mu\text{g DNA)} = \frac{\text{Number of transformants}}{\text{Amount of DNA (}\mu\text{g)}}$$

Method to evaluate gene targeting efficiency