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Transforming yeast

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Yeast ORFans CURE

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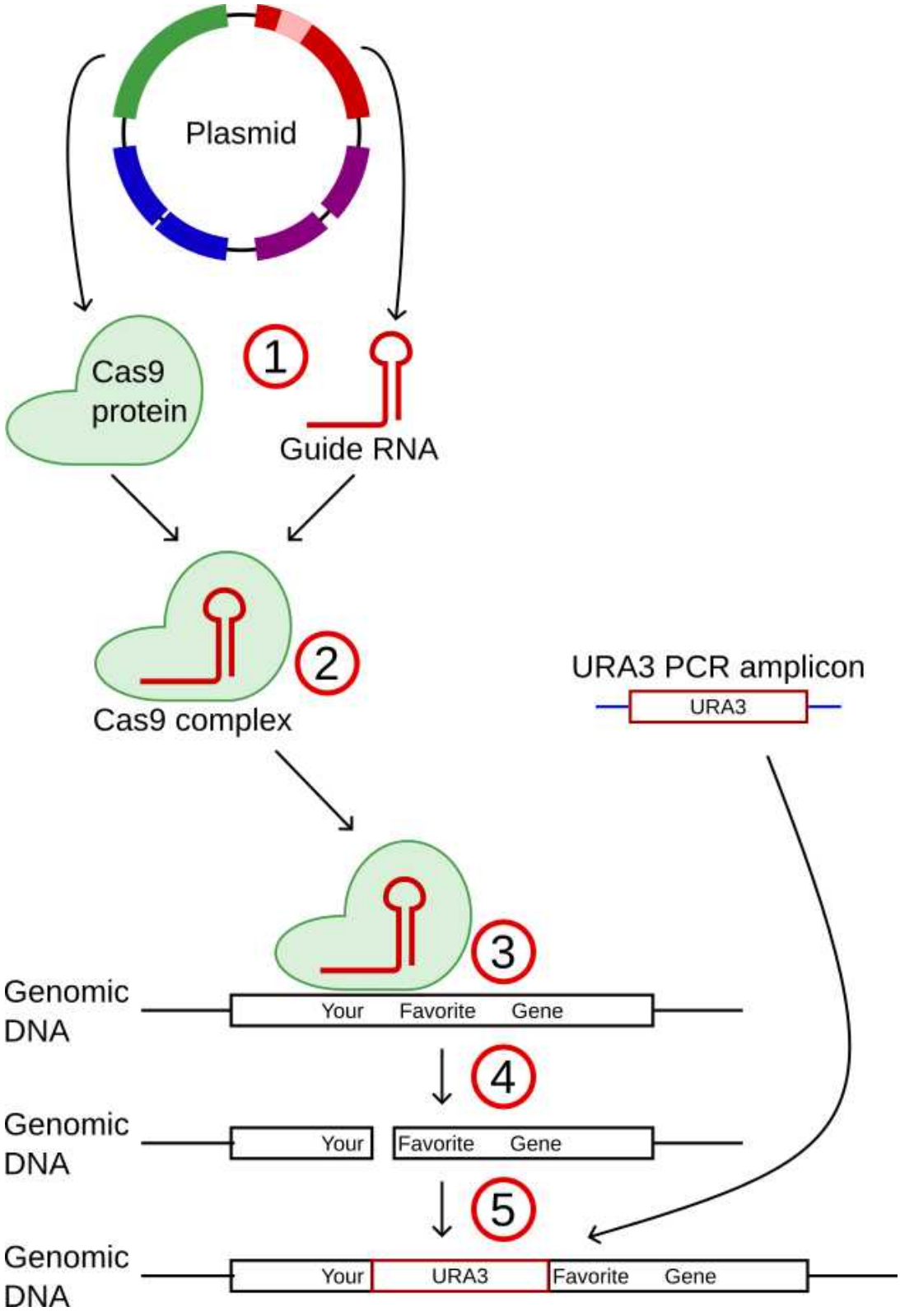
ABSTRACT

In the same way that we transformed E. coli by making them take up a plasmid, we will transform yeast by inducing them to take up both a plasmid (to cut your genomic location) and some linear DNA to repair that location.



PROTOCOL CITATION

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KEYWORDS

yeast, transformation, plasmid, dna

IMAGE ATTRIBUTION

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PARENT PROTOCOLS

In steps of

Transforming Yeast (Instructor Protocol)

MATERIALS TEXT

Equipment

- Hot water bath (or dry bath or incubator) set to **42 °C**
- Vortexer
- Microcentrifuge
- Incubator set to **30 °C**

Materials

- 1 tube of frozen competent yeast cells, stored at **-80 °C**
- Polyethylene Glycol (PEG) 3350 **Electron Microscopy Sciences Catalog #19760** Step 4 solution, **50 Mass / % volume**
- Lithium Acetate Dihydrate **Sigma Aldrich Catalog #L4158** Step 4 solution, **1 Molarity (M)**
- Salmon Sperm DNA **Research Products International Corp Catalog #D52150** Step 4 , **2 mg/mL**
- Plasmid DNA to transform
- URA3 PCR product
- Sterile water
- 1 yeast media plate without uracil
- 1 yeast media plate without leucine
- Glass beads 5 mm **VWR Scientific Catalog #26396-596**

SAFETY WARNINGS

Lithium acetate can cause serious eye and skin irritation. Wear appropriate PPE, including a lab coat, gloves, and safety glasses.

1
- Compute the volume of your Cas9 plasmid that contains **1 µg** of DNA.
- Compute the volume of your URA3 PCR product that contains **500 ng** of DNA.
- If you don't have enough DNA for one or both of these, check with an instructor.

2 Immediately upon retrieving a tube of yeast cells from the **-80 °C** freezer, thaw them rapidly by putting them in the **42 °C** water bath for **00:00:30** 30s

3 Centrifuge **13.000 x g, 00:02:00** . Remove and discard the supernatant. 2m

4 Add the following to the tube of yeast cells in order:

- **260 µL**
  Polyethylene Glycol (PEG) 3350 **Electron Microscopy Sciences Catalog #19760**
  50 Mass / % volume
  Lithium Acetate Dihydrate **Sigma**
- **36 µL** **Aldrich Catalog #L4158**
  1 Molarity (M)
- **50 µL**
  Salmon Sperm DNA **Research Products International Corp Catalog #D52150**
  2 mg/mL
- Enough plasmid DNA to equal **1 µg** of DNA (computed above)
- Enough PCR product to equal **500 ng** of DNA (computed above)

5 Vortex vigorously to resuspend the pellet in the transformation mix.

(This may take from 30 seconds to a minute -- be patient!

6 Incubate in the **42 °C** water bath for **00:30:00** 30m

Use a water bath float so you don't have to stand there!

7 Centrifuge **13000 x g, 00:00:30** . Aspirate the supernatant. 30s

8 Pipette **1 mL** of sterile water into the transformation tube. Stir the pellet with a micropipette tip to break up the cell pellet, then vortex to thoroughly resuspend the pellet.

9 Pour 5-10 glass beads on a **Uracil dropout** plate. Pipette **100 µL** of cells onto it. Shake the plate to spread the cells out.

10 Pour 5-10 glass beads on a **Leucine** plate. Pipette **100 µL** of cells onto it. Shake the plate to spread the cells out.

11 Incubate 48-72 hours **upside down** at **30 °C .**