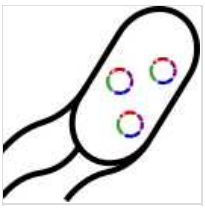

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Transforming E. coli

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Yeast ORFans CURE

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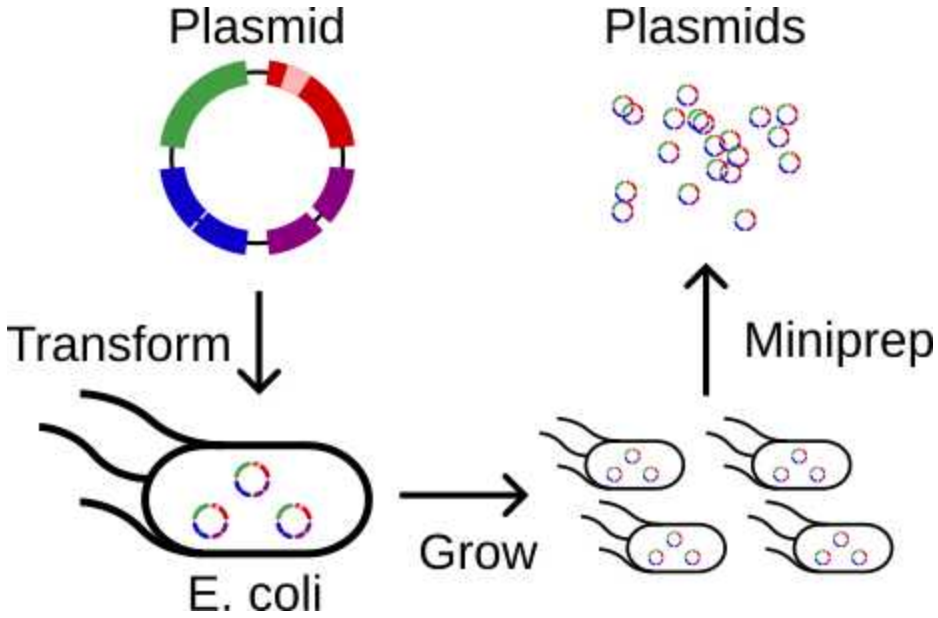
ABSTRACT

Transformation is the process of inducing chemically competent *E. coli* to take up a plasmid. We do this in order to use E. coli as a "DNA copier" -- the bacterium takes up the plasmid, then copies it as it copies its own genomic DNA. We can grow up as many E. coli as we like in a liquid culture -- each of which has 20-50 copies of the plasmid -- then extract the plasmid DNA back out of the bacteria in the culture.



PROTOCOL CITATION

Brian Teague 2022. Transforming E. coli. **protocols.io** https://protocols.io/view/transforming-e-coli-ce4ytgxw

KEYWORDS

E. coli, transformation, plasmid, cloning

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PARENT PROTOCOLS

In steps of

Transforming E. coli (Instructor protocol)

GUIDELINES

Time and temperature are critical parameters here -- make sure to follow the instructions very closely. For example, if the instructions read "heat shock in a water bath at **42 °C** for exactly **00:00:30** ", don't grab your tube out of the ice bucket, wander over to the water bath, get distracted chatting to friends, etc. Instead, bring your ice bucket with your cells to the water bath, put the cells in the water bath for 30 seconds, and then put them immediately back on ice.

MATERIALS TEXT

Equipment

- Water bath set to **42 °C**
- Shaking incubator, set to **200 rpm, 37°C**
- Ice bucket, with ice

Materials

- Ligation to transform
- Positive control plasmid

  SOC Outgrowth Medium - 100 ml **New England**
- **Biolabs Catalog #B9020S** Step 7

  (or homemade SOC)

Swirl the tube of SOC and check to make sure it's clear and **NOT CLOUDY.** It gets contaminated very easily!

  Glass beads 5 mm **VWR**
- **Scientific Catalog #26396-596** Step 10
- LB-Agar + kamamycin plates
- Competent E. coli (stored in the **-80 °C** freezer)

Retrieve the two tubes of chemically competent E. coli cells from the **-80 °C** freezer and place them *immediately* on ice. Incubate the cells on ice 3-4 minutes to thaw.

SAFETY WARNINGS

This protocol creates genetically modified organisms (GMOs) -- in particular, the E. coli bacteria become antibiotic-resistant. Make sure to dispose of contaminated plastics and cultures as instructed.

None of the materials in this lab are hazardous. HOWEVER, we are shedding nucleases -- enzymes that degrade DNA -- all the time. Wear lab coats and gloves to keep your samples nuclease-free.

Transformation 32m 30s

1 Retrieve the two tubes of chemically competent E. coli cells from the **-80 °C** freezer and place them *immediately* on ice. Incubate the cells on ice 3-4 minutes to thaw.

2 Add **1 µL** of DNA from your ligation to a tube of cells. Immediately after adding the DNA to the tube, flick several times to mix, then immediately return to ice.

3 Add **1 µL** of DNA from the transformation control to a *separate* tube of cells. Immediately after adding the DNA to the tube, flick several times to mix, then immediately return to ice.

4 Incubate the cells on ice for **00:30:00** 30m

5 Heat shock the cells for **exactly** **00:00:30** in the **42 °C** water bath. 30s

> Bring your transformations ON ICE to the hot water bath. Move the cells from the ice bucket to the water bath, then BACK to the ice bucket.

6 Incubate the cells on ice for **00:02:00** 2m

7 Add **250 µL**
SOC Outgrowth Medium - 100 ml **New England Biolabs Catalog #B9020S** to each tube.

8 Tape each tube to the platform of an incubating shaker and shake **200 rpm, 37°C, 01:00:00**

Plating 2m

Label the kanamycin selection plates appropriately.

9

There will be a LOT of plates generated by the class -- make sure you can tell which ones are yours and what is on each of them!

I suggest you label the plates *on the bottom*, not on the lid.

10 Glass beads 5 mm **VWR**

Shake ~10 **Scientific Catalog #26396-596** onto each plate.

11 For each transformation, pipette **100 µL** onto the center of the plate.

12 Cover the plates and shake the beads around to spread the cells out.

13 Dispose of the beads by tapping them into the waste container.

14 Incubate the plates **upside down** in an incubator, **37 °C** **Overnight** 2m

Don't incubate for more than 18-24 hours!