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# E. coli transformation

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**Protocol status:** Working
**We use this protocol and it's working**

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## Abstract


- Take tubes with 50 µL of TOP10F′ competent cells from -80°C freezer and place on ice.
- Add 5 µL of each ligation reaction directly to competent cells and mix by tapping gently. **Do not mix cells by pipetting up and down**. Store any remaining ligation at -20°C.
- Incubate cells on ice for 30 min. [while waiting, turn on 42°C water bath]
- Incubate for exactly 30 sec in 42°C water bath. Do not mix or shake.
- Remove vials and place quickly on ice. Store for 2 min on ice.
- Add 500 µL of LB medium (pH. 7.5) to each tube.
- Incubate tubes (taped horizontally to platform) for 60 min at 37°C and 225 rpm.
- Plate 550 µL (variable) of each transformation on LB + AB plates.
- Incubate overnight at 37°C, in the dark.