iGEM Registry Bacterial Transformation

Vinoo,Abigail,Meagan,Tiago Lubiana

Abstract

Protocol for heatshock transforming E.coli competent cells, from strains such as DH5-alpha. These can be made competent in-house or bought from various suppliers.

Transformations are essential to using the DNA Distribution Kits: resuspend the DNA sample in a well, transform the DNA into competent cells, and select single colonies. However, transformations can also be one of the more fickle laboratory techniques.

Read through the entire protocol before starting!

If you have issues with transformations, read through the Troubleshooting page for help.

(adapted from http://parts.igem.org/Help:Protocols/Transformation)

Citation: Vinoo,Abigail,Meagan,Tiago Lubiana iGEM Registry Bacterial Transformation. **protocols.io** dx.doi.org/10.17504/protocols.io.rqsd5we
Published: 16 Jul 2018

Materials

- Resuspended DNA by Contributed by users
- Positive transformation control DNA by Contributed by users
- Competent Cells by Contributed by users
- 1.5mL Microtubes by Contributed by users
- SOC Media by Contributed by users
- Petri plates w/ LB agar and antibiotic by Contributed by users
- Ice & ice bucket by Contributed by users

- Lab Timer by Contributed by users
- 42°C water bath by Contributed by users
- 37°C incubator by Contributed by users
- Microcentrifuge by Contributed by users
- spreader by Contributed by users

Protocol

Step 1.
Get 2 Petri dishes containing solid media with apropriate antibiotics for each construct you want to transform, plus one for your control

Step 2.
Resuspend DNA in selected wells in the Distribution Kit with 10µl of ultrapure water

Step 3.
Label 1.5ml tubes with part name or well location. Fill lab ice bucket with ice, and pre-chill 1.5ml tubes

Step 4.
Thaw competent cells on ice: This may take 10-15min for a 260µl stock.

Step 5.
Pipette 50µl of competent cells into 1.5ml tube. Keep all tubes on ice. Don’t forget a 1.5ml tube for your control.

Step 6.
Pipette 1µl of resuspended DNA into 1.5ml tube

Step 7.
Pipette 1µl of control DNA into the control tube:

Step 8.
Close 1.5ml tubes, incubate on ice for 30min: Tubes may be gently agitated/flicked to mix solution, but return to ice immediately.

Step 9.
Heat shock tubes at 42°C for 45 sec. Place in water bath to ensure the bottoms of the tubes are submerged. Timing is critical.

Step 10.
Pipette 950µl SOC media to each transformation

Step 11.

Incubate at 37°C for 1 hour, shaking at 200-300rpm

Step 12.
Pipette 100µL of each transformation onto petri plates Spread with sterilized spreader or glass beads immediately

Step 13.
Spin down cells at 6800g for 3mins and discard 800µL. Resuspend the cells in the remaining 100µL, and pipette each transformation onto petri plates

Step 14.
Incubate on ice for 5min: Return transformation tubes to ice bucket.

Step 15.
Incubate transformations overnight (14-18hr) at 37°C: Incubate the plates upside down (agar side up).

Step 16.
Pick single colonies: Pick single colonies from transformations: do a colony PCR to verify part size, make glycerol stocks, grow up cell cultures and miniprep.