E. coli Heat Shock Transformation Version 2

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Abstract

This describes a method to transform a plasmid into homemade DH5α cells.

Citation: Alex Rajewski E. coli Heat Shock Transformation. **protocols.io**
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Materials

- Dry Block Heater Z3321 by Promega
- DH5α Competent Cells by Contributed by users
- SOC Media View by Contributed by users

Protocol

Set up
Step 1.

Thaw competent cells on ice. This takes about 10 minutes. Also thaw the plasmid to be transformed.

Set up
Step 2.

Warm SOC media to room temperature.

AMOUNT
250 µl Additional info: SOC media per reaction

NOTES
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LB media can also be used. Anecdotally, we haven't seen a difference between LB and SOC.

Set up
Step 3.

Heat a dry block to 42ºC, a shaker-incubator to 37ºC, and a cabinet-style incubator to 37ºC.

Set up
Step 4.

Label a 1.5mL eppendorf tube for each transformation reaction with the construct name and place it on ice. Label an agar plate with the construct, date, your initials, and plate media type (with antibiotic, if applicable).

EXPECTED RESULTS

Plate: pYPQ131A 13 March 2018 AR LB+Tet

Heat Shock

Step 5.

Add plasmid to the labeled eppendorf tube on ice.

AMOUNT

2 µl Additional info: plasmid

Heat Shock

Step 6.

Add thawed competent cells to the tube with the plasmid and gently swirl with the pipet tip to mix.

AMOUNT

45 µl Additional info: competent cells

NOTES

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DO NOT vortex. Competent cells have very specific and fragile cell membranes that can be damaged by vortexing.

Heat Shock

Step 7.

Incubate the mixture on ice for 30 minutes.

NOTES

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This time is fungible. Longer is better, but as short as 5 minutes will also work.

Heat Shock

Step 8.

Place the mixture in the dry block at 42ºC for 30 seconds

Heat Shock

Step 9.

Incubate the mixture on ice for 2 minutes.

Grow Up

Step 10.

Add SOC to the mixture and shake in an incubator

AMOUNT
250 µl Additional info: SOC per reaction

Grow Up

Step 11.

(Optional) Spin the bacterial broth for 30 seconds at 6000 rpm, removed 180µL of supernatant, and gently resuspend the pellet in the remaining media to concentrate.

Plate

Step 12.

Immerse a plate spreader in 70% ethanol to sterilize and then burn off the excess alcohol.

Plate

Step 13.

Pipet at least 100µL of the broth onto the labeled agar plate and spread it evenly across the surface of the agar with the sterile spreader. Restilerize the spreader.

NOTES
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A larger volume can be used, or you can make two plates with different volumes (e.g. 100 and 50µL) to prevent getting colonies that are too close together.

Plate

Step 14.

Place the prepared plate (lid-down) into a 37ºC incubator overnight (16 hours)

NOTES
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The plates can also be incubated at room temperature for several days (the weekend).

Warnings

Competent cells and any materials that touch them should be disposed of in the biohazardous waste container.