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Recombineering

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[1]In-house protocol

1 Works for me This protocol is published without a DOI.

Eadewunm

PROTOCOL CITATION

Elizabeth Fozo 2020. Recombineering. **protocols.io** https://protocols.io/view/recombineering-bpzhmp36

LICENSE

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DISCLAIMER:

BEFORE STARTING

Template: if you are using pKD4 (kan cassette), you can use 200 ng of DNA for transformation. Plate on Kan 20 ug/mL plates (lower than normal). Pick to Kan 30 plates to verify really resistance.

Dr. Fozo has not had an issue obtaining transformants on Kan 30 initially: only problematic if a weak RBS is driving Kan.

Be careful not to overgrow cells: we have seen a decrease in efficiency if the cells start approaching an OD 600 of 0.7 before you induce at 42.

If you are not using Kan 20 plates, use more DNA, and rescue for 2 hours.

Storing transformation mix: some people find that leaving the mix on the bench overnight increases the number of positive transformants when they plate out the next day.

Template

1 Prepare a PCR product. Typically, 200-600 ng/transformation. See note below. If using a plasmid template, may have to digest using DpnI or another enzyme to remove the additional plasmid

2 Following purification, combine PCR products and ethanol precipitate. Resuspend products in water

I usually combine 2-4 PCR products into 5 ul and use 2.5 ul for a first recombineering attempt.

Strains

3 Grow up 25 ml of a strain of choice at 30 or 32 degrees (do NOT use DY330. Use NM400, NM1100, or some such derivative).

4 When OD600 approximately 0.4, transfer to 42 degrees shaking water bath for 15 – 20 minutes.

5 After 15-20 minutes, place the flask in an icy slurry and swirl. Keep in icy slurry for about 2 minutes.

6 Spin at 4 degrees, 10 minutes, 4150 rpm.

7 Wash cells with 1 ml cold 10% glycerol at 4 degrees, three times

8 Resuspend cells in **100 µl** cold 10% glycerol.

9 Electroporate: use **50 µl** of cells + DNA. Immediately after electroporation, add 950 ul of SOC. Shake at 30 degrees for 1 hour. Plate 1/10 onto one plate, and concentrate the remaining 900 ul and plate to a second plate.