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SLIC Protocol

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1 Works for me dx.doi.org/10.17504/protocols.io.6t3heqn

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

M. Z. Li and S. J. Elledge, "Harnessing homologous recombination in vitro to generate recombinant DNA via SLIC," Nature Methods, vol. 4, no. 3, pp. 251-256, 2007.

1 Measure the DNA concentration (ng/ml) of each assembly piece.

2 Add 1 mg of each assembly piece (including the linearized vector backbone) to a separate 20 ml chew-back reaction mixture as follows:

1 mg assembly piece
+ 0.1 ml 5 U/ml T4 DNA polymerase
+ 2 ml 10X Promega ligase buffer
+ _____ dH_2O to
20 ml

3 Incubate the chew-back reactions at room temperature for 30 minutes (optimal for 20 bp overhangs). Arrest the chew-back with the addition of 2 ml 10 mM dCTP, and place on ice.

4 On ice, add 100 ng of the chewed-back linearized vector backbone (still in the chew-back reaction mixture) and equimolar amounts of the other chewed-back assembly pieces (also still in their respective chew-back reaction mixtures) to a 15 ml total volume assembly reaction mixture as follows:

linearized vector backbone (100 ng)
+ each additional assembly piece (to equimolar with backbone)
+ 1X Promega ligase buffer to
15 ml

5 Incubate the assembly reaction at 37 C for 30 minutes, and then place on ice.

6 Transform 5 ml of the assembly reaction into 100 ml of competent E. coli and/or run a diagnostic agarose gel to check for successful assembly.

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