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SLIC Protocol

In 1 collection

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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.

Protocol status: Working
We use these protocols in our group and they work.

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Troubleshooting

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.