AUG 27, 2019

SLIC Protocol

In 1 collection

N.J. Hillson^{1,2}

¹JBEI; ²LBNL

Michael J Fero

OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.6t3heqn

Protocol Citation: N.J. Hillson
2019. SLIC Protocol.

protocols.io

<https://dx.doi.org/10.17504/protocols.io.6t3heqn>

MANUSCRIPT CITATION:

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.

License: This is an open
access protocol distributed
under the terms of
the [Creative Commons
Attribution License](#), which
permits unrestricted use,
distribution, and reproduction
in any medium, provided the
original author and source are
credited

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

Created: Aug 26, 2019

Last Modified: Aug 27, 2019

PROTOCOL integer ID:

27227

- 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
+ _____ dH₂O 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.