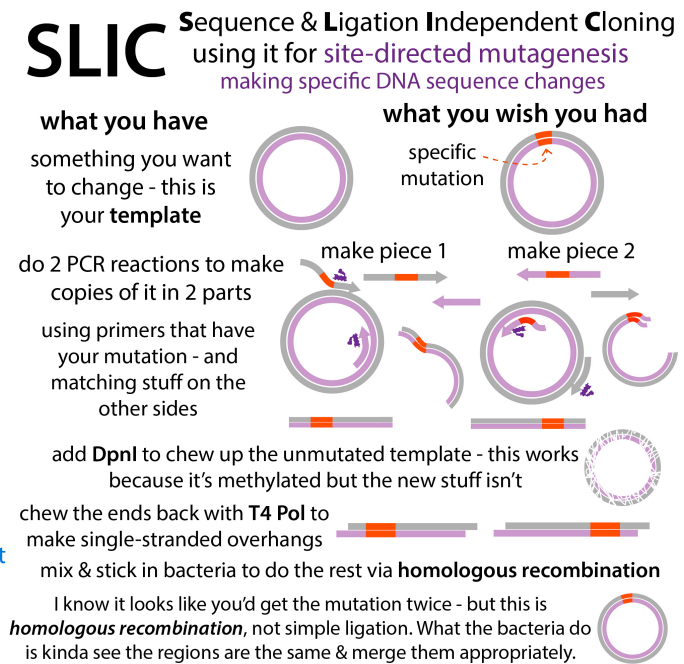
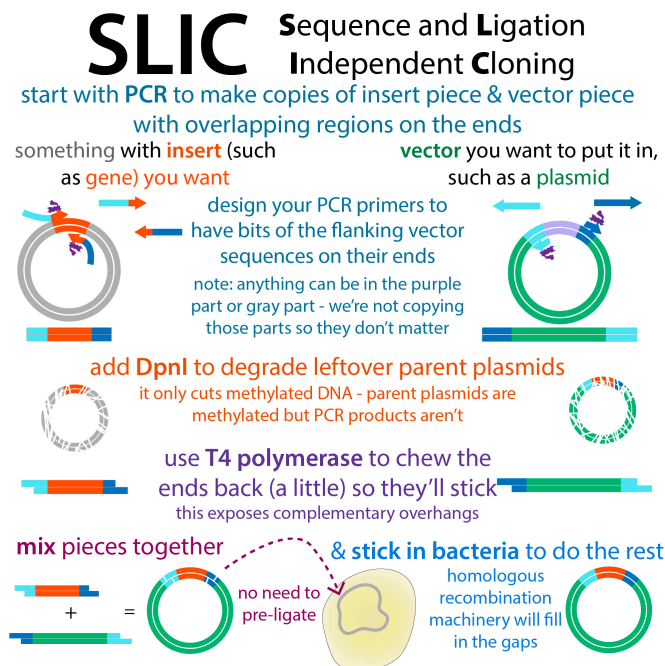


SLIC Cloning Protocol – Bibel lab 2025

Note: adapted from Leemor Joshua-Tor Lab protocol



Design: For typical cloning, you will be doing 2 reactions, one to amplify the vector & one to amplify the insert (using primers that add on sequences which overlap with the vector)

- Design your primers so that your products will have 15-20 nt of overlapping sequence
 - For your **vector**, you can make “generic” vector-specific primers starting at the insertion sites -> can use to amplify just the vector, which you can then SLIC with various inserts
 - These should be ~18-23 nt long, follow typical primer guidelines
 - Check with a tool like amplifX to make sure they don't have strong secondary structure, off-target binding, etc.
 - If they do, try shortening or lengthening (from the vector-side-end) to see if you can improve them
 - For your **insert**, make primers that are ~40 nt long, with 15-20 nt matching insert & 15-20 nt matching end of vector
 - Check and optimize as above
 - Note: the T_m calculated by the software will be misleading since only part of the primer will be bound

PCR reaction:

- This part isn't SLIC specific, so use whatever you're comfortable with, but make sure the polymerase has high fidelity – I typically use Phusion (the High Fidelity (HF) master mix version is a super convenient)
- PCR reaction for insert and vector (remember to adjust the elongation time according to the length of the PCR product), template: 1-10ng
 - I usually use Phusion polymerase, 50 µL total
 - For Phusion, use 15s elongation per kb

PCR Set-up

Details will vary and may need to be optimized for your individual reactions. For example, you can add 1 μL DMSO if you're having problems with low yield and/or nonspecific products. In that case, be sure to adjust the amount of water accordingly.

If using Phusion HF MM, 50 μL reaction

50 μL rxn	
2X Phusion HF MM	25
F primer (10 μM)	2.5
R primer (10 μM)	2.5
template (10 ng/ μL)	1
ddH ₂ O (ultra-pure, nuclease-free water)	19

Make MM of all but template & primers

Add 44 μL MM to template & primers in PCR tubes

If using Phusion HF (not MM)

50 μL rxn	
5X Phusion HF Buffer	10
10 mM dNTPs	1
F primer (10 μM)	2.5
R primer (10 μM)	2.5
template (10 ng/ μL)	1
Phusion Polymerase	0.5
ddH ₂ O (ultra-pure, nuclease-free water)	32.5

Make MM of all but template & primers

Add 44 μL MM to template & primers in PCR tubes

Notes regarding DNA resuspensions and dilutions: I typically do my resuspensions and dilutions in nuclease-free water, but you could also do in a buffer like 10 mM Tris-HCl, pH 8.0.

I like to resuspend my primers to 100 μM . This simplifies calculations. Add 10 μL water or buffer per nmol dried primer for 100 μM .

I like to pre-dilute my templates all to 10 ng/ μL . This simplifies calculations and use of MasterMix. If your template has a very low concentration, you may need to adapt.

PCR Reaction

Although the anneal temp may need to be adjusted for your specific reaction, 68°C is often a good starting point (remembering that the T_m calculated by software will likely be misleading since only part of the primer will be bound). Optimize as needed (increase if non-specific product formation, decrease if no product formation).

<u>Cycle step</u>	<u>Temp (°C)</u>	<u>Time (min:s)</u>	<u>Cycles</u>
Initial denaturation		98 1:00	1
Melt		98 0:10	35
Anneal	68 (or adjusted for T_m)	0:10	
Extend	72	15s/kb	
Final extension		72 10:00	1
Hold (optional: convention says 4-10C, but it isn't needed according to many)	(4-10)*	∞	1

*Source for not needing to hold it cold: <https://www.minipcr.com/four-degree-myth-pcr-stability/>

Agarose gel electrophoresis to check PCR products

- Check the PCR product on a gel (about 3-4 ul)
- A 1% agarose gel is typically good

Dpn I digestion to degrade parent plasmids

- about 2-4 hours (ideally overnight) at 37°C
 - convenient to stick PCR strip in makeshift holder (taped-together pipet tip box inserts) and put in plate incubator
- 1 ul DpnI per 50 ul PCR

PCR Purification/DNA clean-up kit

- Follow kit instructions (there are lots of different kits, qiaquick PCR Purification, Zymo DNA Clean & Concentrate, etc.)
- Elute with 20 ul water

Measure DNA concentration

- Measure the DNA concentration of vector and insert (should be about 50-100 ng/ul)

T4 reaction:

Prepare T4 DNA Polymerase by diluting to 0.5 U/ μ L. For standard NEB T4 DNA Pol, this means diluting it 1:6 in 1x NEB r2.1 (e.g. 1 μ L T4 DNA Pol + 5 μ L 1X NEB2). Note that you will also need to prepare 1X NEB r2.1 from the 10X stock for this dilution)

10x NEB r2.1	2.5
0.5 U/ul T4 DNA polymerase (dilute standard NEB enzyme 1:6 in 1x NEB r2.1)	1.0
DNA (vector or insert, no matter what concentration)	20
Water	2.5
	26.0

- 30 min @ RT
- + 1ul 10 mM dCTP (or any other dNTP, just to stop the T4 reaction!)
- 10 min @ 65 °C (Heat inactivation of T4 DNA polymerase)

SLIC reaction:

Vector 150 – 300 ng	
Insert (according to length) 1:1 (e.g. if your vector is 5 kb & your insert is 1 kb, add 5x nanogram-worth of vector for equal molar amounts)	
	10.0

- 30 min @ RT

Transformation:

- Transform E.coli DH5 alpha cells (or similar, such as XL1 Blue) with the whole 10 ul SLIC reaction
- Typically use heat shock w/competent cells
- Protocol varies depending on cells but I... add SLIC reaction to competent cells on ice -> place in 42°C water bath for 40s -> place back on ice -> add 800 µL LB (or SOC) -> recover in thermal shaker @ 37°C for 30 min – 1 hr (30 min for Amp, 1 hr for Kan, Chlor, Strep)

Plating:

- Plate the entire thing (get plate out to prewarm while cells recover)
 - Spin down the tube to pellet the cells (~10K RPM for 1 min) -> resuspend in 100-150µL LB -> plate on plate with correct antibiotics -> allow to dry a little -> flip over and stick in 37°C plate incubator overnight -> check for colonies in the morning

Resources:

Calculation set-up spreadsheet: https://lmu0-my.sharepoint.com/:x/g/personal/bri_bibel_lmu_edu/EQSCIWehvDxCnCgJ-BZjc9QBFkJOEfCQ6hK6r9c4Wy-KQ?e=0YnlCB

More on SLIC: blog: <https://bit.ly/SLICworkflow> ; YouTube: https://youtu.be/Wy_kCoyBdZc

More on molecular cloning: <http://bit.ly/molecularcloningguide> & <https://youtube.com/playlist?list=PLUWsCDtjESrESdmbna9aUt-VXwaQWMONu>

Here's the original SLIC paper: Li, M.Z., Elledge, S.J. (2012). SLIC: A Method for Sequence- and Ligation-Independent Cloning. In: Peccoud, J. (eds) Gene Synthesis. Methods in Molecular Biology, vol 852. Humana Press. https://doi.org/10.1007/978-1-61779-564-0_5

Here's a nice blog post from Addgene on SLIC: Plasmids 101: Sequence and Ligation Independent Cloning (SLIC), By Mary Gearing, 2015 <https://blog.addgene.org/plasmids-101-sequence-and-ligation-independent-cloning>

Here's a comparison of SLIC, Gibson, and a few other methods: J5 manual, The SLIC, Gibson, CPEC, and SLiCE assembly methods (and GeneArt® Seamless, In-Fusion® Cloning) <https://j5.jbei.org/j5manual/pages/22.html>

More on PCR: <http://bit.ly/pcrtrain> & <https://youtu.be/GZSLfECgW3Q>

PCR playlist: <https://www.youtube.com/playlist?list=PLUWsCDtjESrGDJ0GTWcdeDBCvBdU7FdUy>

Optimizing PCR reactions: blog: <https://bit.ly/pcrspecificity> ; YouTube: <https://youtu.be/vLauaHFixQs>

Promega has a nice guide on optimizing your PCR: <https://www.promega.com/resources/guides/nucleic-acid-analysis/pcr-amplification/#general-considerations-for-pcr-optimization-6f575242-fe99-4fcb-88c5-b205ad7becc7>

More on agarose gel electrophoresis: <https://bit.ly/agarosegelcompare> & <https://youtu.be/vbuxf3rcMxg>

More on nucleic acid spin columns: <http://bit.ly/spincolumns> & <https://youtu.be/fz2OpjxQKKM>

more on transformation & heat shock: blog: <http://bit.ly/transformheatshock> ; YouTube: <https://youtu.be/3C6X2a7xWVw>