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SLIC Cloning Protocol

The sequence and ligation independent cloning (SLIC) method [1] can be used to subclone genes without restriction digestion of the insert. Unlike the related technique, Ligation Independent Cloning (LIC), SLIC does not require specially adapted vectors. The strategy involves PCR amplification of the insert using specific primers that contain regions of homology to the destination vector (see Figure 1A). Long sticky ends are generated by T4 DNA polymerase, which acts as an exonuclease in the absence of dNTPs. The T4-treated insert and vector are incubated together and then transformed directly into *E. coli* to produce the desired expression construct (see Figure 1B).

Simultaneous assembly of multiple inserts is also possible with SLIC (see Figure 1C), which can be a convenient way of introducing fusion tags, creating chimeras, splice variants, etc. The original literature protocols [1] are quite detailed. Their application to subcloning and 3-way SLIC assembly using CSB vectors is described below.

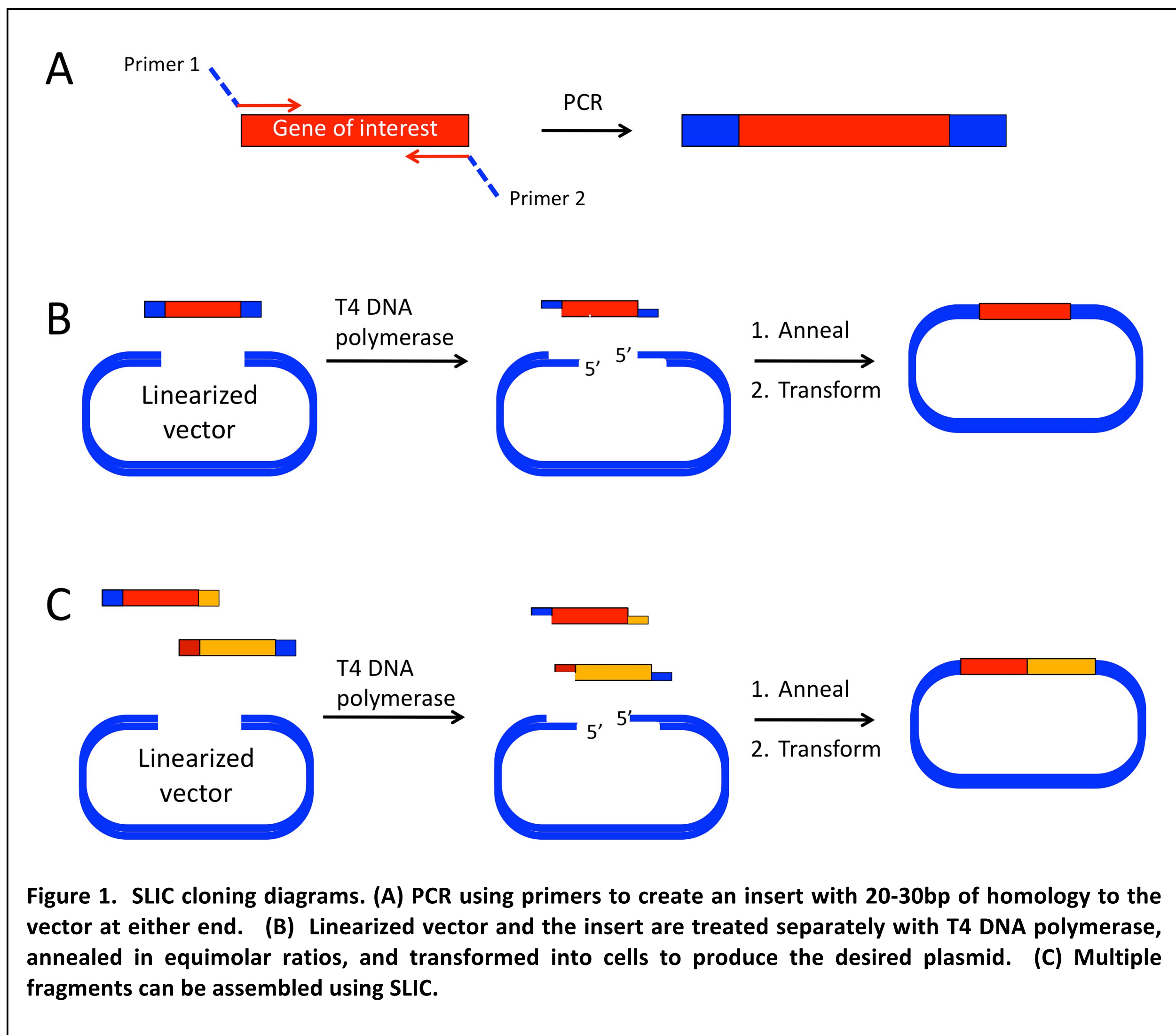

Figure 1. SLIC cloning diagrams. (A) PCR using primers to create an insert with 20-30bp of homology to the vector at either end. (B) Linearized vector and the insert are treated separately with T4 DNA polymerase, annealed in equimolar ratios, and transformed into cells to produce the desired plasmid. (C) Multiple fragments can be assembled using SLIC.

Primer design.

For SLIC cloning, the Li and Elledge paper recommends that insert primers contain 20-30 bp of overlap with the destination vector. We typically use 25bp but have also used 30bp and 20bp using the same volumes and incubation times with good results. All primers were checked using dna analysis software and optimized for stability, annealing temperature, GC clamp, etc.

Example primers for SLIC cloning into BamHI/EcoRI sites of pBG100:

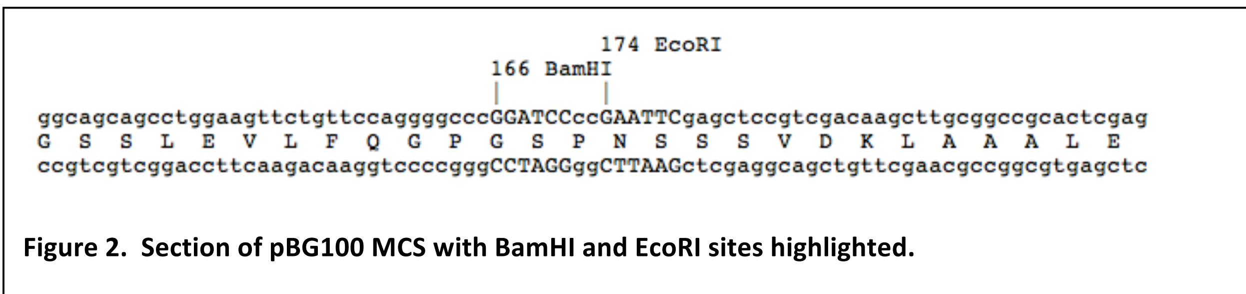

Figure 2. Section of pBG100 MCS with BamHI and EcoRI sites highlighted.

Forward primer (primer 1 in Figure 1A) with 30bp of homology to vector (BamHI site is underlined; gene is in frame with NT fusion tag):
5'-CTGGAAGTTCTGTTCCAGGGGCCCGGATCC*geneofinterest*

Reverse primer (primer 2 in Figure 1A) contained 30bp of homology (EcoRI site is underlined; added stop codon is in bold):
5'-GCCGCAAGCTTGTCGACGGAGCTCGAATTC**TTA***tseretnifoeneg*

NOTE: These primers can be used with other CSB vectors (pAT107b, pAT109, pBG101, pBG102, pBG106, pHD116, pLM302, pLM304, and pNR112) since the dna sequences upstream and downstream of the restriction sites are identical.

SLIC subcloning protocol (1 insert):

I. Set up PCR reaction for the insert using the designed primers. Gel purify the products and quantify DNA.
NOTE: It is important to remove all residual dNTPs, otherwise the T4 polymerase reaction will not work.

II. Perform double digest of vector. Gel purify the double cut vector. Quantify DNA.
NOTE: We typically cut 5ug of DNA. It is important to remove all residual dNTPs. We isolate the double cut vector using a Qiagen gel extract kit, eluting with a minimum of warmed (65°C) elution buffer. It's important to get the vector as concentrated as possible. We found that most of the background in the SLIC reaction comes from uncut vector.

III. T4 DNA polymerase reaction.
Set up separate reactions in 20uL volume for double cut vector and for the insert:

2uL NEB buffer #2 (10X)
500ng DNA
0.2uL BSA (100X)
Nuclease-free H_2O to 19.8uL
0.2uL T4 polymerase (NEB)

1. Mix and leave at room temperature for 30 min.
2. Quench each reaction with 2uL dCTP (10mM).
3. Ice.

IV. Annealing reaction.
Set up reactions in 10uL volume using 150ng T4-treated double cut vector and an equimolar amount of T4-treated PCR insert. Remember to set up a negative control containing vector, but no insert:

1uL T4 DNA ligase buffer (10X)
150 ng T4 DNA pol treated vector
equimolar amount of T4 DNA pol treated insert
Nuclease-free H_2O to 10uL

1. Mix and incubate at 37°C for 30 min.
2. Ice or store at -20°C.

NOTE: We have used 125ng of vector per reaction without problems. According to the Elledge paper, having a higher molar ratio of insert is can improve efficiency. If possible, we will usually set up 3 reactions with 0, 1 and 2 molar equivalents of insert. For ds DNA, to convert µg to pmol: $\mu g \times 10^6 pg/1\ \mu g \times pmol/660pg \times 1/N = pmol$

V. Transformation:

1. Add 2uL of annealing mixture to 25uL XL-1 Blue cells.
2. Ice 30 min.
3. Heat shock 42°C 45 sec.
4. Ice 2 min.
5. Add 225uL SOC medium
6. Incubate 37°C with shaking 1hr
7. Plate all cells on warmed plate w/appropriate antibiotics
8. Incubate overnight 37°C

Do a PCR screen of colonies to identify those containing insert. Miniprep a couple of hits and then have them sequenced. Make sure you sequence the regions upstream and downstream of the insertion sites.

SLIC Assembly Protocol

SLIC allows directional assembly of multiple inserts and vector in a single step (see Figure 3). Li and Elledge claim that as many as 5 overlapping fragments can be assembled simultaneously with high efficiency and 10 can be assembled with reduced efficiency [1]. We've successfully used the technique to assemble a ~2kb insert from 720 and 1200 pieces between the BamHI and EcoRI sites of the CSB vector, pBG100. We've also used it to assemble a concatenated dimeric gene between the NdeI/NotI sites of the CSB vector, pAT107. The published protocol recommends 40 bp of overlap between the PCR fragments, which is introduced in primers 2 & 3 used for PCR (see Figure 3A).

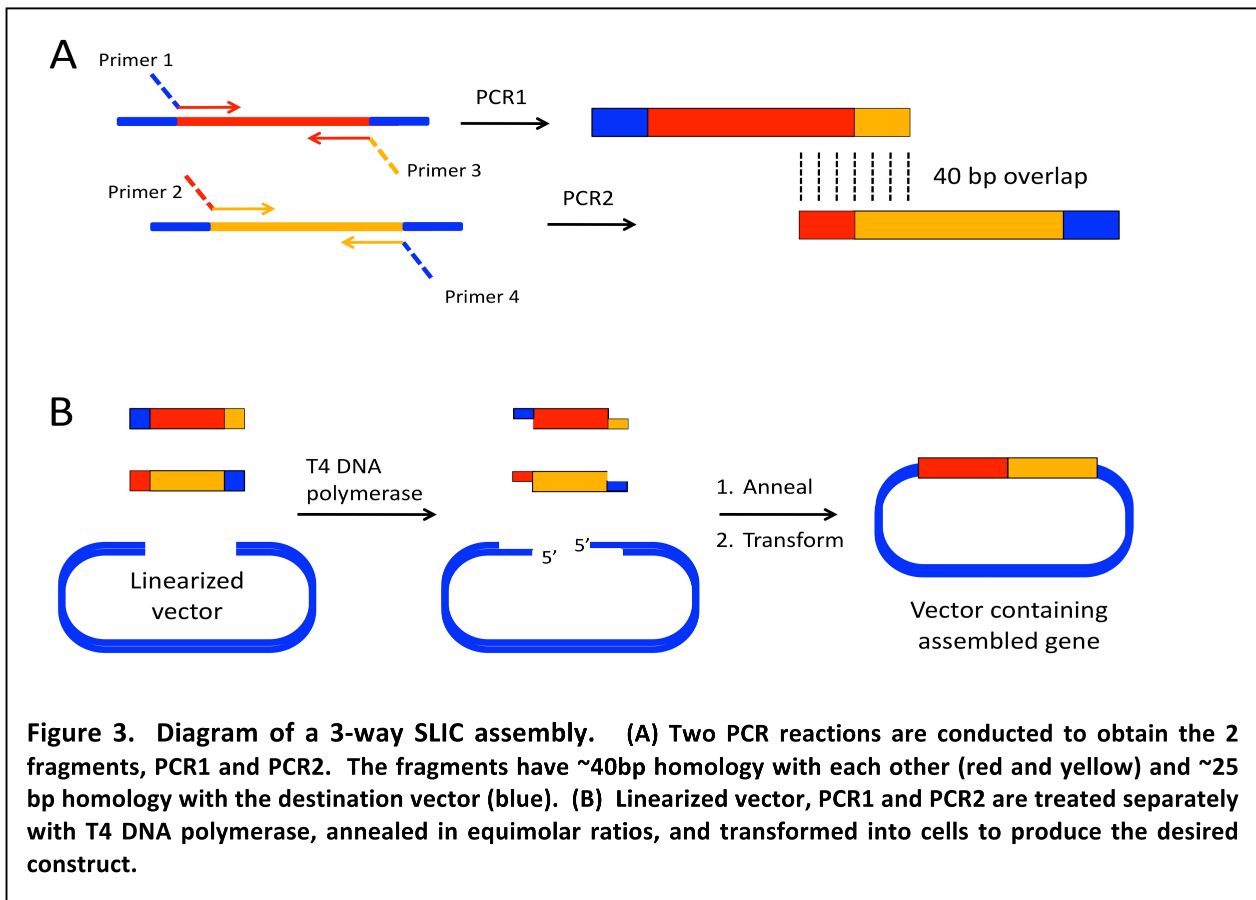

Figure 3. Diagram of a 3-way SLIC assembly. (A) Two PCR reactions are conducted to obtain the 2 fragments, PCR1 and PCR2. The fragments have ~40bp homology with each other (red and yellow) and ~25 bp homology with the destination vector (blue). (B) Linearized vector, PCR1 and PCR2 are treated separately with T4 DNA polymerase, annealed in equimolar ratios, and transformed into cells to produce the desired construct.

For 3-way SLIC assembly, we compared the Li and Elledge protocol to one published by Bieniossek [2] and found that the latter was more efficient, so that is what is detailed below. Steps I, II, and V are the same as above. For the annealing reaction, we used 150ng of vector and 1 molar equivalent of each insert.

For ds DNA, to convert µg to pmol:
$\mu g \times 10^{6} pg/1\ \mu g \times pmol/660pg \times 1/N = pmol$

III. T4 DNA polymerase reaction.
Set up separate reactions in 20uL volume for double cut vector and for each insert. Also run a negative control with vector but no inserts:

2uL NEB buffer #2 (10X)
1uL 100mM DTT
2uL 2M urea
500ng DNA
Nuclease-free H_2O to 19.8uL
1uL T4 polymerase (NEB)

1. Mix and incubate at 23°C for 20 min
2. Arrest by adding 1 µl 500 mM EDTA
3. Heat at 75°C for 20 min

IV. Mixing and Annealing.
Mix 150ng of T4-treated vector with equimolar amounts of T4-treated inserts. Also run a negative control with vector but no inserts:

1. Heat at 65°C for 10 min.
2. Turn off heat block and let mixture cool to RT. This takes ~1.5 hrs.
3. Transform 2uL of cooled annealing mixture into 25uL of XL-1 Blue cells.
4. PCR screen to identify hits, miniprep, send off for sequencing.

NOTE: Our total volumes varied but were kept <20uL.

[1] Li, M.Z. and Elledge, S.J. (2007) Harnessing homologous recombination in vitro to generate recombinant DNA via SLIC. *Nat Methods* 4, 251-256
[2] Bieniossek, C., *et al.* (2009) Automated unrestricted multigene recombineering for multiprotein complex production. *Nat Methods* 6, 447-450