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Plasmid Modification by Annealed Oligo Cloning

Addgene the nonprofit plasmid repository[1]

[1]Addgene

1 Works for me dx.doi.org/10.17504/protocols.io.47tgznn

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ABSTRACT

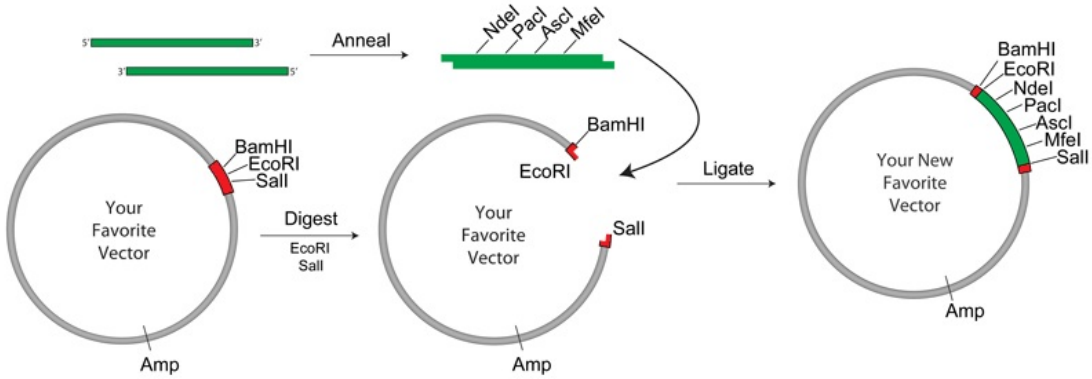
This protocol describes plasmid modification by annealed oligo cloning. To see the full abstract and additional resources, please visit the Addgene protocol page.

EXTERNAL LINK

https://www.addgene.org/protocols/annealed-oligo-cloning/

Designing overlapping oligos

1 Briefly, we will design overlapping oligos that once annealed can be cloned directly into the overhangs generated by restriction digest of existing sites in the original vector.



To add NdeI, PacI, AscI and MfeI sites between the EcoRI and SalI sites of the vector, we design a top oligo with each of the additional sites in tandem (NdeI - CATATG, PacI - TTAATTAA, AscI - GGCGCGCC, MfeI - CAATTG). The bottom oligo will be the reverse compliment so that they can anneal.

Top oligo: 5' - CATATGTTAATTAAGGCGCGCCCAATTG - 3' = 28 bp

Bottom oligo: 3' - GTATACAATTAATTCCGCGCGGGTTAAC - 5' = 28 bp

We also need to include additional bases to complement the overhangs generated when digesting the vector with EcoRI and SalI (see diagram). To do this, we add 5' - AATTC and G - 3' to the top oligo and 3' - G and CAGCT - 5' to the bottom oligo, making our final oligos 34 bp each:

Top oligo: 5' - AATTCCATATGTTAATTAAGGCGCGCCCAATTGG - 3'

Bottom oligo: 3' - GGTATACAATTAATTCCGCGCGGGTTAACCAGCT - 5'

Note, we could leave off the 3' G on each oligo (and the complementary C of the other oligo), but this would destroy the EcoRI and SalI sites in the final vector.

Order the following oligos from your favorite oligo synthesis company:

Top oligo:	5' - AATTCCATATGTTAATTAAGGCGCGCCCAATTGG - 3'
Bottom oligo:	5' - TCGACCAATTGGGCGCGCCTTAATTAACATATGG - 3'

Note, if you plan to phosphatase treat your cut vector it is necessary to use 5'-phosphorylated oligos. This is an option that can be added when ordering them or can be performed enzymatically later.

Digest and Purify Vector

2 While waiting for your oligos to arrive, conduct a restriction digest of **1 µg** of vector with EcoRI and SalI

3 Run an agarose gel and cut out the band containing your vector DNA

4 Gel purify your DNA away from the agarose using a commercially available kit or standard protocol.

Anneal Oligos

5 **Anneal oligos**

The oligos should be resuspended in annealing buffer (10mM Tris, pH7.5-8.0, 50mM NaCL, 1mM EDTA) and mixed in equimolar concentrations. We recommend mixing **2 µg** each in a total volume of **50 µl** - add additional annealing buffer if necessary to get to 50µL. Efficient annealing can be achieved by one of two methods:

5.1 **Method 1**

- Place the mixed oligos in a 1.5mL microfuge tube.
- Place tube in **90 °C** - **95 °C** hot block and leave for **00:03:00** - **00:05:00** .
- Remove the hot block from the heat source (turn off or move block to bench top) allowing for slow cooling to **Room temperature** (~ **00:45:00**).

5.2 **Method 2**

- Place mixed oligos in a PCR tube.
- Place tube in a thermocycler programmed to start at 95 °C for 00:02:00 .
- Then, gradually cool to 25 °C over 00:45:00 .

Ligitation

6 Dilute 5 µl of annealed oligos with 45 µl nuclease-free water and quantify the concentration (should be about 8ng/µl).

7 Mix the annealed oligos with cut vector in molar ratios (vector:insert) between 4:3 and 1:6 in a standard ligation reaction (ex. to ligate an annealed oligo insert of 50bp in length into a 5kb vector, mix 100ng of the vector with 750 µg - 6 ng of annealed oligos).

8 Transform 2 µl - 3 µl into your favorite competent bacteria and plate.

9 Be sure to pick multiple colonies for mini-prepping and verify insert by sequencing.