

# Protocol for Cloning DNA Oligo Cassettes into Plasmid Vectors

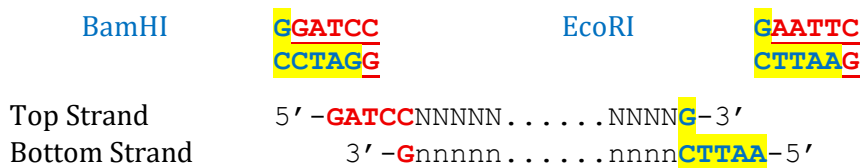
(Commented by TCH @ 06/21/2025)

## A). PURPOSES

- 1). To add more restriction enzyme sites for cloning.
- 2). To change the order of existing enzyme sites for easier cloning manipulations.
- 3). To incorporate specific short sequences to desired locations.

## B). TYPICAL DNA OLIGO CASSETTE DESIGN

(e.g., BamHI ---- EcoRI cassette; both sites are preserved)



## C). PREPARATION OF THE ANNEALED OLIGO CASSETTES

- 1). Dissolve each of oligo (top and bottom strands) in ddH<sub>2</sub>O to make stocks at final concentration of 1.0 µg/µl.
- 2). Transfer 20µl each to a new screw cap 1.7ml microfuge tube (or PCR tubes), and mix well.
- 3). Boil the oligo mixture for 5-10 minutes (or use PCR machine and incubate 95°C for 5min).
- 4). Cool the mixture down at room temperature (usually taking 5-10 min).
- 5). Transfer 10µl of annealed oligo mixture to a new 1.7ml microfuge tube, and add 490µl ddH<sub>2</sub>O (i.e., **1:50 dilution**). This is your annealed oligo cassette solution (serving as an insert) ready for ligation reaction.

## D). PREPARATION OF VECTOR

- 1). Set up restriction enzyme digestion reactions as follows:

Plasmid vector (miniprep DNA)	5.0µl
ddH <sub>2</sub> O	82.0µl
10x Buffer (NEB)	10.0µl
Restriction enzyme #1 (NEB)	3.0µl

- 2). Incubate at 37°C (or specific temp) for 30 min, followed by ethanol precipitation and 70% ethanol washing (**NOTE: PC-8 extraction is optional but usually unnecessary**).

Plasmid vector (post 1 <sup>st</sup> cut)	dry pellet
ddH <sub>2</sub> O	87.0µl
10x Buffer (NEB)	10.0µl
Restriction enzyme #2 (NEB)	3.0µl

- 3). Incubate at 37°C (or specific temp for certain enzymes; please double check with the vendor's website/catalog) for 30 min, followed by ethanol precipitation and 70% ethanol washing.
- 4). Dissolve the **vector DNA in 20-30µl ddH<sub>2</sub>O**. Ready for ligation reaction or kept at -20°C.

## E). LIGATION REACTION AND BACTERIAL TRANSFORMATION

1). Set up ligation reaction as follows:

5x Ligation Buffer (Invitrogen)	3.0µl
ddH <sub>2</sub> O	9.0µl
Vector cut with enzymes #1&#2	<b>1.0µl</b>
Annealed Oligo Cassette (1:50)	<b>1.0µl</b>
T4 DNA Ligase (Invitrogen)	1.0µl

2). Incubate at room temperature for 1 ~ 4hrs.

3). Perform restriction enzyme digestion of the ligation mix to cut background: usually in 100µl reaction with 1-2µl enzyme at 37°C for 10-20min. (**NOTE: you can cut half of the ligation mix and leave another half uncut**).

4). Perform ethanol precipitation and follow the lab's standard electroporation protocol (**NOTE: You just need to use 25%-50% of the digested ligation mix or uncut mix to do electroporation**).

5). You can **directly plate** (without incubation) and/or **plate after incubating** the transformation mix at 37°C for 20min. Follow standard colony PCR protocol to screen for potential positives.

### GENERAL CONSIDERATIONS

- The amount of annealed oligo cassettes used on ligation:** We have tried to use different dilutions of annealed oligo cassettes, ranging from 1:10 to 1:100 dilutions. The common mistake for oligo cassette cloning is to use too much of the oligo cassettes, which will reduce the ligation efficiency due to multimerization among excessive cassettes.
- Cutting background:** While it is always critical to cut background, the oligo cassettes can also suppress cloning background by annealing to the sticky ends of the vector that may be cut by either or both enzymes. Thus, it is not surprising to see fewer colonies than a regular ligation. In fact, the higher the cassette concentration used, the fewer colonies obtained.
- Compatible overhangs:** Some enzymes can produce the same overhangs so you can take advantage to insert an oligo cassette into the sites with compatible overhangs. Please keep in mind the new site cannot be cut by either enzyme. Some commonly used enzymes that share compatible ends include: **BamHI and BglII; Sall and XhoI; XbaI, NheI, AvrII and SpeI**. You can find more from **NEB website**: <https://www.neb.com/en-us/tools-and-resources/selection-charts/compatible-cohesive-ends-and-generation-of-new-restriction-sites>
- Restriction enzyme sites can be retained or destroyed by inserting oligo cassettes:** for example,

BamHI

**GGATCC**  
**CCTAGG**

EcoRI

**GAATTC**  
**CTTAAG**

**BamHI site is destroyed, while EcoRI site is retained.**

5' - **GATC**ANNNNN . . . . . NNNN**G**-3'  
3' - **T**nnnnn . . . . . nnnn**CTTAA**-5'  
("C" can be changed to **A, T or G**)

**BamHI site is retained, while EcoRI site is destroyed.**

5' - **GATCC**NNNNN . . . . . NNNN**C**-3'  
3' - **G**nnnnn . . . . . nnnn**GTTAA**-5'  
("G" can be changed to **C, T or A**)