

Gateway clone primer design

The proper design of attB-attached primer is a crucial point for the BP and LR reaction of Gateway clone and the functional expression of the gene. The following is a simplified way to make your gateway primers.

According to your gateway entry vector(s), choose appropriate attB adapter pair(s).

- 1) attB1: 5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTCXXXXXX(18-25nt)-3' (Added to 5' PCR forward primer)
- 2) attB1: 5'-GGGGCTGCTTTTGTACAAACTTGTXXXXXX(18-25nt)-3' (Added to 3' PCR reverse primer)
- 3) attB2: 5'-GGGGCAGCTTCTTGTACAAAGTGGGAXXXXXX(18-25nt)-3' (Added to 5' PCR forward primer)
- 4) attB2: 5'-GGGGACCACTTTGTACAAGAAAGCTGGGTXXXXXX(18-25nt)-3' (Added to 3' PCR reverse primer)
- 5) attB3: 5'-GGGGCAACTTTGTATAATAAAGTTGTXXXXXX(18-25nt)-3' (18-25nt) Added to 3' PCR reverse primer)
- 6) attB4: 5'-GGGGCAACTTTGTATAGAAAAGTTGGAXXXXXX(18-25nt)-3' (Added to 5' PCR forward primer)

Red: is the sequences of attB sites that function as adapters.

Green: is the the nucleotide(s) added to keep the sequence in the right reading frame.

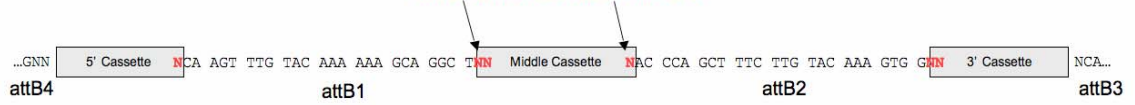
You can change it to other nucleotide(s) if you don't like it (but do not choose stop code).

XXXXXX(18-25nt): Your specific gene sequence.

Highlighted Yellow: If you have universal attB adapters available in your fridge, you can only choose highlighted yellow sequence to do 1st PCR then add the corresponding universal attB primers to do the 2nd PCR.

The Following example (figure) is copied from SEYDOUX LAB website to give an idea how it works, good luck!

Depending on where the START and STOP codons are placed,
 frame may have to be preserved through *att* sites
 (add 2 Ns in the 5' and 1 N in the 3')



Example

In this example, the **ATG** is at the 3' end of the *pie-1* promoter.
 Therefore *attB1*, GFP, *attB2*, and the ORF must all be kept in frame with that initial ATG.
 Note that in this example, both *attB1* and *attB2* will be included in the final fusion protein.

