Cloning Protocol

Cloning Your PCR Product into a p3A Vector

Appending the primer sequences (see below) to your forward and reverse PCR primers for cloning your target gene sequence. **Note that the primers differ for encoding an N-terminal or C- terminal AviTag.** An additional 20 nucleotides of ORF sequence from your target gene are the recommended length of ORF nucleotide sequence to be added to the primers. The ATG start sites for your gene of interest are already included in both primer designs.

Your ORF must NOT contain any SapI recognition sites, as the Electra[1] cloning process utilizes the type IIS enzyme SapI.

PCR Forward primer for C- terminal AviTag:
5'-TACACGTACTTAGTCGCTGAAGCTCTTCT**ATG**….(ORF)….

PCR Reverse Primers for C- terminal AviTag:
5'-AGGTACGAACTCGATTGACGGCTCTTCTGCC….(ORF Reverse Complement)….

*__Notice that the start codon is already designed into the C-terminal forward primer__, which keeps your gene of interest in frame with the AviTag. **Do not** add a translational stop codon to the C-terminal reverse primer, so that the AviTag will be expressed at the end of your gene of interest.

PCR Forward Primer for N- Terminal AviTag:
5'-TACACGTACTTAGTCGCTGAAGCTCTTCTTCT**ATG**….(ORF)….

PCR Reverse Primer for N- Terminal AviTag:
5'-AGGTACGAACTCGATTGACGGCTCTTCT**TTA**….(ORF Reverse Complement)….

*__Notice that the start codon is already designed in the N-terminal forward primer__, which keeps your gene of interest in frame with the AviTag. The stop codon (TAA) is also already in the N-terminal reverse primer as well.

From the Atum protocol for PCR products: (1:1 vector to insert)

Component	Volume (µl)
PCR reaction	20 ng x (bp insert/bp vector)
p3A Vector	1 (20 ng)
Electra Buffer Mix*	2
Electra Enzyme Mix*	1
Sterile ddH_2O	15
Total Volume	20

* *Electra Cloning Kit reagents*

1. Combine components as listed above in single 0.6 ml tube. Incubate at room temperature for 5-20 minutes.

2. Transform 2 µl of each reaction into competent cells.

3. Plate on LB + kanamycin (50 µg/ml)

4. Incubate plates overnight at 37°C. Pick transformants.

[1]Intellectual Property Statement Available online:

www.atum.bio/company/terms-and-conditions