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 start site for your target gene is already included in the 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'-TACACGTACTTAGTCGCTGAAGCTCTTCTATG….(ORF)….

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

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

PCR Reverse Primer for N- Terminal AviTag:

5'-AGGTACGAACTCGATTGACGGCTCTTCTTTA….(ORF Reverse Complement)….

From the Atum protocol for PCR products:

Component	Volume(µl)
PCR reaction	1 (20 ng DNA)
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