

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 Sapl recognition sites, as the Electra¹ cloning process utilizes the type IIS enzyme Sapl.

PCR Forward primer for C-terminal AviTag:
5'-TACACGTAAGCTCTTCTATG....(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'-TACACGTAAGCTCTTCTATG....(ORF)....

PCR Reverse Primer for N-Terminal AviTag:
5'-AGGTACGAACTCGATTGACGGCTCTTCTTAA....(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:

Component	Volume(μl)
PCR reaction	1 (20 ng DNA)
p3A Vector	1 (20 ng)
Electra Buffer Mix*	2
Electra Enzyme Mix*	1
Sterile ddH ₂ O	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 μ l of each reaction into competent cells.
3. Plate on LB + kanamycin (50 μ g/ml)
4. Incubate plates overnight at 37°C. Pick transformants.

¹Intellectual Property Statement Available online:

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