

Primer Resuspension & Sequencing Protocol

Step 1, Add Sterile Water: Carefully open the tube and add 10 ul of dH2O to generate a 10uM stock.

Step 2, Incubate: Close the tube and let it sit for 10 minutes at room temperature, or 4°C overnight.

Step 3, Vortex: Briefly vortex the tube and then do a quick spin to concentrate the liquid at the bottom.

The primer stock is now ready to be added to a DNA sequencing reaction (1ul = 10pmol).

Which primers should I use?

If you are purchasing an empty vector, the sequencing primers will be listed in the "Features" section of the vector's product page. If you are purchasing a ready-to-use TrueORF or Lenti-ORF clone, you must first scroll down to the "Vector" section in the clone product page which will lead you to the vector page containing the primer information. If you need assistance finding the appropriate sequencing primers, please reach out to <u>techsupport@origene.com</u> or call 1-888-267-4436.

Sequencing for TrueClones:

The forward VP1.5) & reverse (XL39) sequencing primers can be purchased here.

DNA sequencing from the 5` end of the cDNA insert should be performed with VP1.5 (5`-GGACTTTCCAAAATGTCG-3`) whose priming site is located ~120 bp upstream of the polylinker. DNA sequencing from the 3` end of the cDNA insert should be performed with XL39 (5`-ATTAGGACAAGGCTGGTGGGG-3`) whose priming site is located ~70 bp downstream of the polylinker. Do not use other common sequencing primers such as M13rev or T7 as these are not always unique in *OriGene vectors*.

To obtain a high-quality sequencing signal, use 1 ul of primer in an automated DNA sequencing reaction containing 100 ng of OriGene's TrueClone plasmid DNA. OriGene used 1 ul of Big Dye[®] v1.1 (Applied Biosystems; Foster City CA) in a 10 ul reaction volume to end-sequence the TrueClone Collection. The alignment of sequences to either the NCBI reference or the TrueClone sequence published on our website will confirm that the correct full-length clone was obtained.