## Electra Cloning

ATUM has developed a simple one-step cloning process that can be performed in a 5-minute bench-top reaction with the fidelity of a restriction based cloning system. This process leaves no cloning scars and does not require sequential vector digestion, purification and ligation steps. The Electra system uses the type IIS restriction enzyme SapI, which recognizes a 7bp non-palindromic recognition sequence and leaves a 3bp 5' overhang after digestion. The Electra Vector™ system is available for R&D applications.

A gene that is provided as a DNA2GO™ gene fragment or a PCR product can be quickly and efficiently moved into any Electra DAUGHTER vector allowing the gene to be tested under different contexts – promoters, ribosome binding sites, C- and N-terminal tags and/or fusions. ATUM has constructed a large collection of bacterial, mammalian and yeast expression vectors compatible with the Electra cloning process.

## Description - Electra Cloning Kit (50 Reactions)

The Electra Cloning kit contains all necessary components to facilitate cloning a gene of interest from a DNA2GO™ gene fragment or a PCR product into an Electra DAUGHTER expression vector.

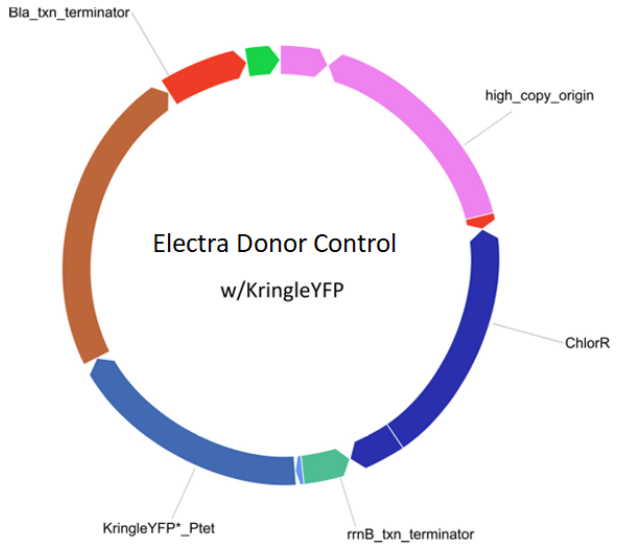
Electra Cloning Kit Components:

**Electra Buffer Mix** is supplied as a 10X mix, to be diluted to 1X in the final reaction mix

**Electra Enzyme Mix** is supplied as a 20X mix, to be diluted to 1X in the final reaction mix. The mix contains SapI and T4 ligase and is formulated for optimal cloning efficiency.

**Electra donor Control** is a plasmid with a Tet promoter and a yellow fluorescent protein (KringleYFP). It allows monitoring of the transfer of KringleYFP into a DAUGHTER expression vector and is seen as yellow colonies when plated with selection antibiotic.

### Electra donor control plasmid



## Storage

Store all kit components at -20°C. Electra Buffer should be aliquoted to avoid multiple freeze thaws.

## Cloning Information

**To clone a PCR product:** we recommend you add the following ends to your primers, as these contain the Electra sites to clone directly into Electra expression vectors. Add 15-20 bp of your ORF to the 3′ primer ends of the forward and reverse primers to amplify your ORF and have it compatible with any of the Electra expression vectors.

- Forward primer:
  5′-TACACGTACTTAGTCGCTGAAGCTCTTCTATG .... (ORF)....-3′
- Reverse primer:
  5′-AGGTACGAACTCGATTGACGGCTCTTCTACC....(ORF Reverse Complement)....-3′

* Your ORF must not contain any SapI recognition sites, since the Electra cloning process utilizes the typeIIs enzyme SapI.

**To clone DNA2GO™ gene fragments**: You can request to have your DNA2GO™ gene fragment designed and synthesized as Electra compatible fragment when you place the order.

The Electra vectors are provided linearized with a 5′TAC and a 3′GGT overhangs. Your gene provided as an Electra compatible DNA2GO™ gene fragment is mixed with the linearized Electra expression vector in the presence of Electra reagent mix for 5 to 20 minutes at room temperature (25°C).

| COMPONENT | VOLUME (µl) |
|---|---|
| DNA2GO™ DNA fragment or Electra donor control*(20ng) | 1 |
| DAUGHTER Vector (pDXXX) (20ng) | 1 |
| Electra Buffer Mix* | 2 |
| Electra Enzyme Mix* | 1 |
| Sterile dd$H_2O$ | 15 |
| Total Volume | 20 |

**Electra cloning kit reagents*

1. Combine components as listed above in single 1.5 ml tube. Incubate at room temperature for 5- 20 minutes.
2. Transform 2 µl of each reaction into competent cells.
3. Plate on LB + selection antibiotic.
4. Incubate plates overnight at 37°C. Pick transformants.