Electra™ Cloning Reagents Kit

Electra Cloning

DNA2.0 has developed a simple one-tube universal 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 PCR or other mutation-inducing amplification. 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 both R&D and commercial applications, and is IP-Free© with no licensing restrictions.

A gene that is provided in a pMOTHER vector can be quickly and efficiently moved into any pDAUGHTER vector allowing the gene to be tested under different contexts – promoters, ribosome binding sites, C- and N-terminal tags and/or fusions. DNA2.0 has constructed a large collection of IP-Free bacterial, mammalian and yeast pDAUGHTER expression vectors. Any vector can be easily converted to function as an Electra vector, and DNA2.0 will assist anyone who wishes to do so.

Description - Electra Reagents Kit (50 Reactions)

The Electra Cloning kit contains all necessary components to facilitate cloning a gene from a pMOTHER vector or a PCR product into an Electra pMOTHER or pDAUGHTER expression vector.

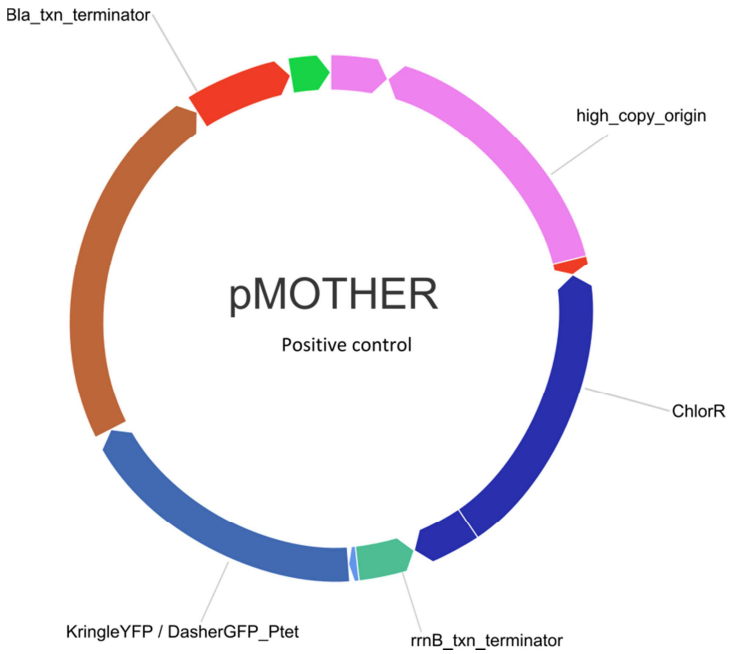
Electra Reagents Kit Components:

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.

Positive Control is a mix of pMOTHER vectors with a Tet promoter and a yellow fluorescent protein (KringleYFP) (for Electra expression pDAUGHTER vectors) and a Tet promoter with a green fluorescent protein (DasherGFP) for Cas9 Electra pDAUGHTERS. It allows monitoring of the transfer of KringleYFP or DasherGFP into a pDAUGHTER expression vector and is seen as yellow or green colonies when plated with selection antibiotic.

pMOTHER Positive Control: Vector map



Storage

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

Cloning Information

To PCR your ORF: we recommend you add the following ends to your primers, as these contain the Electra sites to clone directly into pMOTHER or pDAUGHTER vectors. Add 15-20 bp of your ORF to the 3' primer end to amplify your ORF and have it compatible with any of the Electra pMOTHER or Electra pDAUGHTER expression vectors.

- Forward primer:
  5'-TACACGTACTTAGTCGCTGAAGCTCTTCTATG....(ORF)....-3'
- Reverse primer:
  5'-TAGGTACGAACTCGATTGACGGCTCTTCTACC....(ORF Reverse Complement)....-3'

Electra Reagents kit is used to clone in a PCR product directly into pMOTHER or pDAUGHTER vectors or shuttle ORFs from a pMOTHER vector to pDAUGHTER vectors.

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

The pMOTHER and pDAUGHTER vectors are provided linearized with a 5'TAC and a 3'GGT overhang. Your gene in DNA2.0's MOTHER vector or PCR product is mixed with the linearized pMOTHER or pDAUGHTER vector in the presence of Electra reagent mix for 5 to 20 minutes at room temperature (25°C).

COMPONENT	VOLUME (µl)
MOTHER DNA/PCR product/Positive control*(20ng)	1
MOTHER or DAUGHTER Vector (20ng)	1
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 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.
   3a. Optionally, LB + selection antibiotic + counter- selection - streptomycin at 100 µg/ml (for selection against pMOTHER with rpsL), Teknova Cat # L1148. Streptomycin resistant strain such as DH10B is recommended if using pMother with rpsL counter-selection.
   OR
   3b. YEG+ selection antibiotic + counter-selection - p-chloro phenylalanine at 10mM (for selection against pMOTHER with pheS).

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

Intellectual Property Statement
Available online: www.dna20.com/wp-content/uploads/2013/04/Intellectual_Property_Statement.pdf