

Notes on the use of pEarleyGate Vectors

1. Users should first familiarize themselves with Gateway cloning by visiting the ThermoFisher website and viewing/downloading the appropriate protocols. Tutorials can also be found by searching the internet using your favorite browser.
2. Sequences to be recombined into pEarleyGate vectors need to be flanked by att recombination sites. This can be most easily accomplished by capturing sequences of interest into a Gateway entry vector. For directional cloning, which is necessary to preserve reading frames for fusions with epitope tags or fluorescent proteins, we use the pENTR/D-TOPO entry vector which allows for ligase-free topoisomerase-mediated cloning of sequences amplified by PCR.
3. Note that with N-terminal fusion vectors, you will need to include a stop codon at the end of your open reading frame (ORF) of interest. Conversely, for the C-terminal fusion vectors, you will need to eliminate any natural stop codons at the end of your ORF to allow for translational fusion to the C-terminal epitope tag or fluorescent protein.
4. Clonase mix is ordered from Invitrogen
5. Please obtain pEARLEY GATE vector DNA from the ABRC (www.arabidopsis.org).
6. Unfortunately, the pENTR vector and the pEarleyGate vectors both contain the same bacterial selection marker, namely kanamycin resistance. Therefore, to prevent transformation of bacteria with the pENTR plasmid following the transfer of the sequences of interest into the pEarleyGate vector, simply cut your pENTR vector with a restriction endonuclease that cleaves within the pENTR backbone but does not cut within the sequence of interest or the pEarleyGate vector. We often use Mlu I; this enzyme cuts twice within the pENTR backbone. The digestion is performed prior to the recombination reaction.
7. Perform the recombination reaction using linearized pENTR plasmid according to Invitrogen's clonase protocol. We often gel purify the fragment containing the sequence of interest, but the recombination reaction works OK with cleaved DNA.
8. Select E. coli or A. tumefaciens that has been transformed with pEarleyGate plasmids using kanamycin (50 ug/ml).
9. Plants transformed with T-DNA that was transferred from a pEarleyGate vector can be selected by spraying seedlings with Finale herbicide (AgrEvo Environmental Health, Montvale,

New Jersey) diluted 1/200 with water. Final herbicide, as purchased, is 5.78% (w/v) glufosinate-ammonium (butanoic acid, 2-amino-4-hydroxymethylphosphinyl, monoammonium salt). The BAR gene confers resistance to this herbicide. Because the T-DNA includes the BAR gene, transgenic plants that express this selectable marker gene survive herbicide spraying, whereas non-transgenic plants are killed. Seedlings are typically sprayed twice, first when they are approximately one week old and again when they are two weeks old.