ENFINIA™ Plasmid DNA

Vector Onboarding Acceptance Criteria

ORIGINS

We accept any copy number bacterial origin, but the origin must allow propagation in an F′-containing strain, such as NEBStable or One Shot™ ccdB Survival™ 2 T1 R Competent Cells to enable us to prepare a counter-selectable version of the plasmid. For any bacterial origins that are not compatible with either Stbl3 or NEB10beta for cloning, we require the customer to provide a strain of bacteria and conditions that can propagate this origin. This strain of bacteria must either be commercially available, or the customer must send us an agar stab of the bacterial strain.

ANTIBIOTIC RESISTANCE MARKERS

Vectors with *E. coli* resistance markers for Ampicillin/Carbenicillin, Kanamycin, Spectinomycin, Streptomycin, Tetracycline, and Chloramphenicol are accepted.

INSERTION SITE SPECIFICATION

Choose either an insertion site or restriction enzyme to define the location of your insert sequence. You must supply both the 3′ and 5′ end for whichever selection you choose.

Reference: ENFINIA Plasmid DNA Insertion Site Guide

RESTRICTION SITES

1. <=4 instances of the PaqCI recognition sequence ‘CACCTGC’ or its reverse complement ‘GCAGGTG’ are allowed. If the sequence does not contain PaqCI sites, skip 2 and 3.
2. Within the same plasmid, no more than 1 occurrence of the same 4 base pair (bp) sequence located 4 bp to the 3′ end of a ‘CACCTGC’ sequence or 4 bp to the 5′ end of a ‘GCAGGTG’ sequence *(see diagram below)*.
3. The sequence ‘CACCTGC’ must not be found within 12 bp to the 5′ end of the 5′ insertion site, and the sequence ‘GCAGGTG’ must not be found within 12 bp to the 3′ end of the 3′ insertion site.

5′-CACCTGCNNNNCAGT-N_x-CACCTGCNNNNGTTA-3′ — ✓

5′-CACCTGCNNNNCAGT-N_x-GTTANNNNCACCTGC-3′ — ✓

5′-CACCTGCNNNNCAGT-N_x-CACCTGCNNNNCAGT-3′ — ✗

5′-CACCTGCNNNNCAGT-N_x-CAGTNNNNCACCTGC-3′ — ✗

Note: Elegen strongly recommends avoiding more than 1 occurrence of ‘CACCTGC’ or ‘GCAGGTG’ in the vector backbone if possible.

LINEARIZATION

Unique Type IIp restriction enzyme sites that produce a sticky end must be located within 150 bp to the 5′ and 3′ end of the 5′ and 3′ insertion sites, respectively.

AVOID THE FOLLOWING SEQUENCES

- mazF
- ccdB
- Bacteriophage genes
- Genes that encode toxins from animals, plants, or pathogenic bacteria
- Proteases active in *E. coli*
- Endonucleases active in *E. coli*
- Other sequences that can interfere with the propagation of *E. coli*

PHYSICAL VECTOR SUBMISSION REQUIREMENTS

1. Vector must be submitted in >100 µL of liquid (either nuclease-free water or Low TE), in a micro-centrifuge or PCR strip tube.
2. Vector concentration > 100 ng/µL.
3. Vector mass > 10 µg
4. Vector must be a clonal population from mini-prep.
5. Vector must be circular.

If you have any additional questions regarding the criteria listed, please contact your Elegen sales representative.

INSERTION SITE GUIDELINES

Name	Sequence
AatII	GACGTC
ApaLI	GTGCAC
BamHI	GGATCC
BbsI	GAAGAC
BsaI	GGTCTC
ClaI	ATCGAT
EcoRI	GAATTC
HindIII	AAGCTT
KpnI	GGTACC
NcoI	CCATGG
NdeI	CATATG
NheI	GCTAGC
XhoI	CTCGAG
PaqCI	CACCTGC
PstI	CTGCAG
PvuI	CGATCG
SacI	GAGCTC
SalI	GTCGAC
SphI	GCATGC
SstI	GAGCTC
XbaI	TCTAGA
XmaI	CCCGGG

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