Name: pDR-GFPuniv

Description: Plasmid for in vivo recombination assays. Based on the original pDR-GFP plasmid described in Pierce et al. GenDev (1999) v13p2633.

Use data: Recipient plasmid for (homing) endonuclease target sites in order to assess in vivo activity of an endonuclease. The plasmid contains two non-functional copies of the eGFP gene: the 5' copy is interrupted by the target site of interest and stop codons; the 3' copy is truncated on both 5'- and 3'-end.

pDR-GFP as is does not fulfill any other purpose than to receive (homing) endonuclease target sites. The recognition site for the endonuclease of interest must be cloned into the XhoI/SacI sites in the upstream eGFP gene, thus obliterating the XhoI, KpnI and SacI sites. Successful cleavage of the cloned target site generates a DSB. This triggers in vivo a homologous recombination event with the 3' truncated copy of the eGFP which renders the 5' copy functional. The activity of a given endonuclease can be measured by the number of GFP+ cells generated.

The site that is cloned into pDR-GFP must insert a frameshift into the 5' eGFP-copy and/or contain a stop codon in frame with the upstream sequence of the eGFP gene to assure that the ORF is not functional and no eGFP can be synthesized prior to the DSB repair event. In order to easily screen for the successful insertion of the oligonucleotide pair representing the site of interest, it is useful to integrate a restriction site that is not present in pDR-GFPuniv (e.g. a PvuII site).

Sample oligonucleotide pair:

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                                  PvuII
oligo1 5'-TCGATAGGGATAACAGGGTAATACAGCTGTAAGCT-3'
oligo2 3'-    ATCCCTATTGTCCCATTATGTCGACAT    -5'
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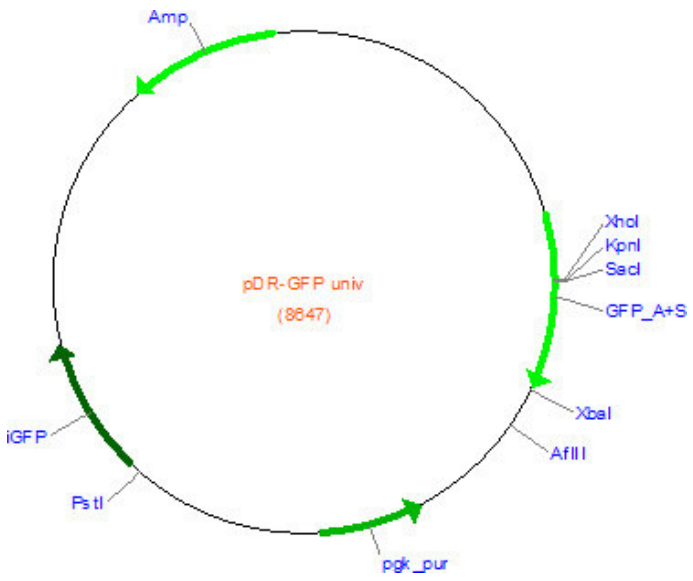

Feature table:

GFP univ.: 1740-2754

Puromycin resistance: 4261-3664

iGFP: 5333-6141

Ampicillin resistance: 8515-7656

MCS:

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                    XhoI  KpnI  SacI
2122   …GCGTGTCCGGCCTCGAGGGTACCGAGCTCACCTACG…   2157
2122   …CGCACAGGCCGGAGCTCCCATGGCTCGAGTGGATGC…   2157
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gtcgacattgattattgactagttattaatagtaatcaattacggggtcattagttcatagcccatatat
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