Resource Summary Report

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LEGO-pDHS-ITDE-AP1x6-DUSP5

RRID:Addgene_217419
Type: Plasmid

Proper Citation

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Plasmid Information

URL: http://www.addgene.org/217419

Proper Citation: RRID:Addgene_217419

Insert Name: Luciferase, GFP

Organism: Homo sapiens

Bacterial Resistance: Ampicillin

Vector Backbone Description: Backbone Marker:Boris Fehse (Addgene #27358); Vector Backbone:LeGO-iG; Vector Types:Mammalian Expression, Lentiviral, Luciferase; Bacterial Resistance:Ampicillin

Comments: This expression vector contains a synthetic enhancer that was designed as an array of transcription factor binding sites predicted to be highly active in acute myeloid leukaemia cells carrying activating mutations in the receptor FLT3 (Assi et al., 2019). This putative enhancer was generated as an oligonucleotide duplex containing binding sites for EGR1, ETS, SRF, CREB, NF1, NF-kB and WT1, inserted into LEGO-pDHS-AP1x6-DUSP5. The closely linked ETS, SRF, and CREB sites are derived from a Serum Response Element in the FOS promoter which is induced by MAPK. LEGO-pDHS-AP1x6-DUSP5 is an inducible lentiviral luciferase expression vector that can has 6 RAS/MAPK-inducible AP-1 sites driving transcription from the human DUSP5 promoter, which is highly active in most acute myeloid leukaemia samples but not in normal myeloid stem cells (Assi et al, 2019). This is a CG island promoter containing Sp1, SRF, ETS and NF-kB sites, and is likely to be activated by signalling downstream of FLT3-ITD mutations (Assi et al, 2019). This vector also contains a myeloid cell chromatin priming element from the human IL3 gene that can potentially diminish chromatin silencing effects by maintaining open chromatin. LEGO-pDHS-AP1x6-DUSP5 was constructed from LEGO-pDHS-AP1x6-GM55 by replacing the minimal human CSF2 promoter with the full-length human DUSP5 promoter. The pDHS was added by inserting into the XbaI site a 274 bp PCR-generated fragment of a DNA segment located 10 kb upstream of the human IL3 gene that maintains open chromatin in myeloid cells. LEGO-AP1x6-GM55 was previously constructed from

LeGO-iG (Weber et al, 2008) (Addgene #27358) by replacing a NotI/XbaI fragment containing the U6 and SFFV promoters with a fragment from the plasmid pAP1-3 (Addgene #71258) that has 3 copies of the MMP3 gene AP-1 site in front of human CSF2 -55 to +28 minimal promoter driving expression of the Luc+ firefly luciferase gene originating from the plasmid pGL3 (Promega), and then duplicating the array of 3 AP-1 sites. The luciferase gene is linked to an IRES that in principle supports co-expression of luciferase and EGFP. The insert has 2 copies of the SV40 transcription termination/polyadenylation signal upstream of the multiple cloning site, which blocks background read-through transcription originating in the vector backbone, which itself can be substantial (Bert et al, 2000). The inserted luciferase gene and SV40 polyA site sequences originate from the plasmid pXPG (Bert et al, 2000) (Addgene #71248). The priming element (pDHS) encompasses RUNX1 and ETS1 binding sites and resembles sites that prime genes for rapid activation in T cells (Bevington et al, 2016)). References: Assi et al. Nat Genet. 2019; 51:151-162. PMID 30420649. Bert et al. Plasmid. 2000 Sep;44(2):173-82. PMID 10964627. Weber et al. Mol Ther. 2008 Apr. 16(4):698-706. PMID 18362927. Bevington et al. Bioessays 2016 Dec 27. doi: 10.1002/bies.201600184. PMID 28026028.

Plasmid Name: LEGO-pDHS-ITDE-AP1x6-DUSP5

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Ratings and Alerts

No rating or validation information has been found for LEGO-pDHS-ITDE-AP1x6-DUSP5.

No alerts have been found for LEGO-pDHS-ITDE-AP1x6-DUSP5.

Data and Source Information

Source: Addgene

Usage and Citation Metrics

We have not found any literature mentions for this resource.