

Resource Summary Report

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Tol2-GgVCL/*TBM

RRID:Addgene_162790

Type: Plasmid

Proper Citation

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Plasmid Information

URL: <http://www.addgene.org/162790>

Proper Citation: RRID:Addgene_162790

Insert Name: Mutated Gallus gallus vinculin (G454V)

Organism: Gallus gallus

Bacterial Resistance: Ampicillin

Vector Backbone Description: Vector Backbone:destination vector pDestTol2pA2(#394); Vector Types:Mammalian Expression, Bacterial Expression; Bacterial Resistance:Ampicillin

Comments: The incorporation of GgVCL and GgVCL/*TBM in a eukaryotic transient expression vector was done according to the following steps: 1) amplification of VCL sequence with Phusion-HF DNA pol and primers with a Kozac initiation sequence or RBS and side restriction enzyme sites (KpnI -FW and EcoRI -rev; ch-VCL custom primers); 2) extension with Taq DNA pol to generate A-ends; 3) Ligation to the T-ended vector TOPO TA #450640 through the topo cloning reaction and dilution in TE (Invitrogen 8019005?); 4) transformation of DH5? (C2987I) and selection with kanamycin and IPTG/Xgal (EU 0012-D); 5) Minipreps (740588.250) from presumed 3 TOPO-WT and 3 TOPO-MUT colonies, DNA quantification and diagnostic digestions with Accl, EcoRI or KpnI; 6) electrophoresis of abundant EcoRI + KpnI digestion products and band cutting; 7) purification of VCL sequence from gel and of EcoRI + KpnI digested and alkaline phosphatase (#EF0651) - treated pME-MCS (#237) with Macherey-Nagel #740609.250 kit; 8) DNA quantification; 9) ligation of each VCL sequence to pME-MCS plasmid using Ligase T4 (NEB MO202T); 10) transformation of DH5? (NEB C2987I) with the respective ligation reactions; minipreps (740588.250) to obtain pME-VCL, DNA quantification and diagnostic digestions with Accl, (KpnI-HF + EcoRI-HF) and BamHI-HF; 11) Tol2 kit LR reaction (Kwan et al 2007, DOI: 10.1002/dvdy.21343,) . Although the kit is originally aimed at zebrafish, it had already been assayed in mammalian cells. The LR reaction involved the use of Gateway LR Clonase II Plus Enzyme Mix (Invitrogen 12538-120) to fuse 4 plasmids in one: the 5'entry

plasmid with a β -actin promoter called p5E-actin (#299), the middle entry plasmid with the VCL insert pME-VCL (wt or mut), the 3'entry plasmid p3E-IRES-nls-GFP (#391) and the destination vector pDestTol2pA2 (#394). The expected LR reaction product is a 14,5 KB eukaryotic expression vector which can be amplified in bacteria. NEB C3019I bacteria were transformed with the LR reaction product. Three ampicillin-resistant clear colonies of each (β -actin- wt or mutVCLIRES nclGFP) were chosen and amplified. Minipreps, DNA quantification and diagnostic digestions with (KpnI+Accl) and Pvu-IIHF were carried. Finally, 200 mL of liquid culture were used to prepare MIDIPREPS (XXX). About 180 μ g good quality DNA of each (Tol2-GgVCL or Tol2- GgVCL/*TBM) expression vectors were obtained and checked by digestion with restriction enzymes.

Plasmid Name: Tol2-GgVCL/*TBM

Relevant Mutation: changed Gly 454 to Val

Record Creation Time: 20220422T221920+0000

Record Last Update: 20260815T080905+0000

Ratings and Alerts

No rating or validation information has been found for Tol2-GgVCL/*TBM.

No alerts have been found for Tol2-GgVCL/*TBM.

Data and Source Information

Source: [Addgene](#)

Usage and Citation Metrics

We have not found any literature mentions for this resource.