Jun 28, 2022

Generating a low-copy overexpression cell line using PhiC31 integration

Goran Tomic[1]

[1]The Francis Crick Institute

dx.doi.org/10.17504/protocols.io.36wgq71kxvk5/v1

Goran Tomic_Protocols

Goran Tomic

ABSTRACT

This is an in-house adaptation of the commerically available Jump-In system by Thermo. It generates clones with a lower overexpression level than those generated by lentivirial delivery and can be carried out in a standard CL1 tissue culture room. The gene of interest in the attB construct is assembled in-house and is driven by the PGK promoter and contains the BGH pA sequence (assembled from pX28 plasmid (Addgene Plasmid #46850), which had the required sequences). pCMVInt plasmid that expresses the PhiC31 integrase was purchased from Addgene (Plasmid #18935). This protocol is made combining the protocol for Jump-In system (ThermoFisher) and phiC31 Integrase Vector System (System Biosciences).

ATTACHMENTS

PhiC31_schematic copy-01.png

DOI

dx.doi.org/10.17504/protocols.io.36wgq71kxvk5/v1

PROTOCOL CITATION

Goran Tomic 2022. Generating a low-copy overexpression cell line using PhiC31 integration. **protocols.io** https://dx.doi.org/10.17504/protocols.io.36wgq71kxvk5/v1

KEYWORDS

phiC31, overexpression, attb

CREATED

Jun 28, 2022

LAST MODIFIED

Jun 28, 2022

PROTOCOL INTEGER ID

65542

MATERIALS TEXT

pCMVInt **addgene Catalog #18935**

BEFORE STARTING

Peform a quick Blasticidin curve to determine the optimal concentration that kills the cells (0-20 ug/mL). Use 24 well plates and try, 0, 2.5, 5, 10, 15, 20 ug/mL Bsd.

1 Day 1: Use a 6-well plate (0.15x10^6 cells per well). Incubate for 24-48 hours. Transfect if the well is 70-90% confluent.

2 Day 2: Make DNA-Lipofectamine™ 3000 complexes in serum-free medium such as Opti-MEM™ Reduced Serum Medium.

For a 6 well plate: 2.5 ug of DNA + 125 uL Opti-MEM + 5 uL P3000 reagent (2 uL/ug DNA). Mix PhiC31 integrase plasmid in a 50:1 ratio (w/w) over the donor i.e. 2500 ng of PhiC31 + 50 ng of GOI/attB plasmid
125 uL OptiMEM + 3.75 uL Lipofectamine 3000
Mix 1:1 and incubate for 15 min at RT
Add 250 uL per well to cells in culture medium

3 Day 3: Split the cells into 10 cm plates at 1:10 and 1:20 ratio and add blasticidin. Observe daily and change blasticidin media every 3-4 days until colonies appear.
Validate the expression of the GOI using qPCR, WB, etc.

N.B. To increase the probability of getting single-copy integration of the targeting expression

Citation: Goran Tomic Generating a low-copy overexpression cell line using PhiC31 integration
https://dx.doi.org/10.17504/protocols.io.36wgq71kxvk5/v1

construct, use the amount of vector that results in less than five drug-resistant colonies in the absence of integrase. Using that amount in the presence of integrase should result in >15 colonies.
It is good to have an empty vector control (to generate Bsd-resistant cells, but without GOI).
Important! We observed that in some clones the expression of the GOI can reduce over time. Best to freeze an early passage of the selected single clones after validation, and check the expression of the GOI before any important experiments.

Citation: Goran Tomic Generating a low-copy overexpression cell line using PhiC31 integration
https://dx.doi.org/10.17504/protocols.io.36wgq71kxvk5/v1