

Insertion of PATC-rich *C. elegans* introns into synthetic transgenes by golden-gate-based cloning

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Method Article

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Abstract

Transgenes are particularly prone to epigenetic silencing in the *C. elegans* germline. Here, we describe a protocol to insert introns containing a class of non-coding DNA named Periodic A_n/T_n Clusters (PATCs) into synthetic transgenes. PATCs can protect transgenes from position-dependent silencing (Position Effect Variegation, PEV) and from silencing in simple extra-chromosomal arrays. Using a set of simple design rules, it is possible to routinely insert up to three PATC-rich introns into a synthetic transgene in a single reaction.

Introduction

Reagents

BsaI-HF restriction enzyme (#R3733, New England Biolabs)

T4 ligase (#L603-HC-L, Enzymatics)

Exonuclease V (#M0345L, NEB)

One Shot Top10 competent cells (#C404010, ThermoFisher)

Zymoclean Gel DNA recovery kit (#D4001, Zymo Research)

Standard LB selective plates with Kanamycin or Ampicillin.

Golden-gate compatible donor vectors with introns (https://www.addgene.org/Christian_Froekjaer-Jensen/)

Equipment

Standard thermocycler

Incubator (37 degrees C)

Procedure

1. Mix plasmids for reaction

Add 150 ng of the synthetic transgene and an equimolar amount of the plasmids containing introns. Add molecular biology quality water to 12 ul. Mix plasmids in a PCR tube placed on ice.

2. Add enzymes to the reaction

Add the following enzymes to the reaction.

1.0 ul BsaI-HF

1.5 ul 10x Ligase buffer (supplied with ligase)

0.5 ul T4 Ligase

for a total volume of 15 ul.

Mix the enzymes in a PCR tube placed on ice.

3. Perform Golden Gate reaction in a PCR machine.

Program a thermocycler for 50x cycles of:

5 min @ 37°C (digestion)

5 min @ 16°C (ligation)

Let the reaction run overnight (it takes ~ 8 hours)

4. Remove background plasmids with recBCD enzyme.

Add the following enzymes to the reaction mix

2 ul 10 mM ATP (supplied with recBCD)

1 ul recBCD enzyme (Exonuclease V or "PlasmidSafe")

2 ul NEB buffer 4 (supplied with recBCD)

0.5 ul BsaI-HF

Incubate in a thermocycler at 37°C for 30 minutes, followed by 80°C for 30 minutes.

Note: recBCD digests linear double-stranded DNA but not closed circular plasmids.

5. Purify the reaction with the gel purification kit.

To clean up the reaction and concentrate the mix, add 200 ul of the ADB buffer from the Zymo gel purification kit. Run over a gel purification column and wash twice with 200 ul of the wash buffer. Make sure to completely eliminate the wash buffer in the last spin by emptying the tube with the flow-through and repeating the spin for 1 minute at the highest speed.

6. Transform Top10 chemically competent bacteria with 2 ul and plate on an antibiotic plate.

Perform standard transformation into Top10 cells. Shake for 1 hour before the transformation. Plate on the appropriate antibiotic resistance plate (Kan or Amp).

7. Pick colonies and digest plasmids.

Pick 2-4 colonies and verify plasmid by restriction enzyme digest.

Troubleshooting

Time Taken

The whole procedure takes approximately two days, including several long incubation steps.

Anticipated Results

References

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