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Working

Protocols involving the pgMAX plasmid

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manabu murakami[1]

[1]Department of Pharmacology, Hirosaki University Graduate School of Medicine

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manabu murakami

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1 Protocols involving the pgMAX plasmid

Manabu Murakami
Department of Pharmacology, Hirosaki University Graduate School of Medicine, Hirosaki, 036-8562, Japan.
Corresponding to: mmura0123@hotmail.co.jp

[Abstract]
The pgMAX system involves direct subcloning of a DNA fragment into a prokaryotic DNA expression plasmid and rapid preparation of a mammalian expression vector.
The complete pgMAX protocol involves four steps.
1. **Subcloning**
Ligation of a blunt-end DNA fragment into the EcoRV site of pgMAX (prokaryotic mode)
2. **Prokaryotic analysis** (*E. coli*)
Plasmid DNA analysis and IPTG induction of protein expression.
3. **Conversion to mammalian mode**
Digestion of the recombinant plasmid with SwaI and PmeI and re-ligation.
4. **Transfection into mammalian cells**
Transient transfection in HEK 293T cells.

Background

The pgMAX system is a single expression plasmid system, which enables simple and rapid construction of prokaryotic and mammalian expression vectors within 1 week. The plasmid consists of two expression modes (a lac operon related to prokaryotic expression and a CMV promoter related to mammalian expression). For efficient selection of the insert-containing recombinant clone, the plasmid contains an inhibitory peptide of DNA synthesis with rare-cutting enzymes (the inhibitory unit; iUnit).

Advantages

This system is rapid, simple and cost-effective. It can be widely used for general gene expression analyses. It is based on standard molecular biological techniques and requires no special methods (1).

Procedure

1. Subcloning

In this critical step, blunt-end DNA (usually a PCR fragment with a proofreading DNA polymerase), the EcoRV-restricted pgMAX plasmid and ligase are mixed and incubated at 16 °C for 30 min, per standard ligation. We prefer to use the Takara DNA Ligation Kit ver. 2.1 for DNA ligation. DNA ligation is followed by a standard transformation procedure using competent cells (2). After 16 h of incubation at 37 °C on

plates containing ampicillin (150 µg/ml) and IPTG (1 mM) (for lac operon induction), colonies were collected. For library construction, EcoRI, NotI and XbaI sites were used for linker-based ligation.

TIPS: Good PCR amplification and DNA purification are mandatory. (following agarose gel *electrophoresis, DNA fragments should be digested and purified).*

2. Prokaryotic analysis

Incubate an inoculated single colony in LB broth (~5 ml) containing ampicillin (150 µg/ml) and IPTG (1 mM). After a 12–16 h incubation at 37 °C with vigorous shaking (~225 rpm), collect 3 ml of the culture for plasmid DNA prep and 0.5 ml for protein analysis.
For the plasmid DNA analysis, purify the plasmid DNA from the cells (3 ml of the overnight culture) by standard plasmid DNA prep (mini-prep).
For the protein analysis, harvest the cells (~0.5 ml of the overnight culture) by centrifugation (14000 rpm in the Eppendorf 5418 centrifuge), re-suspend and incubate in 100 µl cell lysis buffer (1 mM EDTA, 1 mg/ml lysozyme) at room temperature for 15 min (3). For Western blot analysis, load 5 % of the cell lysate.

3. Conversion to mammalian expression mode

After confirmation of insertion of the desired gene, convert the pgMAX plasmid from the prokaryotic to mammalian expression mode. To this end, digest the plasmid (~0.3 µg) with PmeI and SwaI and re-ligate the plasmid using standard techniques. After transformation, cells are plated on LB agar plates containing ampicillin (150 µg/ml) and incubated at 37 °C for 12–16 h. If time is limited, 50 % of the transformants can be directly incubated in LB broth containing ampicillin (150 µg/ml, i.e., the short-cut method). However, the short-cut method can be problematic if any un-cut plasmid remains.

4. **Transfection into mammalian cells**

After plasmid DNA purification (mini-prep), the plasmid DNA is ready for transfection into HEK 293T cells using Lipofectamine (Thermo Fisher Scientific) or X-treme (Sigma-Aldrich)

Details of the pgMAX plasmid

The pgMAX plasmid contains a CMV promoter and poly A sequences for mammalian expression. Located between the CMV promoter and poly A sequences is a lac promoter unit (lac promoter and lac operator), which contains SwaI (at the 5'-terminal end) and PmeI (at the 3'-terminal end) restriction sites, Kozak and Flag tag sequences followed by multiple cloning sites (EcoRI, EcoRV and XhoI) and an inhibitory unit (iUnit) that inhibits DNA synthesis in *E. coli*.

Standard protocols

Vector preparation (subcloning)

PgMAX 1 µg
H_2O up to 17.5 µl
B buffer (Roche, Sigma-Aldrich) 2 µl
EcoRV-HF (New England Biolabs) 0.5 µl
Total 20 µl
37 °C for 20 min
80 °C for 15 min
Cool on ice for 3 min (or store at −20 °C)

TIPS: EcoRV-HF from New England Biolabs can be heat-inactivated.

Ligation

Blunt-end DNA 1.0 µl (usually ~0.2 µg)
Vector 0.5 µl
Ligase 1.5 µl (Takara DNA Ligation Kit ver. 2.1)
Total 3.0 µl
16 °C for 30 min

Transformation

Prepare competent cells according to the Inoue Protocol (2). High-efficiency ultra-competent cells are usually not necessary.

References

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Sequence of pgMAX

GACGGATCGGGAGATCTCCCGATCCCCTATGGTCGACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGTATCTGCTCCCTGCTTGT
GTGTTGGAGGTCGCTGAGTAGTGCGCGAGCAAAATTTAAGCTACAACAAGGCAAGGCTTGACCGACAATTGCATGAAGAATCTGCTTAGGGTTAG
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