Reporter Vectors – For Promoter Studies

Application Guide

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Introduction

The pRMT reporter vectors are designed for promoter studies, testing promoter or putative promoter activities under different conditions using luciferase as a reporter (pRMT-Luc) or GFP as a reporter (pRMT-tGFP). The reporter genes in the vector have no promoter (promoterless reporter vectors), but there are multiple cloning sites (MCS) to clone your promoter of interest; the activity of your sequence will drive the reporter expression.

Features:

- *Low background*, reporter genes are not transcribed in the promoterless vectors
- *Two reporter options,* luciferase or GFP
- *Mammalian selection marker,* Neomycin resistant gene for stable selection

pRMT-Luc, the Luciferase Reporter Vector

Package Contents and Related Products

The following components are included:

- One (1) vial containing pRMT-Luc vector (SKU: PR100001), 10 ug lyophilized plasmid DNA*.
- Certificate of Analysis

** The cDNA clone is shipped at room temperature, but should be kept at -20℃ for long-term storage. If properly stored, clones are guaranteed to be stable for 12 months.*

Related Optional Reagents

Restriction enzymes and buffers

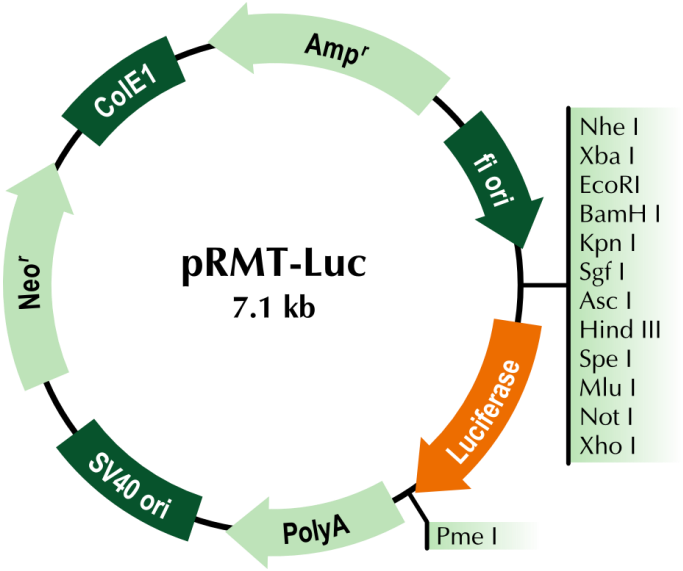
Sgf I/ASIS I from Fermentas

Mlu I from Fermentas or New England Biolabs
Nuclease free water
T4 DNA ligase and buffer
LB agar plates with ampicillin, 100 μg/ml
LB broth (10 g/L Tryptone, 5 g/L Yeast Extract, 10 g/L NaCl. Adjust pH to 7.0 with 1 N NaOH)
DNA purification reagents

Notice to purchaser

This product is for research use only. Use in and/or for diagnostics and therapeutics is strictly prohibited. By opening and using the product, the purchaser agrees to the following: The plasmids may not be distributed, resold, modified for resale or used to manufacture commercial products without prior written approval from OriGene Technologies, Inc. If you do not agree to the above conditions, please return the UNOPENED product to OriGene Technologies, Inc. within ten (10) days of receipt for a full refund.

Fig. 1 pRMT-Luc vector map and MCS



Nhe I — EcoR I — BamH I — Kpn I

GCTAGCTCTAGAAGAATTTTGTAATACGACTCACTATAGGGCGGCCGGGAATTCGTCGACTGGATCCGGTACC

Sgf I — Asc I — Hind III — Spe I — Mlu I

GAGGAGATCTGCCGCCGCGATCGCCGGCGCGCCAGATCTCAAGCTTAACTAGTTAGCGGACCGACGCGTAC

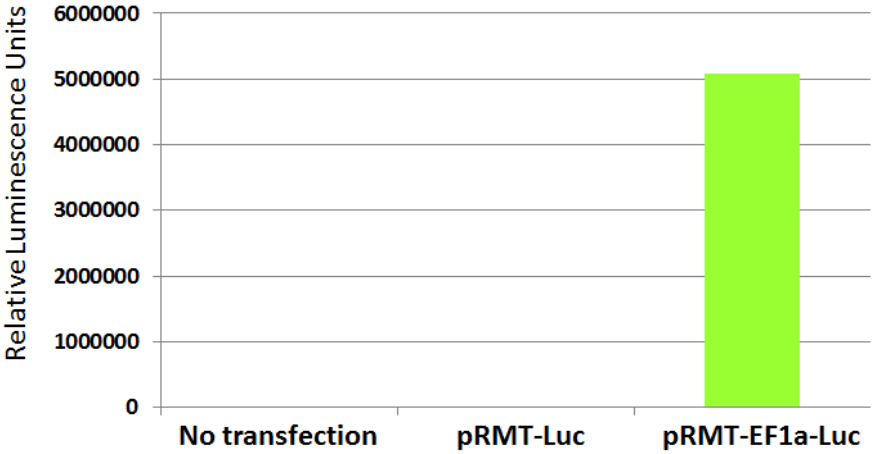
Not I — Xho I

GCGGCCGCTCGAG

pRMT-Luc validation data

To validate the vector, a human EF1a promoter was cloned into Nhe1 and Sgf1 sites to create pRMT-EF1a-luc expression vector. A promoter-less control vector (pRMT-Luc) and pRMT-EF1a-Luc were transfected to HEK293T cells in a 96 well plate using MegaTran 1.0. After 72 hours, Lucifearse activities were assayed using the Luciferase assay kit (cat# PR300001)

Fig. 2 Luciferase data of pRMT-EF1a-Luc



The data above shows the promoterless reporter vector, pRMT-Luc does not have much luciferase activity, very low background in the vector.

Cloning the promoter of interest into pRMT-Luc vector

As show in the vector map of pRMT-Luc, there is MCS 5' end of luciferase gene. The promoter of interest can be cloned at the MCS site and the promoter will drive the luciferase expression. You can then study the promoter activities under different conditions.

pRMT-tGFP, the GFP reporter vector

Package Contents and Related Products

The following components are included:

- One (1) vial containing pRMT-tGFP vector (SKU: PR100002), 10 ug lyophilized plasmid DNA*.
- Certificate of Analysis

*

The cDNA clone is shipped at room temperature, but should be kept at -20°C for long-term storage. If properly stored, clones are guaranteed to be stable for 12 months.

Related Optional Reagents

Restriction enzymes and buffers
Sgf I/ASIS I from Fermentas
Mlu I from Fermentas or New England Biolabs Nuclease free water
T4 DNA ligase and buffer
LB agar plates with ampicillin, 100 μg/ml
LB broth (10 g/L Tryptone, 5 g/L Yeast Extract, 10 g/L NaCl. Adjust pH to 7.0 with 1 N NaOH)
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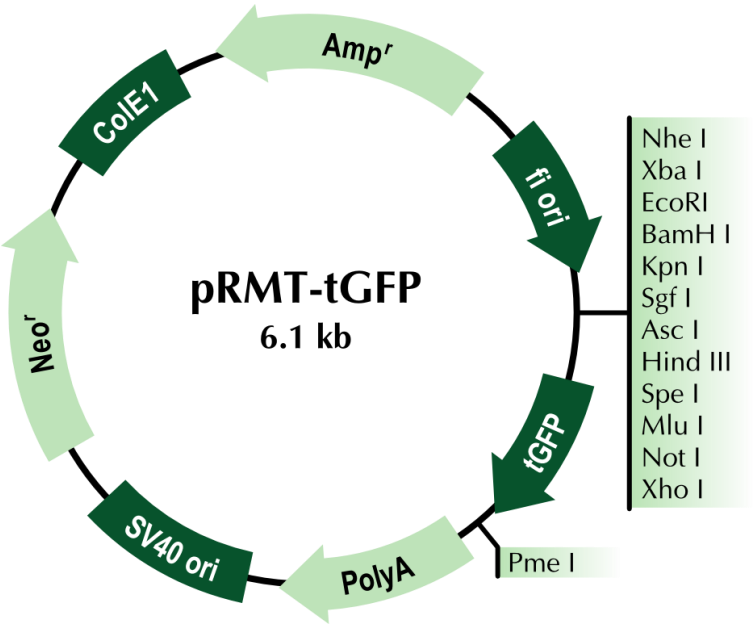
products without prior written approval from OriGene Technologies, Inc. If you do not agree to the above conditions, please return the UNOPENED product to OriGene Technologies, Inc. within ten (10) days of receipt for a full refund.

Fig. 3 pRMT-tGFP vector map and MCS



Nhe I — EcoR I — BamH I — Kpn I

GCTAGCTCTAGAAGAATTTTGTAATACGACTCACTATAGGGCGGCCGGGAATTCGTCGACTGGATCCGGTACC

Sgf I — Asc I — Hind III — Spe I — Mlu I

GAGGAGATCTGCCGCCGCGATCGCCGGCGCGCCAGATCTCAAGCTTAACTAGTTAGCGGACCGACGCGTAC

Not I — Xho I

GCGGCCGCTCGAG

Cloning the promoter of interest into pRMT-tGFP vector

As show in the vector map of pRMT-tGFP, there is MCS 5' end of GFP gene. The promoter of interest can be cloned at the MCS; the promoter will drive tGFP expression. Therefore, you can study the promoter under different conditions using the fluorescence.

Luciferase assay protocol

The protocol shown below is from OriGene's firefly luciferase assay kit (cat# PR300001). Please follow the manufacturer's protocol if you use a different kit.

Preparation of Cell Lysates

A. Preparation of Firefly Luciferase Lysis Buffer

Prepare 1X firefly luciferase lysis buffer by adding 1 volume of 5X firefly luciferase lysis buffer to 4 volumes of dH_2O and mixing well. 1X lysis buffer may be stored at 4°C for up to one month. Store 5X firefly luciferase lysis buffer at –20°C.

B. Lysis of Cells Cultured in Multiwell Plates

1) Remove growth medium from cultured cells and gently add a sufficient volume of phosphate buffered saline (PBS) to wash the surface of the culture vessel. Add 1X firefly lysis buffer to each well using the volume recommended below for each type of culture plate:

Wells/plate	Lysis buffer/well
6 well	500 uL
12 well	250 uL
24 well	100 uL
48 well	65 uL
96 well	20 uL

2) Place the culture plates on a rocking platform or orbital shaker with gentle rocking/shaking to ensure complete and even coverage of the cell monolayer with 1X firefly luciferase lysis buffer. Rock the culture plates at room temperature for 15 minutes.

Note: Cultures that are overgrown are often more resistant to complete lysis and typically require an increased volume of firefly luciferase lysis buffer and/or an extended treatment period to ensure complete lysis. Lifting cells from the plate will facilitate the process of cell lysis.

Transfer the lysate to a tube or vial. Optional: the lysate can be cleared by centrifugation for 30 seconds at top speed in a refrigerated microcentrifuge and transferred into a new tube. Place at 4°C for until ready to assay. Store lysates at -20^{o}C or -70^{o}C if assay will not be performed on the same day.

Firefly Luciferase Assay

A. Preparation of Firefly Luciferase Working Solution

1) Prepare an adequate volume of working solution to

perform the desired number of firefly luciferase assays (100 uL working solution per assay). Thaw a bottle of firefly luciferase assay buffer and pipette a desired volume (5 mL or 50 mL) from the bottle into a clean container.

2) Dissolve the supplied D-luciferin in the firefly luciferase assay buffer from step 1 at a final concentration of 0.2 mg/mL. Dissolve one vial of D-luciferin (component 99907, 1 mg/vial) in 5 mL assay buffer. Firefly luciferase working solution (D-luciferin + firefly luciferase assay buffer) should be prepared fresh and used within a day.

Note: D-luciferin in assay buffer has limited stability. If you need less than 5 mL or 50 mL luciferase working solution as described in step 2, you may dissolve D-luciferin in dH_2O as 10X or 50X stock solution and store it in aliquots at -20^{o}C or below for repeated use. The D-luciferin stock solution should be stable for at least one month, depending on the frequency of freeze-thaw cycles. The required volume of working solution can be prepared by diluting the stock solution in firefly luciferase assay buffer to a final concentration of 0.2 mg/mL D-luciferin.

B. Standard Protocol

For manual luminometer:

1) Set up luminometer with appropriate parameters (delay time, integration time, sensitivity, etc.).
2) Add 100 uL of firefly luciferase working solution to the luminometer tube.
3) Add 20 uL of cell lysate and mix quickly by vortexing or flicking the tube with a finger.
4) Place tube in luminometer and initiate

measurement. Luminescence is normally integrated over 10 seconds without delay. Other integration times may also be used.

5) If the luminometer is not connected to a printer or computer, record the firefly luciferase activity measurement.
6) Discard the reaction tube, and proceed to the next firefly luciferase reaction.

For luminometer with injector:

1) Format the luminometer so that the injector dispenses 100 uL. Prime the injector with firefly luciferase working solution.
2) For each reaction, carefully add 20 uL of cell lysate to an individual luminometer tube or to the wells of a multiwell plate.
3) Place the samples in a luminometer.
4) Initiate measurement. This will cause firefly luciferase working solution to be injected into the reaction vessel and the measurement to be subsequently taken. Luminescence is normally integrated over 10 seconds without delay. Other integration times also may be used.
5) Record the firefly luciferase activity measurement.
6) If using a single tube luminometer, discard the reaction tube, and proceed to the next firefly luciferase reaction. If using a plate luminometer, the luminometer will automatically begin injecting firefly luciferase working solution into the next well indicated on the luminometer plate.

References

1. Alam, J. and J.L. Cook. 1990. Reporter genes: Application to the study of mammalian gene transcription. Anal. Biochem. 188:245-254.
2. Bronstein, I., et al. 1994. Chemiluminescent and bioluminescent reporter gene assays. Anal. Biochem. 219:169-181.
3. Gould, S.J. and S. Subramani. 1988. Firefly luciferase as a tool in molecular and cell biology. Anal. Biochem. 175:5-13.
4. Brasier, A.R., et al. 1989. Optimized use of the Firefly luciferase assay as a reporter gene in mammalian cell lines. BioTechniques. 7:1116-1122.