Dharmacon™
RNAi, Gene Expression & Gene Editing

E. coli Promoter Collection

This protocol is for *E. coli* Promoter Collection.

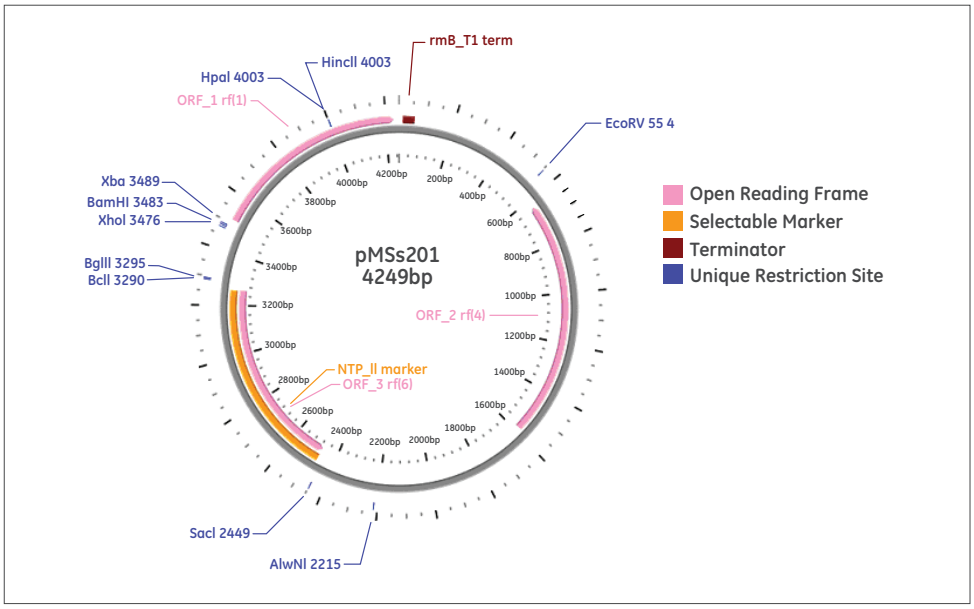

Figure 1. pMS201 vector map.

Table 1. Antibiotic resistances conveyed by pMS201.

Antibiotic	Concentration	Utility
Kanamycin	25 µg/mL	Bacterial selection marker

Product Description:

Researchers at the Weizmann Institute of Science have produced a collection of *E. coli* strains that enables monitoring of gene expression at high temporal resolution in living cells. Each of the reporter strains has a bright, fast-folding green fluorescent protein (GFP) fused to a full-length copy of an *E. coli* promoter in a low-copy plasmid. This collection includes more than 1900 promoters (out of 2500 in the entire genome) for *E. coli* K12 strain MG1655 and enables measurement of gene expression at a resolution of minutes with high accuracy and reproducibility. Performing experiments in a multi-well fluorimeter using FACS or time-lapse fluorescence microscopy has the necessary sensitivity to measure gene expression in individual cells.

Protocol I - Replication:

Materials for replication

- 2x LB broth (low salt)
- Peptone, granulated, 2 kg - Difco
- Yeast Extract, 500 g, granulated
- Glycerol
- Kanamycin

2x LB broth (low salt) medium preparation

2x LB broth	20 g/L
Peptone	10 g/L
Yeast Extract	5 g/L

Appropriate antibiotic(s) at recommended concentration(s)

Item	Vendor	Cat #
2x LB broth (low salt)	Thermo Scientific	BP1427-500
Peptone, graulated, 2 kg-Difco	Thermo Scientific	BP9725-2
Yeast Extract, 500 g granulated	Thermo Scientific	BP1422-500
NaCl	Thermo Scientific	BP3581
Glycerol	Thermo Scientific	BP2291
Kanamycin, 5 g	Thermo Scientific	BP906-5

For archive replication, grow all clones at 37 °C in 2x LB broth (low salt) medium plus 25 µg/mL kanamycin. Prepare medium with 8% glycerol* and the appropriate antibiotics.

***Glycerol should be omitted from the medium if you are culturing for plasmid preparation. If making copies of the constructs for long-term storage at -80 °C, 8% glycerol is required.**

Freeze at -80 °C for long term storage. Avoid long periods of storage at room temperature or higher in order to control background recombination products.

Protocol II - Plasmid Preparation:

Materials for replication

For plasmid preparation, grow all clones at 37 °C in 2x LB broth (low salt) medium plus 25 µg/mL kanamycin.

Most plasmid mini-prep kits recommend a culture volume of 1-10 mL for good yield. For these constructs, 5 mL of culture can be used for one plasmid mini-prep generally producing 5-10 µg of plasmid DNA.

1. Upon receiving your glycerol stock(s), store at -80 °C until ready to begin.
2. To prepare plasmid DNA, first thaw your glycerol stock culture and pulse vortex to resuspend any *E. coli* that may have settled to the bottom of the tube.
3. Take a 10 µL inoculum from the glycerol stock into 3-5 mL of 2x LB broth (low salt) with 25 µg/mL kanamycin. Return the glycerol stock(s) to -80 °C.
   Note: If a larger culture volume is desired, incubate the 3-5 mL culture for 8 hours at 37 °C with shaking and use as a starter inoculum. Dilute the starter culture 1:500-1:1000 into the larger volume.
4. Incubate at 37 °C for 18-19 hours with vigorous shaking.
5. Pellet the 3-5 mL culture and begin preparation of plasmid DNA.
6. Run 3-5 µL of the plasmid DNA on a 1% agarose gel. pMS201 without ORF is 4260 bps.

What clones are part of my collection?

A CD containing the data for this collection will be shipped with each collection. This data file can be downloaded from the *E. coli* Promoter product page on our website.

What antibiotic should I use?

You should grow all *E.coli* Promoter clones in 2x LB broth (low salt) with 25 µg/mL kanamycin for archive replication.

References:

1. Kalir S *et al* (2001). Ordering genes in a flagella pathway by analysis of expression kinetics from living bacteria. *Science* 292, 2080–2083.
2. Ronen M *et al* (2002). Assigning numbers to the arrows: parameterizing a gene regulation network by using accurate expression kinetics. *Proc. Natl. Acad. Sci.* USA 99, 10555–10560.
3. Setty *et al* (2003). *Proc. Natl. Acad. Sci.* USA 100, 7702–7707.
4. Zaslaver A *et al* (2004). Just-in-time transcription program in metabolic pathways. *Nature Genetics* 36, 486–491.
5. Zaslaver A, Anat Bren, Michal Ronen, Shalev Itzkovitz, Ilya Kikoin, Seagull Shavit, Wolfram Liebermeister, Michael G Surette & Uri Alon (2006). A comprehensive library of fluorescent transcriptional reporters for *Escherichia coli*. *Nature Methods* 3, 623–628.

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