PROTOCOL

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E. coli promoter collection

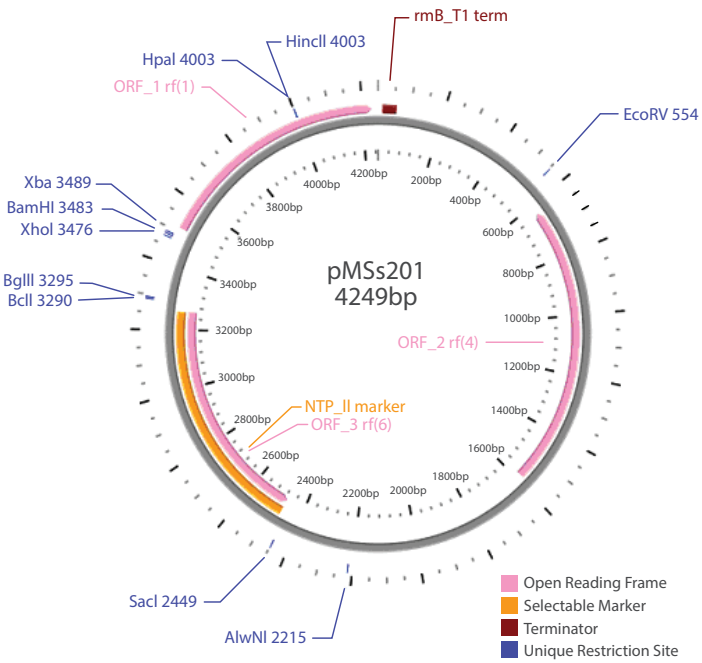

Figure 1. pMS201 vector map.

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

1 x LB broth	10 g/L
Peptone	10 g/L
Yeast Extract	5 g/L

Appropriate antibiotic(s) at recommended concentration(s)

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.

> 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 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 USB drive 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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