TECHNICAL MANUAL

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INSPIRED CELL SOLUTIONS

Dharmacon™ *C. elegans* promoter clones and library

Catalog #: PCE1181, PCE1182

Product description

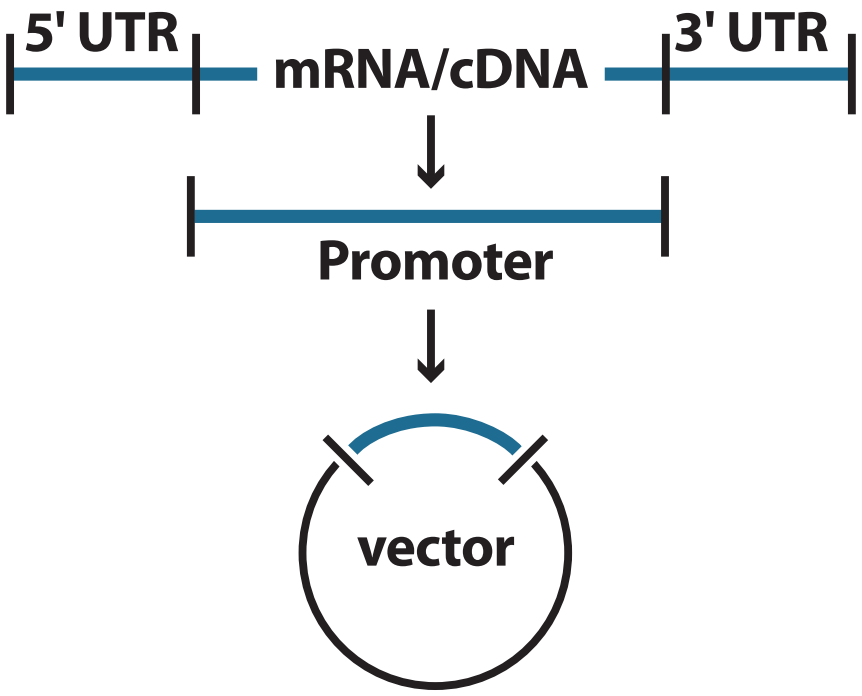
The *C. elegans* Promoter Library includes clones containing the predicted promoters for thousands of genes. For each gene, the "promoter" is defined as the upstream intergenic region (IGR), specifically the region of DNA that spans from the ATG of the open reading frame (ORF) to the extremity of the closest 5' ORF. For the production of the Promoter Library an upper size limit of 2 kb for the PCR fragments was selected to ensure high cloning efficiency. This size restriction implies that regulatory elements located more than 2 kb upstream of the ATG will not be included. However, Dupuy *et. al*[1] note that many of the published expression patterns derived from small "promoters" encompass only a fraction of the actual IGR, suggesting that the inclusion of the entire IGR is not always necessary to obtain specific expression. Moreover, sequence comparison between *C. elegans* and *C. briggsae* shows a dramatic decrease in the level of homology 1,500 bp upstream of the ATG for most genes with a long IGR. Thus, the Promoter Library should result in the inclusion of most upstream cis-regulatory elements. Additionally, several predicted intergenic regions are extremely small (the smallest IGR is only 3 bp), prompting Dupuy to set a lower size limit of 300 bp.

Shipping and storage

Individual clones are shipped at room temperature and may be stored for up to one week at +4 °C. They may stored indefinitely at –80 °C.

Plates are shipped on dry ice and should be stored at –80 °C.

To allow any CO_2 that may have dissolved into the media from the dry ice in shipping to dissipate, please store plates at -80°C for at least 48 hours before thawing.



Glycerol stock

Culture clones are provided in LB broth with 8% glycerol, and kanamycin at a concentration of 100 µg/mL.

Replication of individual clones

Once the clone has been streak isolated and the identity of the strain has been confirmed, we recommend making a stock of the pure culture. Grow the pure culture in LB broth + the appropriate antibiotic. Transfer 920 µL of culture into a polypropylene tube and add 80 µL sterile glycerol to make an 8% glycerol freezing solution. Vortex the culture to evenly mix the glycerol throughout the culture. The culture can be stored indefinitely at -80°C.

Replication of 96-well plates

Prepare Target Plates

- Dispense ~160 µl of sterile LB media into 96-well microtiter plates. The LB should be supplemented with 8% glycerol and the appropriate antibiotic.

Prepare Source Plates

- Remove the foil seals from the source plates. Removing the seals while the source plates are frozen will minimize cross-contamination.
- Thaw the source plates with the lids on. Wipe any condensation underneath the lid with a Kimwipe dampened with alcohol.

Replicate

- Gently place a disposable replicator into the thawed source plate and lightly move the replicator around inside the well to mix the culture. Make sure to scrape the bottom of the plate of the well.
- Gently remove the replicator from the source plate and gently place the replicator into the target plate. Gently move the replicator back and forth in the target plate to transfer cells.
- Discard the replicator.
- Place the lids back on the source plates and target plates.
- Seal the source plates, being mindful to avoid cross contamination.
- Repeat this process until all plates have been replicated.
- Return the source plates to the –80 °C freezer.
- Place the inoculated target plates in a 37 °C incubator. Incubate the plates for 12–24 hours.

References

1. Denis Dupuy, Qianru Li, Bart Deplancke, Mike Boxem, Tong Hao, Philippe Lamesch, Reynaldo Sequerra, Stephanie Bozak, Lynn Doucette- Stamm, Ian A. Hope, David E. Hill, Albertha J.M. Walhout, and Marc Vidal. *Caenorhabditis elegans Promoterome* **1.1**: a Gateway to Localizome Projects.

FAQS/troubleshooting

We provide certain clone resources developed by leading academic laboratories. Many of these resources address the needs of specialized research communities not served by other commercial entities. In order to provide these as a public resource, we depend on the contributing academic laboratories for quality control.

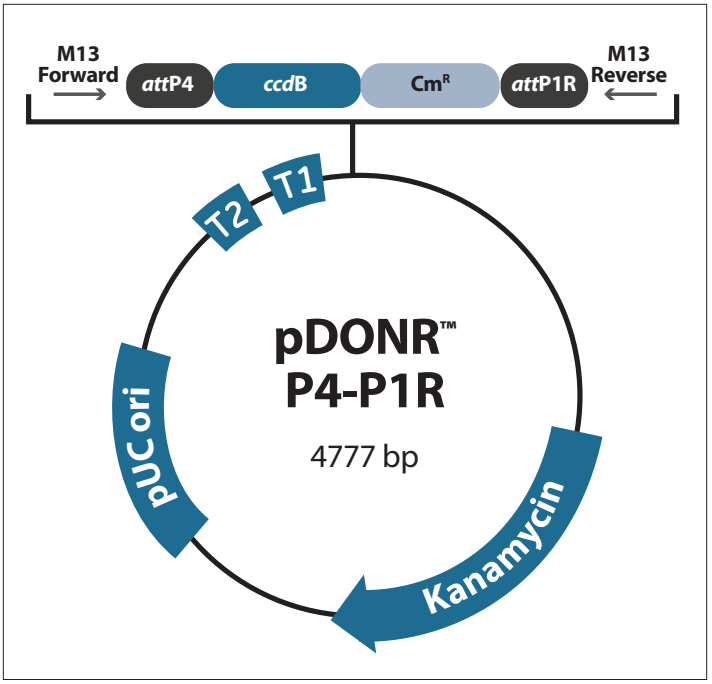


Figure 2. Thermo Scientific™ pDONR™ P4-P1R vector map
rrnB T2 transcription termination sequence: bases 268-295 (c)
rrnB T1 transcription termination sequence: bases 427-470 (c)
M13 Forward (-20) priming site: bases 537-552
attP4 recombination site: bases 593-824 (c)
ccdB gene: bases 1181-1486 (c)
Chloramphenicol resistance gene: bases 1828-2487 (c)
attP1R recombination site: bases 2748-2979 (c)
M13 Reverse priming site: bases 3042-3058
Kanamycin resistance gene: bases 3171-3980
pUC origin: bases 4101-4774
(c) = complementary strand

Useful Websites

WorfDB, the *C. elegans* ORFeome Cloning Project Database
worfdb.dfci.harvard.edu

WormBase web site
wormbase.org

If you have any questions, contact

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