pORF-mcs
An expression vector containing a multiple cloning site.
Catalog # porf-mcs
For research use only
Version # 05G27-SV

PRODUCT INFORMATION
Content:
- 1 disk of lyophilized GT100 E. coli bacteria transformed by pORF-mcs.
- GT100 genotype is: F-, merA, Δ(mrr-hsdRMS-mcrBC), Ø80lacZΔM15, ΔlacX74, recA1, endA1.
- 4 pouches of E. coli Fast-Media® Amp.

Storage and stability:
- Products are shipped at room temperature.
- Transformed bacteria should be stored at -20°C and are stable up to 1 year.
- Store E. coli Fast-Media® Amp at room temperature. Fast-Media® pouches are stable 18 months when stored properly.

Quality control:
- Plasmid construct has been confirmed by restriction analysis and sequencing.
- Bacteria have been lyophilized, and their viability upon resuspension has been verified.

GENERAL PRODUCT USE
pORF-mcs is a ready-made expression vector containing the hybrid EF1α-HTLV promoter and a multiple cloning site.

pORF-mcs may be used for:
Cloning in a gene of interest. Five unique restriction sites comprise the MCS facilitating cloning of genes. Cloned genes will be under the control of the EF1α-HTLV promoter.
As an “empty” control vector. Since pORF-mcs does not contain a therapeutic gene, it can be used in conjunction with other vectors of the pORF family to serve as an experimental control.

PLASMID FEATURES
• EF-1α / HTLV hybrid promoter is a composite promoter comprised of the Elongation Factor-1α (EF-1α) promoter1 and 5’ untranslated region of the Human T-Cell Leukemia Virus (HTLV). EF-1α utilizes type 2 promoter that encodes for a “housekeeping” gene. The promoter is stronger than CMV and is expressed at high levels in all cell cycles and lower levels during G0 phase. The promoter is also non-tissue specific; it is highly expressed in all cell types. The R segment and part of the U5 sequence (R-U5’) of the HTLV Type 1 Long Terminal Repeat has been coupled to the EF-1α promoter to enhance stability of DNA and RNA. This modification not only increases steady state transcription, but also significantly increases translation efficiency possibly through mRNA stabilization.
• Intronic UTR: InvivoGen utilizes an inducible promoter for the second transcriptional unit that is spliced out as an intron in mammalian cells. LacI expression causes overproduction of Lac repressor protein acting on the bacterial promoter to repress the expression of the gene. This safeguard is essential when the second transcription gene product is toxic to bacterial promoter to repress the expression of the gene. This safeguard is necessary to completely dissolve the powder into solution. But be careful to avoid overboiling and volume loss.

METHODS
Growth of pORF-transformed bacteria:
Use sterile conditions to do the following:
1- Resuspend the lyophilized E. coli by adding 1 ml of LB medium in the tube containing the disk. Let sit for 5 minutes. Mix gently by inverting the tube several times.
2- Streak bacteria taken from this suspension on an ampicillin LB agar plate prepared with the E. coli Fast-Media® Amp agar provided (see below).
3- Place the plate in an incubator at 37°C overnight.
4- Isolate a single colony and grow the bacteria in LB supplemented with ampicillin using the Fast-Media® Amp liquid provided (see below).
5- Extract the pORF plasmid DNA using the method of your choice.

Selection of bacteria with E. coli Fast-Media Amp:
E. coli Fast-Media® Amp is a new, fast and convenient way to prepare liquid and solid media for bacterial culture by using only a microwave. E. coli Fast-Media® Amp is a TB (liquid) or LB (solid) based medium with ampicillin, and contains stabilizers.
E. coli Fast-Media® Amp can be ordered separately (catalog code # fas-am-l, fas-am-s, fas-am-x).

Method:
1- Pour the contents of a pouch into a clean borosilicate glass bottle or flask.
2- Add 200 ml of distilled water to the flask.
3- Heat in a microwave on MEDIUM power setting (about 400Watts), until bubbles start appearing (approximately 3 minutes).
4- Swirl gently to mix the preparation.
5- Reheat the media for 30 seconds and gently swirl again. Repeat as necessary to completely dissolve the powder into solution. But be careful to avoid overboiling and volume loss.
6- Let agar medium cool to 37°C before pouring plates. Let liquid media cool to 37°C before seeding bacteria.

Note: Do not reheat solidified Fast-Media® as the antibiotic will be permanently destroyed by the procedure.

TECHNICAL SUPPORT
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