pWHERE Kit
An optimized vector for mouse and rat transgenesis
Catalog # kwhere
For research use only
Version # 03G18-SV

PRODUCT INFORMATION

Content:
- 20 µg of pWHERE is provided as lyophilized DNA.
- 10 µg of pWHERE Control with the rat EF-1α promoter provided as lyophilized DNA.
- 4 pouches of E. coli Fast-Media® Amp (2 pouches for liquid and 2 for solid media)

Storage and Stability:
- Product is shipped at room temperature.
- Lyophilized DNA should be resuspended upon receipt and stored at -20°C (see Methods). Lyophilized DNA is stable 12 months at -20°C. Resuspended DNA is stable more than one year at -20°C. Avoid repeated freeze-thaw cycles.
- Bacteria should be stored at -20°C. Bacteria are stable up to one year when properly stored.
- Store E. coli Fast-Media® Amp at room temperature. Fast-Media® is stable 18 months when stored properly.

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

GENERAL PRODUCT USE

The pWHERE plasmid was designed for studies of temporal expression and tissue distribution of your promoter of interest, cloned within an insulated LacZ cassette, in transgenic mice and rats.

A multiple cloning site (MCS) has been added upstream of the LacZ gene for convenient cloning of your promoter of interest. The MCS contains several restriction sites that are compatible with many other enzymes, thus facilitating cloning. Furthermore, the E. coli region is flanked on either side by the well cutting 8 bp-recognizing restriction enzyme Pac I that enables linearization and easy excision of the E. coli region.

PLASMID FEATURES

- **mH19 insulators** on either side of the lacZ transcription unit. Both insulators are expected to protect the integrated transcriptional LacZ unit from negative as well as positive influences from neighboring sequences. Insulator elements can be functionally identified by their ability to shield promoters from regulators in a position-dependent manner or by their ability to protect adjacent transgenes from position effects. The fragment of the differential methylated region (DMR) located between the mouse Igf2 and H19 acts as a powerful insulator.

The enhancer blocking activity of the DMD fragment is dependent upon four responsive elements to the vertebrate enhancer-blocking protein CTCF. Two mouse DMD fragments have been introduced in opposite orientation in the pWHERE plasmid to insulate your promoter of interest cloned upstream of the new CpG-free LacZ gene from the 5’ and 3’ adjacent regions at the integrated site in transgenic mice.

- **MCS:** The multiple cloning site, located downstream of the mH19 insulator, contains the following restriction sites:
  - Sda I, Avr II, Bam HI, Xho I, Sma I and Nco I
  - Sda I is compatible with Nsi I and Psi I
  - Avr II is compatible with Nhe I, Spe I and Xba I
  - Bam HI is compatible with Bgl II and Bcl I
  - Xho I is compatible with Ava I and Sal I
  - Nco I is compatible with Bsp HI and Bsp LU111
  - Sma I is compatible with any blunt end restriction enzyme.

- **rEF-1α promoter (pWHERE Control):** EF-1α is one of the most abundant proteins in eukaryotic cells and is expressed in almost all kinds of mammalian cells. The promoter of this ‘housekeeping’ gene exhibits a strong activity, higher than viral promoters such as SV40 and RSV promoters and, on the contrary to the CMV promoter, yields persistent expression of the transgene in vivo.

- **pMB1 ori:** a minimal E. coli origin of replication to limit vector size but with the same activity as the longer Ori.

- **Amp:** The ampicillin resistance gene allows the selection of transformed E. coli carrying a pWHERE plasmid.

- **LacZ-ΔCpG NLS:** The E. coli lacZ gene codes for the enzyme β-galactosidase which catalyzes the hydrolysis of the substrate X-Gal to produce a blue color that is easily visualized under a microscope. A nuclear localization signal of SV40 large T has been inserted in the 5’ end of the lacZ gene to allow the targeting of the chimeric protein to the nucleus. To reduce the immunogenicity of this bacterial gene, InvivoGen has engineered a synthetic lacZnls gene that is entirely free of CpG motifs, whereas the wild type lacZ gene contains 298 CpG dinucleotides.

- **EF1 pAn:** is a strong polyadenylation signal. InvivoGen uses a sequence starting after the stop codon of the EF1 cDNA and finishing after a bent structure rich in GT.

EXPERIMENTAL OUTLINE

- Clone your promoter into pWHERE mcs
- Select and isolate recombinant pWHERE
- Linearize recombinant pWHERE with Pac I
- Purify Pac I/Pac I fragment containing your transgene
- Prepare DNA for microinjection
- Generate transgenic lines

TECHNICAL SUPPORT
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METHODS

Plasmid resuspension:
Quickly spin the tube containing the lyophilized plasmid to pellet the DNA. To obtain a plasmid solution at 1µg/µl, resuspend the DNA in 20µl of sterile H2O. Store resuspended plasmid at -20°C.

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 liquid or solid based medium with ampicillin and contains stabilizers. *E. coli* Fast-Media® Amp can be ordered separately (catalog code # fas-am-b, fas-am-s).

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). **Do not heat a closed container. Do not autoclave Fast-Media®.**
4- Swirl gently to mix the preparation. **Be careful, the bottle and media are hot, use heatproof pads or gloves and care when handling.**
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 45˚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.

Pac I linearization of recombinant pWHERE:
1- Digest 10 µg recombinant pWHERE plasmid with 1 to 5 units of Pac I restriction enzyme.
**Note:** Pac I may be purchased from New England Biolabs and used at 0.1-0.5 unit per µg plasmid DNA.
2- Incubate at 37˚C for 1-2 hours.
3- Purify the fragment containing the LacZ expression cassette by agarose gel following your usual protocol.

References: