Biomyx Technology
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pHTS Reporter Vectors

Signal Transduction Pathway-Specific Luciferase Reporter Vectors for Establishing Stable Cell Lines

Catalog Numbers:

Hygromycin Resistant Vectors

pHTS-CRE	P2100
pHTS-GAS	P2105
pHTS-ISRE	P2110
pHTS-NFAT	P2115
pHTS-NFκB	P2120
pHTS-AP1	P2125
pHTS-MCS	P2130

Neomycin Resistant Vectors

pHTS-Neo-CRE	P2102
pHTS-Neo-GAS	P2107
pHTS-Neo-ISRE	P2112
pHTS-Neo-NFAT	P2117
pHTS-Neo-NFκB	P2122
pHTS-Neo-AP1	P2127
pHTS-Neo-MCS	P2132

About pHTS Vectors

Biomyx' firefly luciferase reporter vectors with *cis*-enhancer elements are designed for quick generation of stable cell lines to assess *in vivo* activation of signal transduction pathways that converge at the respective *cis*-enhancer elements although they can also be used for transient assays. This group of vectors covers signal transduction pathways converging at several common enhancer elements including CRE (cyclic AMP response element), GAS (interferon γ–activated sequence), ISRE (interferon-stimulated response element), AP1 (activator protein 1), NFAT (nuclear factor of activated T cells) and NFκB (binding sites for nuclear factor κB). Cloning vectors (pHTS-MCS, Cat# P2130 or pHTS-Neo-MCS, Cat# P2132) are also available so that one can create synthetic promoters to monitor new signaling pathways of interest.

Materials Provided:

Each kit contains:

1. pHTS plasmid: 40μg in 80μl TE (pH7.5), 0.5mg/ml.
2. pHTS-MCS plasmid: 10μg in 20μl TE (pH7.5), 0.5mg/ml.
3. Luc-B1: 0.5 nmole in 50 ul TE at 10 pmole/μl (~60 ng/μl). Sequencing primer that anneals to nt4875-4852 in pHTS-MCS with sequence is: 5'-gca gtt gct ctc cag cgg ttc cat-3'.
4. Product Information Sheet.

Receiving and Storage:

Upon receiving, spin the vials briefly in a microcentrifuge to collect the contents. Store the products at 2-8^{0}C if used immediately or, store at –20^{0}C for extended storage.

Propagate the Plasmids:

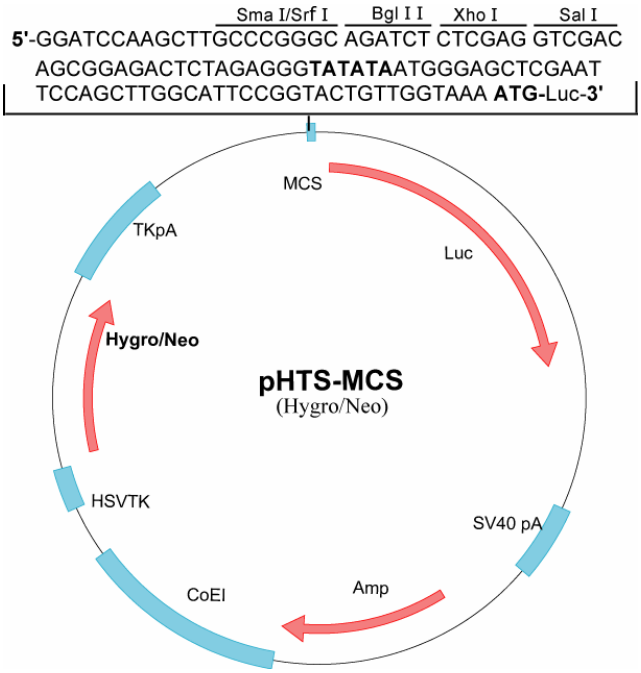
All pHTS vectors carry ColE1 origin and confer ampicillin resistance to transformed bacteria at 50 to 100μg/ml.

Eukaryotic Selection:

The pHTS vectors are available with either the hygromycin or neomycin phosphotransferase expression cassette for mammalian cell selection for stable cell lines with hygromycin or Geneticine® (G418), respectively. A killing curve with various hygromycin or G418 concentrations should be determined empirically for each cell lines. Concentrations of hygromycin ranging from 50 to 500μg/ml or higher have been reported for mammalian cells/cell lines selections. For G418 selection, 300ug/ml to 1mg/ml G418 may be used for mammalian cell selection.

Circular Map of pHTS-MCS:

The complete nucleotide sequences of pHTS vectors can be downloaded from www.biomyx.net .



Size of Plasmids:

The total length of pHTS-MCS is 7716 base pairs, and pHTS-Neo-MCS is 7473 base pairs.

Sequences of Enhancer Elements

All pHTS vectors have the same basic structure as pHTS-MCS except that the MCS sequence is replaced with tandem repeats of different enhancer elements as shown below. For example, the junction of the synthetic promoter and luciferase in pHTS-NFκB has the following sequence.

```
5′-GGATCCAAGCTAGGGGACTTTCCGCTGGGGACTTTCCGCTGGGGACTTTCCGC
TGGGGACTTTCCGCTGGGGACTTTCCGCGGTGACTCTAGAGGGTATATAATGGAAG
CTCGAATTCCAGCTTGGCATTCCGGTACTGTTGGTAAAATG(Luciferase)-3′
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Sequences of Enhancer Elements (continued)

Vector	Sequence of Enhancer Element
pHTS-AP1	(**TGACTAA**)6
pHTS-GAS	(**AGTTTCATATTACTCTAAATC**)4
pHTS-ISRE	(**TAG TTT CAC TTT CCC**)5
pHTS-CRE	(AGCC **TGACGTCA** GAG)4
pHTS-NFAT	(**GGAGGAAAAACTGTTTCATACAGAAGGCGT**)4
pHTS-NFkB	(T **GGGGACTTTCC** GC)5

(Consensus sequences of the respective enhancer elements are underlined; subscripts indicate the number of tandem repeats of the sequence within the brackets in the vector.)

Inserting new enhancer elements into pHTS-MCS

Cloning of your own *cis*-response elements: Complementary oligos of chosen *cis*-response elements may be synthesized and annealed with appropriate overhangs to be cloned in between Sma I and SalI of pHTS-MCS. For example, the following annealed dsDNA can be inserted into the SalI site of pHTS-MCS to create a reporter vector that is responsive to TGF-β.

```
5′-TCGATCTCAATCCACAATCTCGGAGTATGTCTAGACTGACAATG    -3′
3′-    AGAGTTAGGTGTTAGAGCCTCATACAGATCTGACTGTTACAGCT-5′
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Transfection and selection of mammalian cells

The provided plasmids are highly purified and ready for transfection into mammalian cells with any standard transfection methods such as LipofectAmine®, electroporation or Calcium Phosphate Precipitation. Refer to the manufacturers' instruction manuals for details of these transfection methods. Clones that harbor integrated pHTS vectors can be selected from the resulting transfected cells with hygromycin to establish stable cell lines.

Luciferase Activity Assay

There're several commercial sources for reagents prepared for luciferase extraction and activity assay (e.g. Promega, Stratagene). The following protocol is provided for quick reference only.

1. Remove media from cell and rinse twice with PBS and remove residual PBS.
2. Add 1x Lysis Buffer (e.g. 400μl per well of a six-well plate, see below for buffer components). Incubate the plate for 15 minutes at room temperature (RT) with gentle rocketing.
3. The lysates could be used in luciferase assay or be transferred to microcentrifuge tubes and stored at -80^{0}C.
4. Mix 5-20μl of cell lysate with 100μl of 1X Luciferase Assay Buffer in an appropriate tube (e.g. Falcon® 2054 polystyrene tube). All reagents should be brought to RT for assay.
5. Measure the light emission with a luminometer with an integration time of 10-30 seconds.

Buffers for Luciferase Activity Assay: *(Final concentrations are shown)*

Lysis Buffer (5X)		Assay Buffer (1X)	
40mM	Tricine (pH7.8)	40mM	Tricine (pH7.8)
50mM	NaCl	0.5mM	ATP
2mM	EDTA	10mM	$MgSO_4$
1mM	$MgSO_4$	0.5mM	EDTA
5mM	DTT	10mM	DTT
1%	Triton® X-100	0.5mM	Coenzyme A
		0.5mM	luciferin

Key References

1. Boulikas T. (1995) Phosphorylation of transcription factors and control of the cell cycle. *Critical Review in Eukaryotic Gene Expression* 5: 1-77
2. Decker, T. et al., 1991. Cytoplasmic activation of GAF, an IFN-γ-regulated DNA binding factor. *EMBO J.,* 10: 927-932
3. Fiering, S. et al., 1990. Single cell assay of a transcription factor reveals a threshold in transcription activated by signals emanating from the T-cell antigen receptor. Genes Dev., 4: 1823-1834.
4. Galien R, Emanoil-Ravier R, Mercier G. (1994) Differential effects of c-jun and CREB on c-AMP response element activation by Ha-ras. *Oncogene* 9: 1101-1108
5. Karin, M. and Hunter, T. (1995) Transcriptional control by protein phosphorylation: signal transmission from the cell surface to the nucleus. *Curr. Biol*. 5: 747-757
6. Levy, DE., Kessler, DS, Pine, R, Reich, N. and Darnell, JE, Jr., 1998, Interferon-induced nuclear factors that bind a shared promoter element correlate with positive and negative transcriptional control. *Genes Devel. 2: 383-393)*.
7. Treisman, R. (1996) Regulation of transcription by MAP kinase cascades. *Current Opinion in Cell Biol*. 8: 205-215
8. Wingender, E. (1990) Transcription regulating proteins and their recognition sequences.*Critical Review in Eukaryotic Gene Expression* 1: 11-48

Notes

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