R^3 RAS Pathway Clone Collection #1

Ras Reference Reagents Program, Protein Expression Laboratory
Cancer Research Technology Program, Leidos Biomedical Research Inc.
Frederick National Laboratory for Cancer Research, Frederick, MD, 21702, USA

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Overview

The R^3 KRAS Pathway Clone Collection #1 is a set of vectors for use with the Gateway cloning platform (Life Technologies, Carlsbad, CA) to permit construction of expression vectors for in vitro and in vivo research. While these clones can be used for standard Gateway cloning, their utility is greatly enhanced with the use of the FNLCR Combinatorial Cloning Platform (CCP) which allows simplified construction of vectors with different elements. Some uses of this system might be:

- Construction of protein expression constructs with various fusion tags
- Generation of expression constructs with different promoters for in vivo expression
- Production of clones with fluorescent tags for localization experiments
- Generation of constructs for making mutant cell lines or transgenic animals
- Production of vectors for shRNA or miRNA delivery

The advantage of the CCP is in the exquisite specificity of the Multisite Gateway reactions, which permit linkage of multiple elements in a directional fashion, and which involve no additional DNA amplification, thus ensuring the accuracy of the DNA sequence of the final products without the need for additional sequencing. There is also no need for restriction-based cloning processes which have a high rate of failure and may require optimization depending on the sites available in a given clone.

The KRAS Pathway clone collection contains a series of RAS pathway genes in two separate formats. The "closed" format contains a Kozak translational initiation sequence and ATG start site at the 5' end, and a stop codon at the 3' end. These constructs can be used to make native or amino-terminally tagged constructs using Gateway vectors or the CCP. The "open" format has the same 5' sequence, but lacks a stop codon and is in frame with the Gateway attB2 site at the 3' end. This allows production of C-terminal fusions using Gateway vectors or the CCP. These clones have been completely sequence validated and functionally tested in Gateway reactions to ensure proper performance.

Vectors included:

All clones are in a standard attL1-attL2 Gateway Entry vector (pDonr255) and contain a 5' Kozak translation initiation sequence prior to the ATG start codon. Genbank RefSeq identifiers are listed below to identify the specific isoforms of the genes in these clones.

R702-E01	Hs.SOS1	NM_005633.3	closed
R702-E02	Hs.SOS1	NM_005633.3	open
R702-E03	Hs.SOS2	NM_006939.2	closed
R702-E04	Hs.SOS2	NM_006939.2	open

R702-E05	Hs.RASGRP1	NM_005739.3	closed
R702-E06	Hs.RASGRP1	NM_005739.3	open
R702-E07	Hs.RASGRP2	NM_153819.1	closed
R702-E08	Hs.RASGRP2	NM_153819.1	open
R702-E09	Hs.RASGRP3	NM_001139488.1	closed
R702-E10	Hs.RASGRP3	NM_001139488.1	open
R702-E11	Hs.RASGEF1A	NM_145313.2	closed
R702-E12	Hs.RASGEF1A	NM_145313.2	open
R702-E13	Hs.KNDC1	NM_152643.6	closed
R702-E14	Hs.KNDC1	NM_152643.6	open
R702-E15	Hs.RASA1	NM_002890.2	closed
R702-E16	Hs.RASA1	NM_002890.2	open
R702-E17	Hs.SYNGAP1	NM_006772.2	closed
R702-E18	Hs.SYNGAP1	NM_006772.2	open
R702-E19	Hs.RASAL1	NM_001193520.1	closed
R702-E20	Hs.RASAL1	NM_001193520.1	open
R702-E21	Hs.RASA4	NM_006989.5	closed
R702-E22	Hs.RASA4	NM_006989.5	open
R702-E23	Hs.BRAF	NM_004333.4	closed
R702-E24	Hs.BRAF	NM_004333.4	open
R702-E25	Hs.RAF1	NM_002880.3	closed
R702-E26	Hs.RAF1	NM_002880.3	open
R702-E27	Hs.ARAF	NM_001654.4	closed
R702-E28	Hs.ARAF	NM_001654.4	open
R702-E29	Hs.PDE6D	NM_002601.3	closed
R702-E30	Hs.PDE6D	NM_002601.3	open

Materials Available:

Miniprep plasmid DNA for all Entry clones is provided, and concentrations are noted on the vials. In addition, glycerol stocks of E. coli DH10B cells containing the plasmids are provided. Gateway Entry clones are in the pDonr255 backbone and should be propagated with 50 ug/ml spectinomycin to ensure plasmid stability.

Contact Information

For additional information on the system or to get more information on the CCP, please contact Dominic Esposito, Director, Protein Expression Laboratory, Frederick National Laboratory at 301-846-7376 or dom.esposito@nih.gov

For technical questions about the system, please contact Carissa Grose, Protein Expression Laboratory, Frederick National Laboratory at 301-360-3427 or grosecl@mail.nih.gov

Gateway Recombinational Cloning

For more information on the Gateway system, please see Hartley JL, Temple GF, and Brasch MA. (2000) "DNA cloning using in vitro site-specific recombination." *Genome Res.* **10**, 1788-1795.

For more information on the FNLCR Combinatorial Cloning Platform (CCP), please see Wall VA, et. al. (2014) "Combinatorial assembly of clone libraries using site-specific recombination." *Meth. Mol. Biol.* **1116**, 193-208.