R^3 KRAS Entry Clone Collection #1

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Overview

The R^3 KRAS Entry Clone Collection #1 is a set of vectors for use with the Gateway Cloning Platform (Life Technologies, Carlsbad, CA) to permit construction of KRAS 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 Entry clone collection contains wildtype and mutant KRAS 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 aminoterminally 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.

R750-E01	Hs.KRAS4b	closed
R750-E02	Hs.KRAS4b	open
R750-E03	Hs.KRAS4b G12C	closed
R750-E04	Hs.KRAS4b G12C	open
R750-E05	Hs.KRAS4b G12D	closed

R750-E06	Hs.KRAS4b G12D	open
R750-E07	Hs.KRAS4b G12V	closed
R750-E08	Hs.KRAS4b G12V	open
R750-E09	Hs.KRAS4b G13D	closed
R750-E10	Hs.KRAS4b G13D	open
R750-E11	Hs.KRAS4b Q61L	closed
R750-E12	Hs.KRAS4b Q61L	open
R750-E13	Hs.KRAS4b Q61R	closed
R750-E14	Hs.KRAS4b Q61R	open
R750-E15	Hs.KRAS4b S181A	closed
R750-E16	Hs.KRAS4b S181A	open
R750-E17	Hs.KRAS4b S181D	closed
R750-E18	Hs.KRAS4b S181D	open
R750-E19	Hs.KRAS4b S181E	closed
R750-E20	Hs.KRAS4b S181E	open
R750-E21	Hs.KRAS4b C185S	closed
R750-E22	Hs.KRAS4b C185S	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.