CLONING OF AN OVEREXPRESSION VECTOR FOR THE RARE AND UNCHARACHTERIZED KRAS MUTANT R164L

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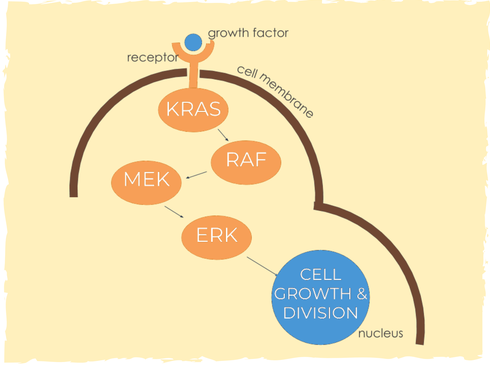
Background

Hannan and Weinberg (2000) define cancer as an illness brought about by cascading sets of phenotypic expressions induced by various alterations in genetic sequences. One of the more commonly studied oncogenes is that of Kras, an important and active isoform of the Ras protein family.



This protein functions in the signaling transduction responsible for regulating proliferation, senescence, differentiation, and survival of a cell (Kießling et. al, 2011). Thus, the formation of certain types of cancer such as lung and colorectal hinges on mutations arising in the DNA coding for Kras (Di Fore et. al., 2007).

However, most cancer tests and therapies are target-specific to more common mutations of KRAS (Janakiraman et. al., 2010), which may lead to erroneous prognosis and therapeutic prescription causing more harm to patients with rare mutations. Thus, understanding the function of non-hotspot mutations is becoming more important.

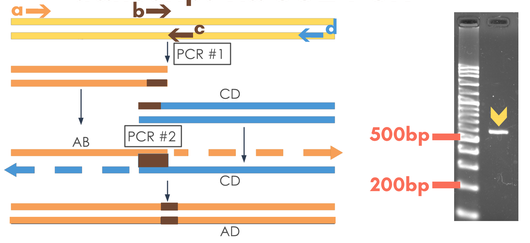
The KRAS mutation at c.491G>T that causes an amino acid change of arginine to leucine was selected from the Catalog of Somatic Mutations in Cancer or COSMIC database (http://www.sanger.ac.uk/cosmic). Studies regarding this mutation is still lacking. The functional characterization for this is yet to be done.

Creating gene copies is first necessary before proceeding with characterization. This study aimed to clone the KRAS transcript with mutation R164L into the pTargeT mammalian expression vector by amplification, ligation, and transformation. The success of the cloning shall be followed by functional characterization of this mutation.

This study shall be followed by functional characterization assays (i.e. proliferation, migration, and cytoskeletal organization) that will determine the probability of malignancy of KRAS mutant R164L.

Methodology + Results

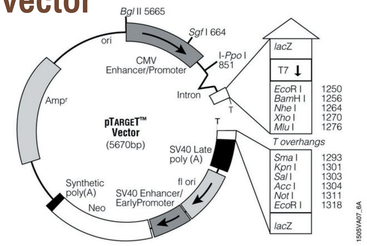
1 Construction of a mutant transcript via SOE-PCR



SOE uses 2 primer pairs consisting of one wildtype and one mutagenic primer. This diagram shows a and d as flanking primers and c and b as mutagenic primers.

Fig 1. Results of SOE-PCR. Confirmatory Gel Electrophoresis after amplification of full mutant transcript. Excpected band size: 600 bp.

2 TA ligation into pTarget vector



Map of pTarget vector. pTarget works with TA cloning making it compatible with any gene sequence with 3'-A overhangs that anneal with the 3'-T overhangs in the vector.

3 Transformation of *E. coli*

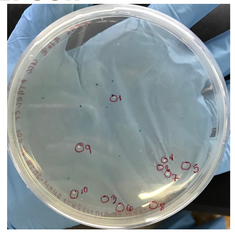


Fig 2. Blue-White screening results after ligation and transformation. The colonies with positive insert (white colonies) were subject to alkaline lysis extraction.

4 Resitriction Enzyme digestion via *Eco*RI

Fig 3. RE Digest results showing successful cloning. Three bands can be found in the lanes of clones with positive inserts. The highest band at 5kbp is the linearized vector, and the lower two bands (500bp and 200bp) are for the insert containing an internal RE site for EcoR1. The multiple cloning sites are also accounted in the total insert length, 700bp, of the resulting bands. (KRAS is only 600 bp)

5 Sequencing and alignment

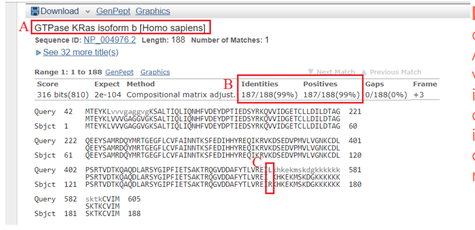


Fig 4. Protein Sequence alignment through Blastx. Aimed amino acid sequence was obtained (A), and identities matched (B) despite an unintended mutation found in the nucleotide sequence, and only the aimed amino acid mutation was obtained (C).

Conclusion and learnings

The overexpression vector for the gene encoding for KRAS contain the noncanonical mutation R164L was successfully cloned. The resulting clones shall now be utilized through various functional characterization assays. Various Molecular Biological concepts such as cancer mutations and MolBio techniques were learned throughout this study.

Acknowledgements

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