Synthetic Biology Tools for p53 and KRAS Genes Co-expression

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Abstract

Controlling cancer related genes and their interactions is a promising direction in cancer research, diagnostics, and treatment. Heterologous gene expression technique facilitates the expression of exogenous gene of interest within a host-regulated genetic module (i.e. plasmid DNA in bacteria).

Whether keeping the gene intact, modifying the gene, turning off the gene of interest, or co-expressing it with another gene of interest, the impact of each can be precisely detected and characterized in the system described in this project. Here we design a two-gene expression system with a reliable detection mechanism (fluorescent imaging and PCR) for two cancer related genes, *p53* and *KRAS*, labelled with green and red fluorescent proteins, GFP and RFP, respectively.

The technique itself and the assumptions made after its experimental testing can be applied in developing novel diagnostic and treatment methods, as well as provide new insights in pancreatic cancer biology.

Introduction

In the modern society, around 38% of world's population is under risk of developing cancer. Cancer may be *benign*, meaning it does not spread to surrounding tissues, or *malignant*, during which cancer cells may break off from the initial tumor and spread into other tissues, forming secondary tumors. Cancer may arise from DNA damage due to environmental exposure, inherited mutations from parents, or a combination of other factors during a person's lifetime. The genetic changes that lead to cancer affect three main types of genes: *proto-oncogenes*, *tumor suppressor genes*, and *DNA repair genes*[1]. Tumor suppressor genes are involved in protecting cells from developing cancer, however when altered these genes release tumor control and cells become more vulnerable to **carcinogens** – any factors stimulating **malignant transformation** of cells. Tumor suppressor gene networks create cancer 'safety net' and therefore can be promising targets for cancer treatment, that are still very little studied. In this project we explore the genetic background of pancreatic cancer by investigating the interaction between the two tumor suppressor genes – p53 and KRAS[2,3].

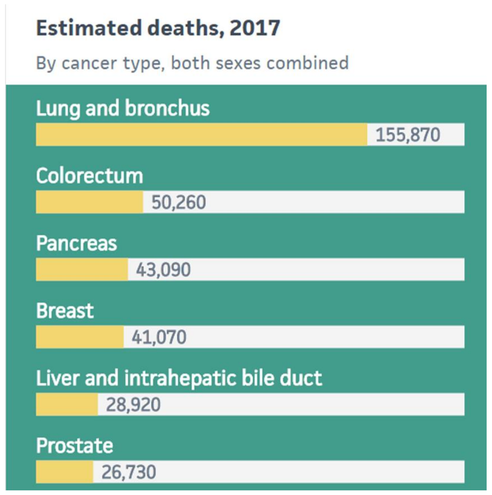


Figure 1. Top-three cancer patient statistics, sexes combined, in 2017.

Pancreatic cancer localizes primarily in the pancreatic tissues, however is known to spread rapidly to nearby organs and is very rarely detected on the early stages. Pancreatic cancer is most commonly caused by acquired genetic mutations. Based on the pancreas anatomy, pancreatic cancers can be **exocrine** (*pancreatic adenocarcinoma* being the most common type*)* or **endocrine** (a.k.a. islet cell tumors). Symptoms of pancreatic adenocarcinoma include weight loss, jaundice, bowel obstruction, and itchy skin. Various methods are used to treat cancers, and the most common ones are **surgery**, **chemotherapy**, **targeted therapy**, and **immunotherapy**. These treatments are not always precise or effective. The literature suggests that such precision can be gained by manipulating tumor development at the genetic level.

Many cancers arise due to mutations in genes, also known as genetic mutations. There are two types of genetic mutations:

- *acquired mutations* (most common cause of cancer and occur from damage to genes in a particular cell; cancers that occur due to acquired mutations are called **sporadic cancers**. Most common are mutations in p53 gene)
- *germline mutations* (they occur in a sperm or egg cell and are passed directly from a parent to a child at time of conception; the mutations from the initial sperm or cell are copied into every cell within the body. Most common ones include mutations in *BRCA1* and *BRCA2* that initiate breast cancer)

Introduction of a gene or a certain part of a gene in a host organism which does not naturally have this gene is called heterologous gene expression. This is most commonly achieved through recombinant DNA technology[4]. Heterologous expression allows for isolating the gene from its native environment for detailed study, as well as the prediction of its genetic mutations not yet observed.

Materials and Methods

In this project we combined molecular and cell biology laboratory techniques with *in-silico* design of gene expression platforms. **AddGene** (https://www.addgene.org/) was used as a resource for the DNA sequences of the genes of interest (*p53, KRAS, EGFP*) as well as other gene expression modules (promoters, terminators, origins of replication, selection markers etc.). We used **SnapGene, SnapGene Viewer**, and **ApE Plasmid Editor** Software for plasmid design and functional simulations.

In this study we used NEB 10-beta *E. coli* cells for all molecular cloning procedures. The plasmid DNA containing *yEGFP* gene of interest was extracted from bacterial cells following the QIAprep Miniprep Protocol. To confirm the presence of *yEGFP* gene sequence in the plasmid we amplified it by polymerase chain reaction (PCR) using LR1.3.3 forward and LR1.3.4 reverse (Table 1). We used Bio-Rad C1000 Touch Thermal Cycler for PCR.

LR1.3.3 GTGGAGGAGAACCCTGGACCTATGTCTAAAGGTGAAGAATTA
LR1.3.4 GTTGATTGCGGCCGCACCGGTTTATTTGTACAATTCATCCAT

Table 1. Primers for *yEGFP* amplification by PCR.

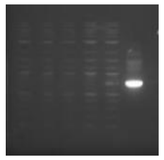
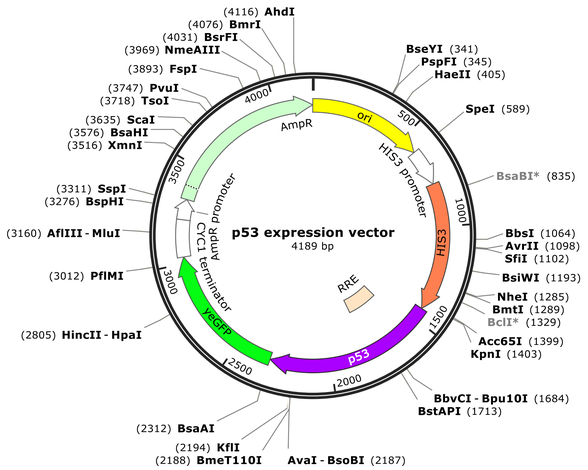
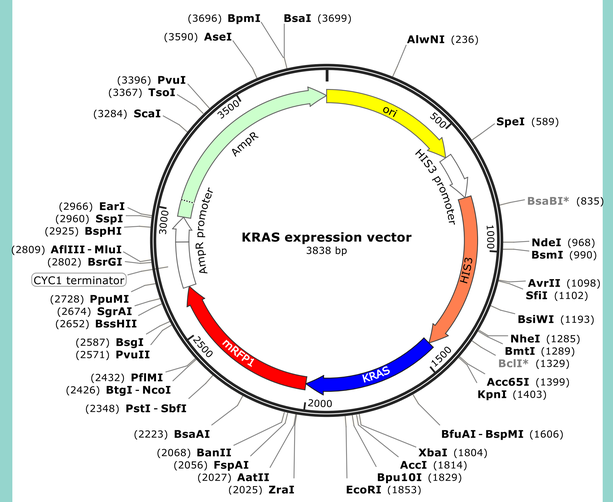


Figure 2. Agarose Gel Electrophoresis of *yEGFP* gene sequence amplified by PCR

Results





Conclusions

This combination of multiple genes may include two native copies, one mutant and one native copy, two mutant copies, or one mutant and knock-out copy (i.e., produced by CRISPR/Cas9 technology). The clone fragment will have encoded for a putative (function not known) homologue for the construction of gene regulation. This will allow for the construction of plasmids suitable for the isolation of certain genetic markers, and immediate applications may lead to the expression of such biological compounds within the host.

Utilizing the techniques of gel electrophoresis and the gene editing software, SnapGene, we addressed a system developed for the controlled co-expression of *P53* and *KRAS*. We designed a two-gene expression system with fluorescent imaging and PCR for both of these cancer related genes. Our next steps will be to use this research for treatments for pancreatic cancer.

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