Engineering a Plasmid for Use in Telomerase Detection

Abstract

The amount of telomerase in select areas of a person's body can indicate the presence of cancer cells. This project aimed to design a plasmid that can provide a visual sign of active telomerase in cells to help with early cancer detection. Due to the complexity of the project, a model of the plasmid was designed using the bio-engineering site Benchling. A product using these plasmids could be groundbreaking for cancer detection, particularly its financial accessibility and use in home environments. Using modified E.coli to detect cancer could allow for an early-detection system, saving lives in a healthy and cheap format.

Overview

Background Information: Telomeres are located on the ends of chromosomes to allow for cell division and to prevent the chromosomes from unraveling. Each time a cell divides, the telomeres shorten slightly because a tiny piece of DNA breaks off. Telomerase is an enzyme that helps prevent the telomeres from shortening by adding DNA to the ends of telomeres.

Existing Research: Research has shown that both too-low and too-high levels of telomerase are harmful to humans. Lower levels are linked to higher rates of infection and disease (3x the risk), and heart failure (8x the risk). Higher levels are linked to greater risk of cancer and immortalization of cancerous cells.

Issue: Knowing the level of telomerase contained within a person's body –especially in a safe, fairly uninvasive, and efficient manner– can help prevent illness; hence, the aim of this study.

Design Goal

Design a vector plasmid that produces a reporter protein that binds with the active site of the enzyme *telomerase* and outputs *GFP* (green fluorescent protein) if the binding is successful.

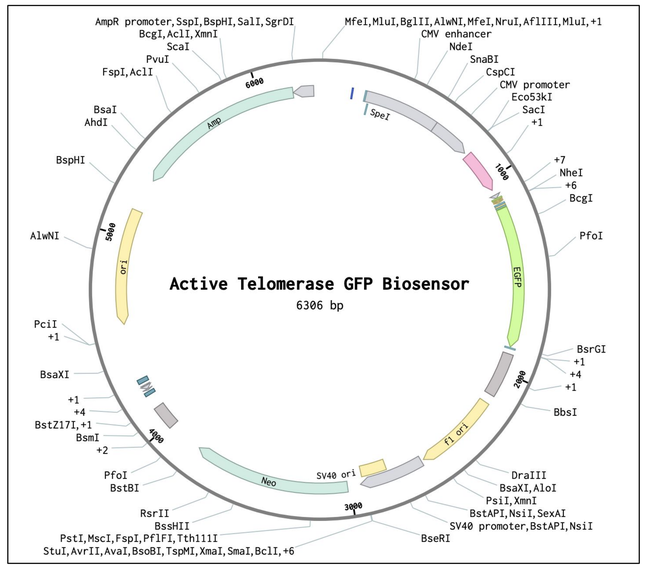
Theoretical Methods

1. Plate cells
   a. Incubate AGK293 cells
   b. Detatch cells from plates
      i. Add trypsin - it cleans the protein that adheres the cells to a plate
   c. Add 6 mL of media
   d. Put cells in a sterile container
      i. One confluent 10 cm dish of cells is typically enough to plate ~2 24-well plates
   e. Count cells using hemocytometer
   f. Count four grids worth of cells, and then divide by 400 to get the number of cells per mL (units are millions of cells per mL)
   g. Dilute cells based on the type of plate being using, the desired volume per well (typically the minimum volume), and the number of wells needed
2. Prepare transfection solutions
   a. Prepare two tubes (with equal volume)
      i. Tube with bebs buffer
      ii. Tube with water, CaCl2 (calcium chloride), DNA
   b. Wait 8-12 hours for accuracy
3. Transfection
   a. Gently pipette & drop DNA into Hebs solution (about 1 drop/second)
   b. Incubate for 3 minutes at room temperature (20°-22° C)
   c. Add transfection mixture to well plates by rapidly pipetting
4. Wait 24 hours, aspirate & replace media
5. Harvest
   d. 24 hours after media change, aspirate media
   e. Lift cells with 200uL PBS+EDTA, transfer to 96 well plate
   f. Spin plate down at 500xg for 5 min to pellet cells, decant supernatant
   g. Put 96 well plate on ice until loading into flow cytometer
   h. Resuspend cells in 200uL PBS+BSA

Design Choices

- sv40 Enhancer Sequence
  - Increases the likelihood of the plasmid encountering telomerase
- TRF1 Binding Sequence
  - Included 3 times
  - Creates telomere-specific protein
- Added to a plasmid that already had EGFP (green fluorescent protein) as a base:
  - Color for detection; produced when the plasmid encounters telomerase

Our Plasmid



*Above figure created in Benchling by exhibitors

Pieces of the Plasmid

- Cytomegalovirus (CMV) promoter - strong eukaryotic promoter for mammalian cells from the human cytomegalovirus that allows for constitutive general gene expression
- CMV enhancer - boosts the efficiency of the CMV promoter by recruiting other proteins and transcription factors that help increase the gene output (boost transcription)
- T7 promoter - from the T7 bacteriophage and used for in vitro transcription and general expression; has a constitutive expression but requires T7 RNA polymerase
- Kozak u100 - crucial for the initiation of the translation process in mammalian cells; shows the start of a gene of interest and signals the start of transcription for that gene
- Restriction enzymes:
  - Two main types: clean cut or sticky ended
- bGH poly(A) signal - signals the sequences for the RNA level
- sv40 promoter - eukaryotic promoter that allows for constitutive general gene expression
  - Used for mammalian transcription
  - Increases the chance that the plasmid will be able to enter into the nuclear, so it increases the chance the plasmid will run into telomerase
- Ampicillin-resistance gene (Amp) - antibiotic commonly used for medicinal purposes, so the resistance gene is to ensure that the plasmid doesn't get eliminated
- Neomycin-resistance gene function - antibiotic that is commonly used for medicinal purposes, so the resistance gene is to ensure that the plasmid doesn't get eliminated.

References

Scan the QR code below using a cellphone for a list of all of our linked references!

Scan the QR code below using a cellphone for a link to our vector plasmid!