Construction of Plasmids Containing Site-Specific DNA Damages

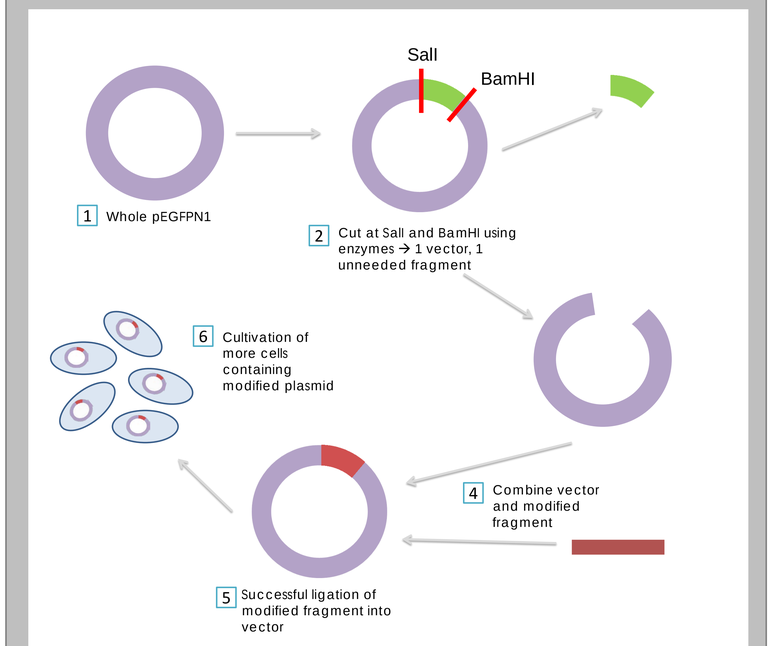
Zixin Chen, Liang Xu, Jenny Chong, and dong wang

Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, 9500 Gilman Drive, La Jolla, California

the abstract

The preparation of plasmids containing site-specific damage has been hampered by low yields and impurity. Because of this, their importance and usage, as well as their effect in gene expression, is not yet clearly understood. This project entails an incorporation of a modified fragment into a pEGFPN1 plasmid. This is achieved by cutting the plasmid to create two non-palindromic sticky ends, and by using a low concentration of the linearized plasmids and a slight excess of the insert, we can produce a maximum yield of modified plasmids. The plasmids are then purified to ensure that the insert is correctly oriented and to certify a single insert's incorporation.

an overview



We start off with a whole, circular vector of pEGFPN1 (1). Using enzymes that cut at sites SalI and BamHI (2), two fragments were obtained – the vector we would use for the next few steps, illustrated in purple, and the unneeded fragment illustrated in green that would be replaced by the damaged insert (4). To produce more of this plasmid, we inserted this plasmid into cells and grew colonies of them (6).

used references

·M. Enoiu, *et al.*, Construction of Plasmids Containing Site-Specific DNA Interstrand Cross-Links for Biochemical and Cell Biological Studies. *DNA Repair and Protocols*, 15. (2012)
·Guainazzi A, Sharer OD, Using synthetic DNA interstrand crosslinks to elucidate repair pathways and identifynew therapeutic targets for cancer chemotherapy. Cell Mol Life Sci 76: 3683-3697 (2012)

the structure of pEGFPN1

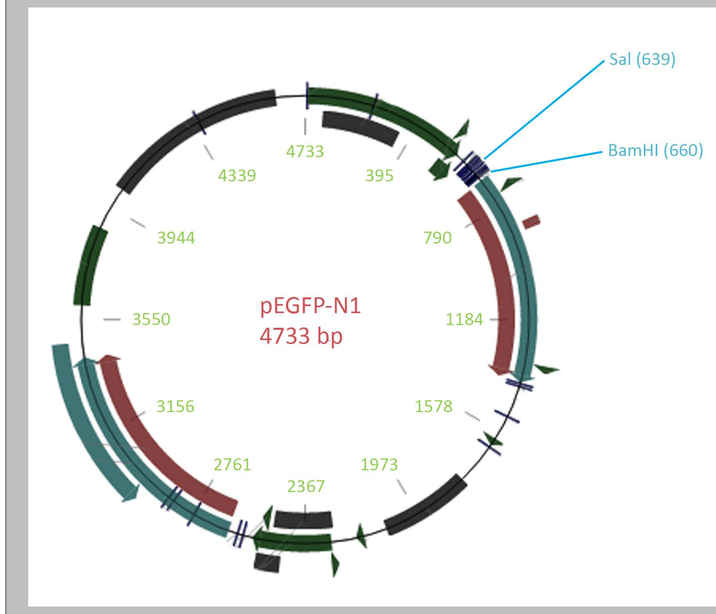
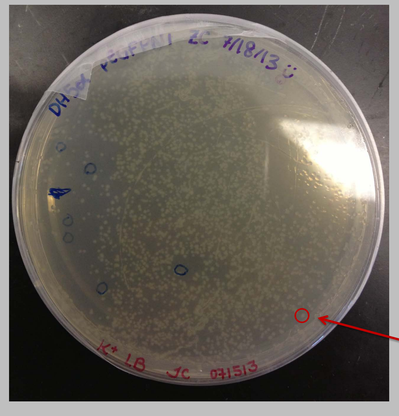


DIAGRAM OF VECTOR pEGFPN1 :
Enzymes are used to cut pEGFPN1 at sites SalI (639) and BamHI (660), in blue.

pEGFPN1 colonies

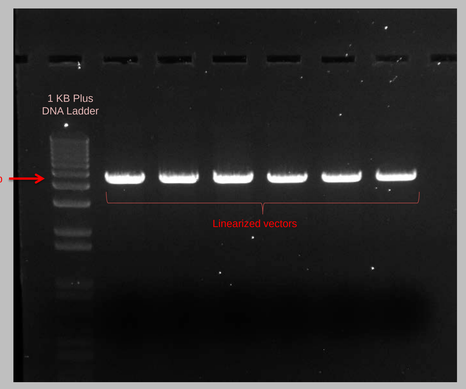


MATERIALS:
- DH5α cells
- 0.5µL pEGFPN1 plasmid
- K+ LB petri dishes
- 500µL LB broth

CONDITIONS:
- Mixture incubated for 1 hr
- 80µL in petri dish
- Petri dish in incubator o/n

The mini-prep procedure involved using buffers and the centrifuge to obtain the modified plasmids within the cells. We then tested the concentration to see how much of the plasmids were yielded.

gel run on double digestion



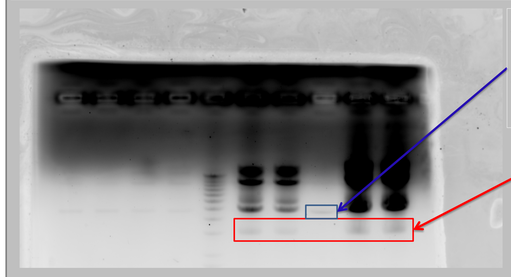
MATERIALS
- 75µL 10x B3.1
- 79µL pEGFPN1
- 15µL SalI enzyme
- 15µL BamHI enzyme

Incubate in 37°C hot water bath 2 hr

GEL ELECTROPHORESIS
- 1% agarose gel
- Run for ~45 mins

To ensure we obtained the correct length for the longer, excised vector, we ran the samples through a gel. The ladder in the first lane serves as an indicator of the number of base pairs. In this case, the vector we needed was 4.8k bp, and the ladder indicates that the vectors are indeed at 4.8k bp. The other fragment of the cut plasmid is too small to show up, which is why only one band is seen in each lane.

gel run on ligation



The bands boxed in red indicate closed, circular plasmids, and evidence of these means that the ligation was a success. After extracting the samples out of the gel, the circular plasmids were purified to ensure the correct orientation and incorporation of the modified fragment.

some future directions

Obtaining enough samples of the plasmid containing a modified vector as well as successfully ligating the modified vector was the purpose of this project. After gathering enough samples, the next step would be to investigate how these modified plasmids affect gene expression in mammalian cells – and how the cell itself would respond to this damage.

the conclusion

The modified fragment was successfully incorporated into the pEGFPN1 plasmid. The protocol described is very useful for introducing fragments that normally cannot be emplified by cells, in the case where plasmids must be artificially generated. The significance of this project is its versatility for generating site-specific damages or modifications, and this is a powerful tool for a variety of studies.