Design a Biosensor for Small Molecules in Bacterial Systems

Recombinant DNA Technology - Vihaang

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Aim

Create a vitamin B12 sensor by cloning or genetically modifying E. coli strain. Insert PQE60 GFP plasmid into mt DH5 Alpha E. coli strain.

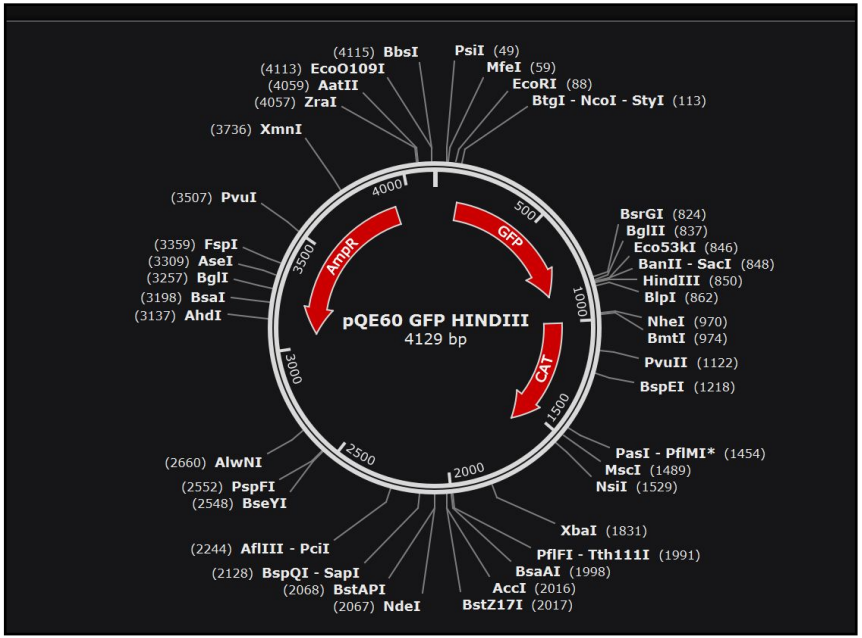
Gene of Interest

eutR and peutS genes from DH5α E. coli Strain.

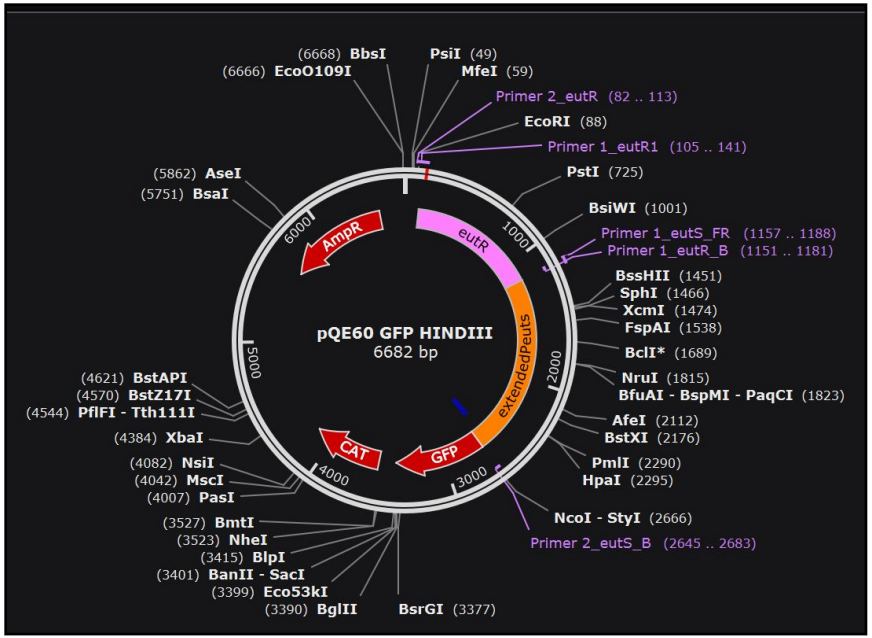
Vector Selection

Modified PQE60 plasmid with GFP gene genetically modified to produce recombinant DNA.

Construct Design



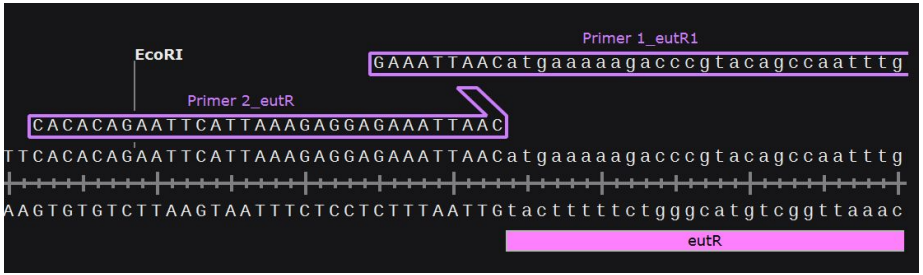
[pQE60 GFP Hindiii.dna]

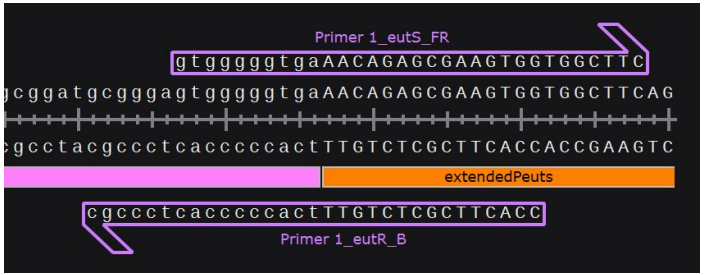


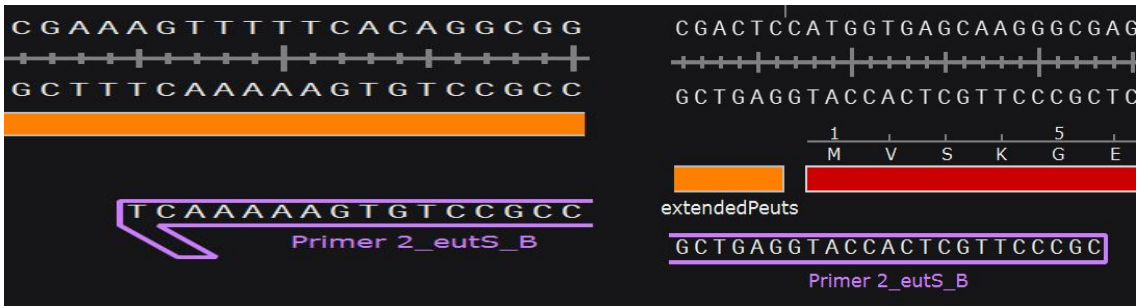
[Insilico construct Map of PQE60 GFP, peutS and eutR]

Insilico Construction

Primers designed using Snapgene for amplification and gene insertion.







[Primers from pQE60 eutR peuts GFP]

Vector Preparation

Plasmid Isolation

- Culture E. coli in LB medium overnight.
- Harvest cells, discard supernatant.
- Resuspend pellets in GTE(Glucose + Tris - HCl + EDTA) and lysis buffer.
- Precipitate DNA with isopropanol.
- Dissolve DNA in TE buffer.

Host Cell Preparation

Competent

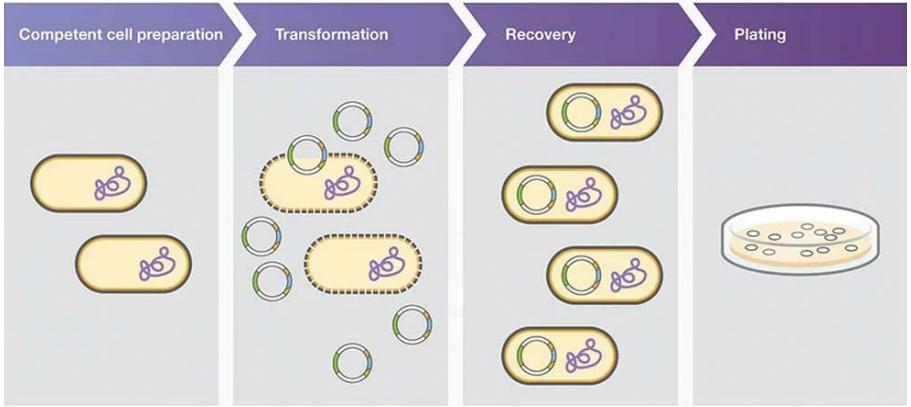
- Resuspend DH5 Alpha cells in CaCl2.

Transformation

- Mix with plasmid DNA, heat shock.

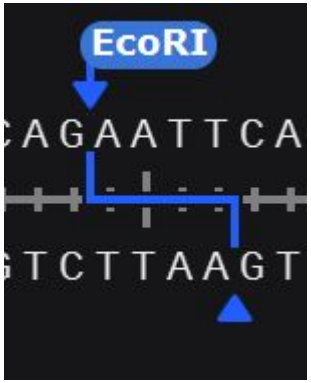
Recovery

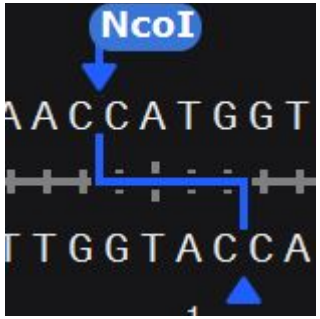
- Plate onto LA media with antibiotics. (Ampicillin)
- Incubate overnight.

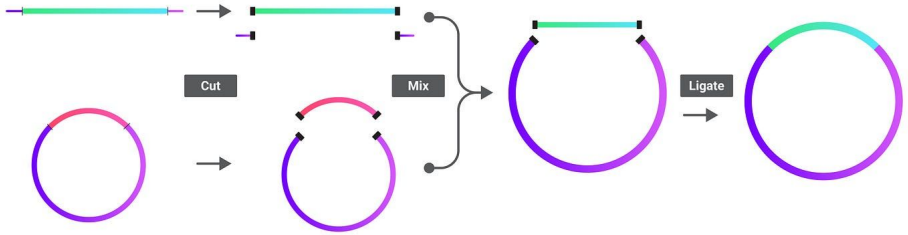


Digestion and Ligation

- EcoRI and NcoI restriction enzymes used for plasmid vector and gene digestion.
- Cohesive(sticky) ends created for ligation.







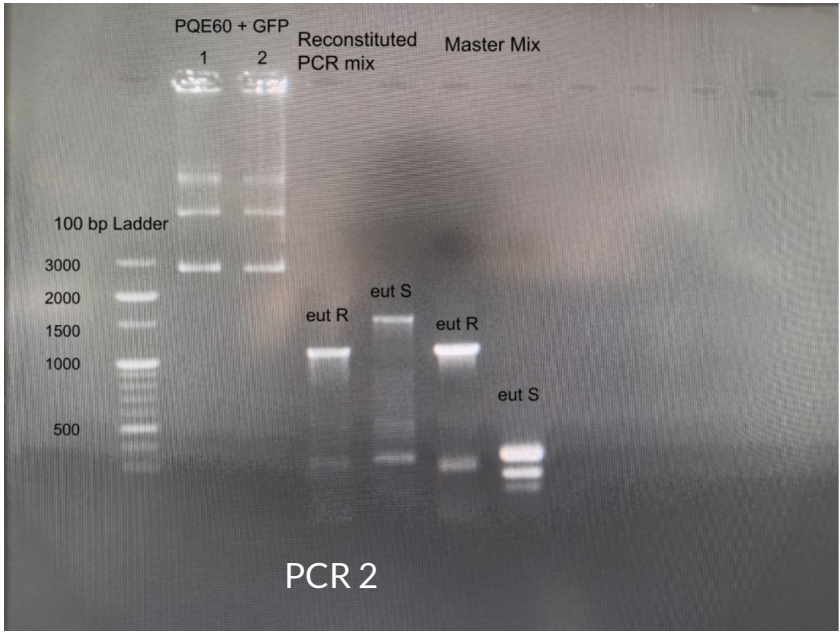
Troubleshooting PCR

Observations

PCR 1 was fail, possible reasons would be Mastermix has a problem.

The PCR 2 is a where we see a issue in mastermix in eut S gene and our reconstructed mix has worked well.

PCR 1

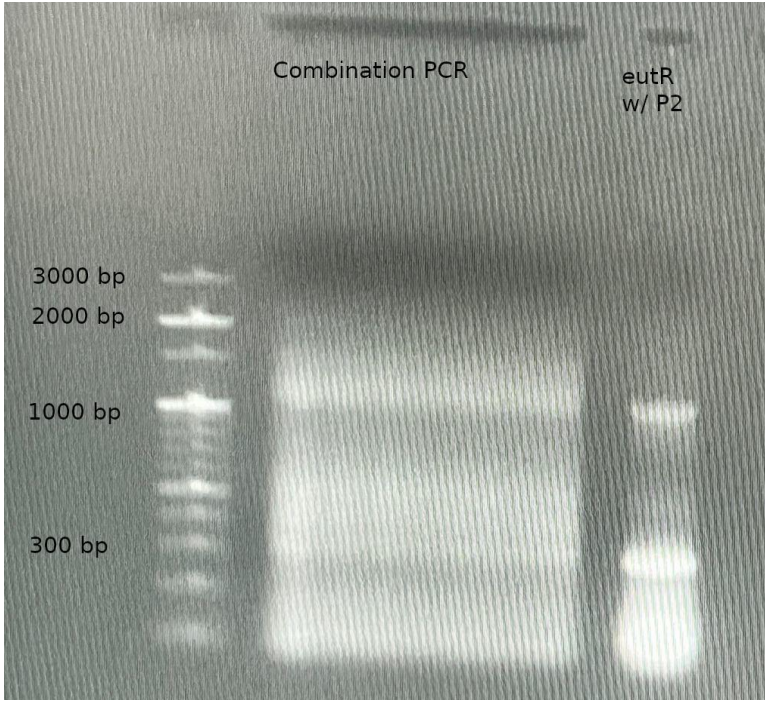


PCR 2

Combined PCR

Combined PCR failed:

- Reasons may be due to mismatch between designed primers and DH5 alpha sequence.
    - The actual length should be 2553 bp.
- eut R is shown successfully amplified and there are some non specific binding at 300 bp.



Screening

Expression of GFP using IPTG

- We successfully observed fluorescence that means the GFP is produced.
- The added IPTG has removed the repressor and activated the transcription of the plasmid.