8/1/17- Preparation of hCG

Upon further inspection of the alkaline phosphatase enzyme, we realized there may be a more efficient detection mechanism to use and the trouble we may come across while fusing such a large enzyme with Factor C which is already large on its own. The Human Chorionic Gonadotropin (hCG) is the hormone that produces a positive pregnancy test. We decided to produce the hCG and fuse it with Factor C. A pregnancy test can be inserted in the solution if there is a positive result on the test then Factor C has undergone autocatalysis. Our reasoning for using hCG is that it is smaller. We will only have to immobilize the factor c instead of the Factor C and BCIP. The hCG part was located in the registry (BBa_K732001 designed by: iGEM_VGEM, Joshua Fass) however the part experience indicates that it fails. So intend to improve upon the part.

AIM: Before the fusion step, the hCG needs to be placed into the PSB1C3 plasmid, so that the hCG can be submitted. In order to add the hCG to add to the plasmid restriction sites need to be added this was done by adding the prefix and suffix through PCR.

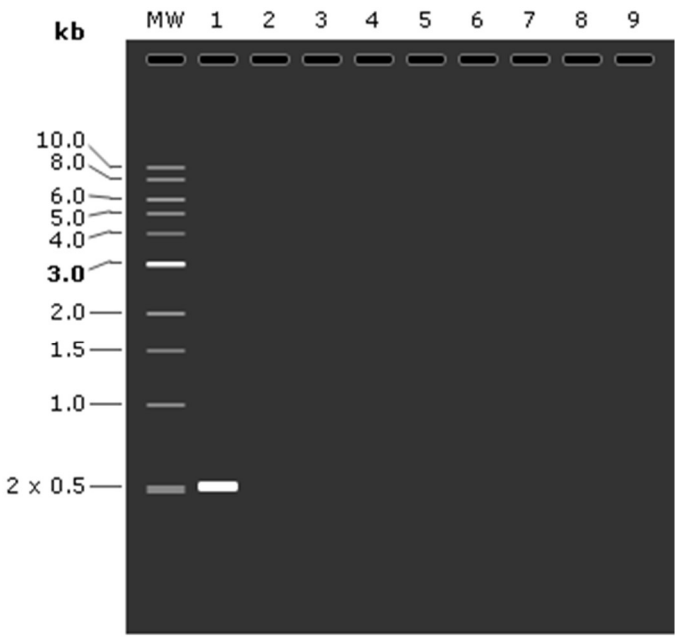
Materials:

- Qiagen Master mix (2x)
- Prefix Primer
- Suffix Primer
- hCG DNA (diluted to 10ng/ul)
- Nuclease free H20

Protocol:

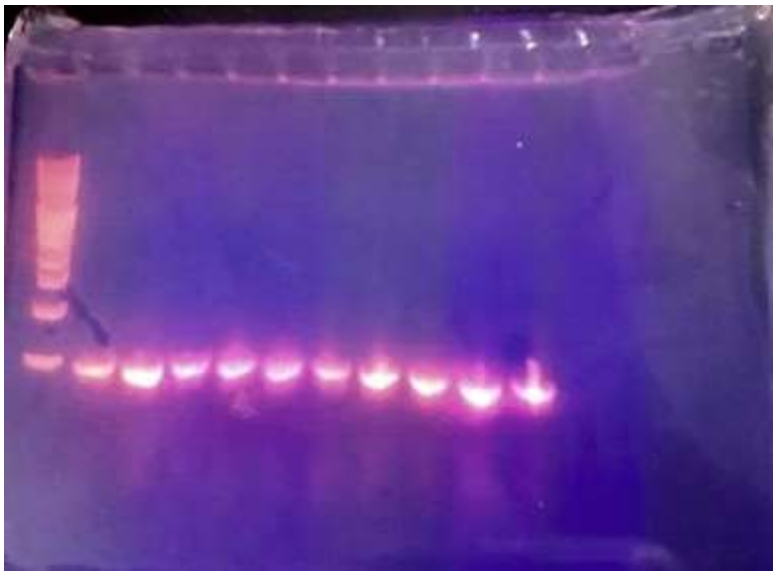
1. Create a 12x cocktail for a 20ul reaction
    a. 120ul Mastermix
    b. 12ul Prefix Primer
    c. 12ul Suffix Primer
    d. 12ul DNA
    e. 84ul H20
2. Pipette 20ul to 10 tubes
3. Place in thermocycler and run normal PCR cycles
4. Diagnostics: Gel Electrophoresis to verify the reaction was a success
    a. Add 5ul of New england biolabs 1kb Ladder with 1ul of dye (6x)
    b. 5ul of each reaction 1-10 with 1ul of dye
    c. Gel: 1% agarose, .7g agarose, 70ml of 1x TAE buffer, 7ul gel red

Results:

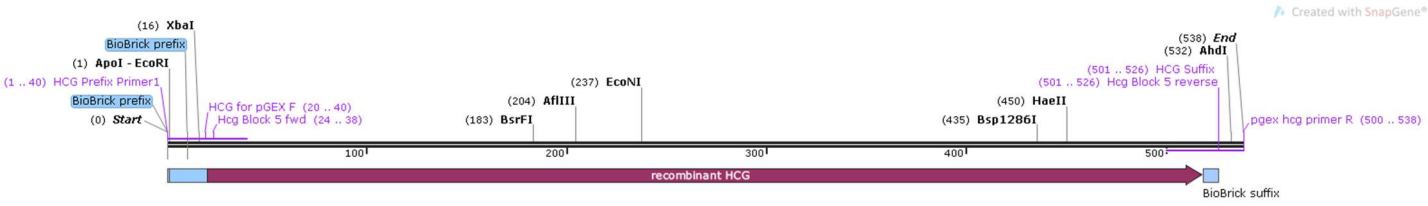


1.0 % agarose

Simulated agarose gel, the expected results from the PCR.



Actual gel: the bands are bright and in line with the correct band on the molecular weight ladder.
Wells contain samples as follows:
MW Ladder, sample 1-10 in order.



Amplified
538 bp