06/12/17

Monday, June 12, 2017 9:17 AM

Martin & Salma (9AM-12PM) & Ayesha (10-1)

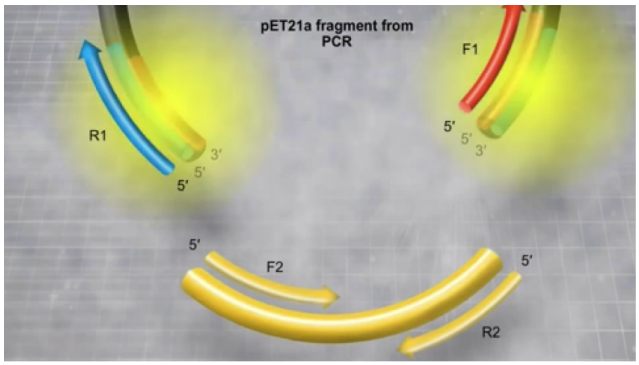
- Checked our IPTG+Kan plates for growth of BL21+pet/cJblue ligated product, saw growth but no blue colonies (cJblue was not expressed)
- Probably just the vector backbone (pet28) just ligated to itself
- Either the digest failed or the plasmid ligated to itself, this is always a risk you run when conducting this protocol
    - Usually when we see only one colony this is the case but we should always test it just to confirm
    - When not doing double digest we usually treat with phosphatase so that the 5' phosphates are removed (this prevents T4 ligase from re-ligated the plasmid to itself)
    - When looking at the plasmid map for Pet28, it is highly probable that the RBS was cut out when using the restriction enzymes, NotI and XbaI. This offers another explanation for why expression failed.

- No lab work today, researched possible plasmid backbone vectors (replacement for pet28)
    - **JOE:** Highly inducible by IPTG, Cam resistance: http://parts.igem.org/Part:BBa_J04500
    - In 2017 distribution kit (Plate 3, 19J)

Conditions that should be met when designing primers for Gibson Assembly:

- Design nucleotide overhangs for correct fragment annealing
    - Use 15-40 bp overlaps that exhibit Tm greater than 48 deg Celsius.

- Increased overlap, increased efficiency, Use less DNA
- We can either
    - Split the overlap region between the forward and reverse primer
      -OR-
    - Add overlap region to only the forward primer
- We need to design 2 forward primers and 2 reverse primers **(if we are amplifying the plasmid)**



- Overhang should be generated from the first bp of the 3 prime end regardless of the type of overhang.

- The overlap region of the forward primer for the gene of interest should line up with the 3 prime end of the overhang and extend back until Tm is greater than 48 deg. Celsius.
- Primers should end in GC base pair. Take up or drop base pair if boundaries end in GC.

Reverse primer design

Sequences that we need to know:

- BioBrick prefix sequence
  - Available here: http://parts.igem.org/Help:BioBrick_Prefix_and_Suffix
- NotI Sequence
  - Available here: https://www.neb.com/products/R0189-noti
- XbaI Sequence
  - Available here: https://www.neb.com/products/R0145-xbai