Ligation Calculations for flu in plasmid pSB2K3

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Length of flu operon insert: 3382 bp

Length of pSB2K3 vector: 4408 bp

General formula for calculating the amount of insert to vector

$$\text{Insert mass in ng} = (\text{excess amount})\ \text{x}\ \frac{\text{insert length in bp}}{\text{vector length in bp}}\ \text{x}\ (\text{vector mass in ng})$$

Where:

- The excess amount is typically 6
- Vector mass should be 10 ng for T4 DNA ligase (not the quick ligase we have in the ERB) for a 10 uL reaction
- 10 ng/ (X ng/uL) where X is the reading from the nanodrop, this gives you the volume of vector to add to the reaction in uL

An example for this ligation

$$\text{Insert mass in ng} = (6)\ \text{x}\ \frac{3382\ \text{bp}}{4408\ \text{bp}}\ \text{x}\ (10\ \text{ng}) =\ 46\ \text{ng of insert}$$

And the amount of insert to be added in uL is 46 ng/ (Y ng/uL) where Y is the reading from the nanodrop

The amounts to add to the reaction mixture are as follows from openwetware (http://openwetware.org/wiki/DNA_Ligation)

10µL Ligation Mix

Larger ligation mixes are also commonly used

- 1.0 µL 10X T4 ligase buffer
- 6:1 molar ratio of insert to vector (~10ng vector)
    - Since 500 ng of DNA is present in a 50 uL digest, typically 1 uL of digest needs to be added
- Add (8.5 - vector and insert volume)µl ddH_2O
- 0.5 µL T4 Ligase

The DNA concentration after purifying the digest

Since the DNA concentration of the flu operon gel extraction was not picked up by the nanodrop, no water will be added to the ligation mixture

The exact ligations that were performed today were

Ligation #1

- 1.0 uL of T4 ligase buffer (vortexed until white precipitate dissolved)
- 1 uL PCR purified KAN backbone in EB buffer
- 7.5 uL unpurified flu digest
- 0.5 uL T4 ligase

Ligation #2

- 1.0 uL of T4 ligase buffer
- 1 uL PCR purified KAN backbone in EB buffer
- 4.5 uL ultra pure water
- 3 uL unpurified flu digest
- 0.5 uL T4 ligase

Unsure if ligation will work using unpurified digest, however NEB buffer 2 and the T4 ligase buffer seem to be made of similar salts so it will be attempted with different concentrations of insert