Ligation Calculations for flu in plasmid pSB2K3

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Length of flu operon insert:3382 bp

Lenth of pSB2K3 vector: 4408 bp

General formula for calculating the amount of insert to vector

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

Where:

- The excess amount is typically 3 or 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 gives you the volume of vector to add to the reaction in uL

For this ligation

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

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 open wetware (http://openwetware.org/wiki/DNA_Ligation):

- 1.0 μL 10X T4 ligase buffer (NOT QUICK LIGASE)
- 6:1 molar ratio of insert to vector (~10ng vector)
- Add (8.5 - vector and insert volume)μl ddH$_2$O
- 0.5 μL T4 Ligase (NOT QUICK LIGASE)