Moving Insert to iGEM Vector

Restriction Digestion to Remove Insert from PGEM-T

Reagent	Volume (ul)
Miniprep product (500 ng/ul)	1*
10x BSA	2.5
10x Buffer 3	2.5
EcoRI (undiluted)	0.5**
PstI (undiluted)	0.5**
H2O	12.5
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	25 ul

Incubate 37 deg Celsius; 1 - 2 hours

Incubate 65 C, 20 minutes

*The final concentration of the DNA in this reaction should be 20ng/ul. The volume of DNA (minprep product) you add to your reaction will be dependent upon the concentration of that DNA. For example: 250 ng/ul x 2ul = 500ng… 500ng/25ul = 20ng/ul). If you have more or less DNA, the volume added should be adjusted such that the final concentration of DNA in the digestion is 20 ng/ul. If you have questions about this – ask! *If your DNA is under 40ng/ul, concentrate it before digesting.*

**Can you accurately pipette 0.5 ul with the pipettes you're using? No; so make a cocktail. Only digesting one reaction? Instead of pipetting directly into the reaction, mix equal small volumes of EcoRI and PstI together, then use 1ul of that. Alternatively, make a cocktail for two reactions and only use half of it.

Example:

- Miniprep product concentration = 100ng/ul
  Use 5ul product to obtain, and adjust the water, such that the total reaction volume is still 25 ul
  100ng/ul x 5ul = 500ng…500ng/25ul = 20ng/ul

- Or, work backwards:
  Desired concentration: 20ng/ul in 25ul ; (20ng/ul)x(25ul) = 500ng
  Concentration of your DNA: 100ng/ul; (500ng)/(100ng/ul) = 5ul
  Check your units – they should all cancel to leave you only with the units you're going after.
  $$\frac{\text{(Desired concentration of DNA ng/ul) x (Volume of reaction ul)}}{\text{(Concentration of your DNA ng/ul)}} = \text{Volume to use (ul)}$$

Ligation into predigested iGEM Vector

Equal masses of insert and vector should be added to the ligation mix. The iGEM vectors that are currently predigested are at 20ng/ul and your Miniprep digest is at 20ng/ul.

Reagent	Volume (ul)
2X Ligase Buffer	10
Miniprep digest (20 ng/ul)	5
Digested Vector (**Kan Resistance**) (20 ng/ul)	5
T4 DNA Ligase	1

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Incubate 4C, overnight

Transform 10 ul of ligation into 50ul DH5α Competent Cells

Plate cells on LB Kan (not LB Carb!)

Incubate 37C overnight

Check colonies for insert of correct size (and the correct vector) by miniprep and digest.