Ligation

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Where

On the bench

Material needed

Quantity	What
Per ligation 10 μl	
1μl	T4 ligase buffer
0.5μl	T4 ligase
	DNA fragments you want to ligate

Steps

1. Determine the concentration of your fragments and the length in bp.
2. Heat inactivate the fragments (to avoid the restriction enzymes to re-cut the DNA after ligation). You can use either a water bath or the 80° incubator. Whatever fits the required temperature. Note, that if you use the incubator you should add 5 minutes to the required time since heating with air is less efficient (than heating in the water bath).
3. Formula for the mass of the insert: $\text{mass insert} = \text{mass vector} \cdot \frac{\text{length insert}}{\text{length vector}} \cdot \text{ratio}$. "Ratio" corresponds to the ratio of insert to vector that is desired.
4. The mass of the vector should be between 10 - 50 ng. So for example use 25 ng. The ratio insert:vector should be between 1:1 and 5:1. Aim for a 1:1 concentration but make sure it is not lower!! Calculate (with the formula above) the mass of the insert and then the volume (by using the measured concentration). If the volume of DNA and buffer exceeds 10 μl (this can happen if the concentration is very low) then you have to reduce the mass of the vector (as stated above the mass of the vector can be between 10 and 50 ng).
5. Chose values that are easy to pipette!! E.g. if you have 0.34μl and 0.44μ for the insert you can chose 0.5 μl for both since the ration may be more than 1:1. Make sure you don't chose a lower value!
6. Calculate the amount of destilled water required such that your mix in the end has a volume of 10 μl.
7. Add the DNA and the water first.
8. Take the Buffer out of the freezer and defrost it. Keep it on ice. After use put back into fridge immediately (contains ATP and will degrade!).
9. Take the ligase out of the freezer just before use since it is very sensitive. Put back immediately!!
10. Do a short centrifuge and keep the tubes at room temperature (1-2 hours).
11. Remark: Alternatively you can put the tubes into the PCR machine and keep them at 16°. Wait for at least four hours in this case. This method is more accurate since the ligase degrades less.

Frequently used values

- **Length of plasmids and inserts:** plasmidCm (pSB1C3) 2072 bp, plasmidAmp (pSB1A3) 2157 bp, KanR(p1003) 967 bp, AmpR(p1002) 943 bp, CmR(p1004) 769 bp, TetR(p1005) 1283 pb, Base Vector (BBa_I51020) 2432 bp, Asaia Origin 1391 bp
- **Temperatures for heat inactivation:** EcoRI HF: 65°for 20 min, PstI: 80° for 20 min, XbaI: 65° for 20 min, SpeI: 80° for 20 min