GIBSON ASSEMBLY

The Gibson Assembly reaction is an easy way to link different DNA fragments.

In order to do this reaction DNA fragments should be prepared in a way to obtain the desired product.

In the following paper I will talk about this reaction in the way we used it. Please note that it can be improved and modified as needed.

PREPARING VECTOR:

- Linearize the vector within the site of insertion (it will be removed during PCR) using restriction enzymes or PCR or both.
- If you use primers, you need a reverse and a forward primer, both adding overhangs to the linearized plasmid. These overhangs should be at least 30 nucleotides long and complementary to the insert ends.
- If you use a restriction enzyme, you will have to amplify your linear plasmid with the same primers.

PREPARING INSERT:

- If you have your insert in a plasmid you first need to amplify its sequence from the plasmid by PCR. Make sure that the end of the amplified sequence is complementary to the overhangs added to the linearized vector.
- If you already have a linear insert, you can directly use it.

For these two previous steps of preparation, you can refer to the PCR protocol if you are using primers to isolate your sequences.

You can also add the overhangs on the insert and not on the vector. This is especially useful if the insert is very short.

MAKING THE GIBSON ASSEMBLY:

For all our experiment we used the NEB Gibson assembly kit and protocol that you can directly find below on pages 10 to 11:

https://www.neb.com/~/media/Catalog/All-Products/0AA961B294E444AFBEDD5C4A904C76E6/Datacards%20or%20Manuals/manualE2611.pdf

IMPORTANT:

- Befor each Gibson Reaction we did a DpnI digest, as is recomended in the protocol. This restriction enzyme degrades methylated DNA. As only your PCR template is methylated it will be digested and thus you will have much less carry over during the Assembly reaction.

- The pmol calculation for this GA reaction is very important so in the following section I will show you how we did it based on the formula you can find in page 11 of the pdf file above.

1. Choose your vector quantity (ex: 100ng -> xµl) based on NEB range and the nanodrop concentration of your sample.
2. Calculate the amount of pmol of your vector based on the quantity you chose in step 1.) and the number of base pairs in your vector to verify if you are in the appropriate range of 0.02-0.5 pmols.
3. Then triple the pmols number you obtained for the vector to have the amount in pmols of insert you should add to the reaction.
4. Do the pmol calculation backwards to calculate the amount of insert you have to add, based on the amount of pmol you obtained in step 3.) and the number of base pairs in your insert.
5. Then add xµl vector + yµl insert +10µl NEB GA 2x mix + H2O up to 20µl.
6. Incubate at 50°C for 60min.