1 Folding DNA-Origami

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Material:

1. Three full pipette packages (small)
2. Multichannel pipette
3. Parafilm sized like the ordered plates
4. scaffold p7560 (end concentration(ec):10nM)
5. staples (ec: 100nM)
6. 1x TE (Tris EDTA)
7. $MgCl_2$ (ec: 0,1M)
8. Water

Before starting the experiment be sure about the concentrations and volumes one has to deploy. Therefore go through the following calculations:

$\Longrightarrow$ Calculate concentrations of the different staples by using the following formula:

$$c_1 \cdot V_1 = c_2 \cdot V_2$$

$$c_2 = \frac{c_1 \cdot V_1}{V_2}$$

Thereby $V_1 = 10\mu l$

- CORE concentration $c_2 = \frac{50\frac{pmol}{\mu l}}{135} = 0,37\mu M$
- SUGAR concentration $c_2 = \frac{50\frac{pmol}{\mu l}}{24} = 208\mu M$
- CONNECTOR concentration $c_2 = \frac{50\frac{pmol}{\mu l}}{9} = 5,5\mu M$
- FLUOROPHORE concentration $c_2 = \frac{50\frac{pmol}{\mu l}}{24} = 208\mu M$

Now we know how the staples are concentrated in 10 μl and with a start concentration of 50 $\frac{pmol}{\mu l}$. To achieve our desired end concentration (c_2) in 50 μl (V_2)(see brackets under material), one has to calculate the required volume.

- CORE volume $V_1 = \frac{50\mu l \cdot 100nM}{370nM} = 13,5\mu l$
- SUGAR+FLUOROPHORE volume $V_1 = \frac{50\mu l \cdot 100nM}{2080nM} = 2,4\mu l$
- CONNECTOR volume $V_1 = \frac{50\mu l \cdot 100nM}{5500nM} = 0,9\mu l$

Additional, the experiment is done under different Magnesium concentrations. The concentration of $MgCl_2$ is enhanced from 10mM to 20mM by steps of 2mM. With this information one has to calculate the required Volume for $MgCl_2$:

$$V_1 = \frac{c_2 \cdot V_2}{c_1}$$

The required Volume (V_1) of $MgCl_2$ with the start concentration of ($c_1 = 0,1M = 100mM$) and the desired end volume $V_2 = 50\mu l$ concentrated to 10mM is:

$$V_1 = \frac{10mM \cdot 50\mu l}{100mM} = 5\mu l$$

Repeat this calculation for the all the $MgCl_2$ concentrations.

Tabelle 1: Magnesium concentration and required Volume

Conc [mM]	10	12	14	16	18	20
$V_1 MgCl_2$ [μl]	5	6	7	8	9	10
H_2O [μl]	25.6	24.6	23.6	22.6	21.6	20.6

Because our end volume should be $50\mu l$, one has to stock up the Eppis with water. Now calculations are finished.

Prepare the stock solutions:

1. Start concentration of the DNA-sequences in the plates is always 50 $\frac{pmol}{\mu l}$.
2. Carefully open the plates.
3. Transfer the samples from the plate to the parafilm (multichannel pipette $10\mu l$)(Attention: Change the parafilm if other samples are used. One Parafilm for each sample!
4. Use the $1000\mu l$ pipette in order to bring the small droplets together to one big and absorb it with the pipette. Be careful to not loose any sample solution. Transfer the big droplet into an Eppi.

   WARNING: In this experiment the first Core sample got contaminated because of a minor amount that was on the gloves of the experimenter.
5. After transferring the $10\mu l$ sample volume in an Eppi, close the plate again with a new lid (drawer under the bench. Be careful that all holes of the plate are covered with the lid.
6. Repeat these steps with all the different samples.
7. Put the scaffold out of the freezer and wait a bit till it is unfreezed. Scaffold an staples should be in the ratio of 1:10. Attention: Avoid any mechanical stress to the scaffold! Never vortex or centrifugate!

8. Take one eppi for each staple flavour and transfer $5\mu l$ of the scaffold solution in each eppi.

9. In order to get the required 1x TE buffer, we transfer $5\mu l$ of the 10x TE buffer to each eppi.

10. in order to get 0,1M $MgCl_2$, we dilute the 1M $MgCl_2$ with water in the ratio of 1:10. Therefore mix $100\mu l$ $MgCl_2$ with $900\mu l$ H_2O.

11. Now the following ingredients should be in each eppi regarding the table above: Scaffold, staples, 1x TE, $MgCl_2$ (0,1M). Stock the solution up with H_2O.

    Attention: Because adding the staples SUGAR+FLUOROPHORE have been forgotten, the calculations concerning the water amount are not right. Thus the there is a fault concerning the magnesium concentration of about 10%. !Do not vortex the samples anymore, because the scaffold has already been transferred to the eppis!

12. Use the small red eppis for the Thermo Cycler (drawer under the bench).

13. We put the samples in the Thermo cycler in the following order:

    10 | 12 | 14 | 16 | 18 | 20

14. Choose the program Opti65

15. Close the Thermo cycler by carefully cut off the lid till small resistance is occurring. Don't push further. Otherwise one can break the eppis!