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iGEM TU/e 2014
Biomedical Engineering

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Rolling Circle Amplification on cell membrane

General protocol and instructions on how to verify using FACS

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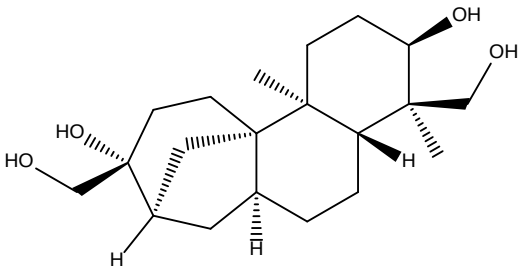
Title
Rolling Circle Amplification on cell membrane

1 Required materials

- Prepare a batch off 10^8 cells/mL expressing COMPx
- DNA-PEG_4-DBCO in dH_2O at 300 µM
- Solution of circular template in dH_2O or purer water
- Dissolve 1 mg of aphidicolin in 148 µL DMSO



Aphidicolin
MW: 338.48

2 Attaching DNA to cells

- Add 200 µL of cells with 2.2 µL of DBCO-PEG_4-DNA in a tube (DBCO:COMPx = 200.5:1)
- Make sure you vortex the cells well before and after adding the DBCO-compound
- React the tube for 1h in shaking block at 4°C and 500rpm
- Spin down the cells for 5 min at 13,400 rpm and discard the supernatant
- Resuspend with 1 mL ice cold PBS
- Spin down the cells for 5 min at 13,400 rpm and discard the supernatant

3 Rolling Circle Amplification

Prepare the following tube

Component	**Quantity/mass/final concentration**	**Volume (µL)**
H_2O	To 50 µL	xx µL
Circular DNA	50 nM	yy µL
DNTPs	5 mM	25 µL
10x Phi29 reaction buffer	1x	5 µL
Annealed DNA	50 nM	5 µL
Phi29 (100 U)	1 U/µL	5 µL
Total		50 µL

Put the tube in the heat shaking block on 30 °C for the desired time (advised is 4 hours). Add 2.1 µL of the aphidicolin stock solution (final concentration ~400 µM) to stop the reaction after the appropriate time.

4 Verification with FACS

- Spin down the cells for 5 min at 13,400 rpm and discard the supernatant
- Resuspend tube 2 in 200 µL ice cold PBS and add 10 µL of fluorescent primer stock (final concentration 16.7µM) solution and let primer anneal for 1 hour in heat shaking block at 4°C, shaking at 350 rpm.
- Spin down the cells for 5 min at 13,400 rpm and discard the supernatant
- Keep pellet in the fridge until FACS
- Just before FACS resuspend the cells in 200 µL ice cold PBS