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iGEM TU/e 2015
Biomedical Engineering

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Preparing FACS samples

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Table of contents

1 Preparing FACS samples

Estimated bench time: 90 minutes
Estimated total time: 3 hours
Purpose: Preparing the bacteria (after protein expression) for a FACS measurement. The bacteria will be extracted from the culture media and a fluorescent dye is added to covalently bind to the proteins.

1.1 Materials

- 1,5 ml cuvettes
- 5 mM DBCO-PEG_4-TAMRA
- Cell Density Meter (OD600)
- ddH_2O
- Eppendorf tubes
- MiniSpin centrifuge
- PBS-0.5%BSA
- Pipettes and tips
- Shaking block
- Tabletop centrifuge
- Tin foil

1.2 Setup & Protocols

- Spin down the cells in the tabletop centrifuge for 15 minutes at 3,000 xg and 4°C.
- Discard supernatant.
- Resuspend with 1 ml PBS-0.5%BSA and transfer to a 1.5 ml Eppendorf tube.
- Spin down the cells in the MiniSpin centrifuge for 1 minute at 13,400 rpm.
- Discard supernatant
- Resuspend with 1 ml PBS-0.5%BSA.
- Perform an OD measurement on a 20x dilution of the culture sample.
  OD measurement (OD600):
  - Blank: 950 µl ddH_2O and 50 µl PBS-0.5%BSA
  - Sample: 950 µl ddH_2O and 50 µl PBS-0.5%BSA
- Multiply the OD with 20.
- Calculate the amount of cells in the culture using the Agilent Technologies website. [1]
- Make a dilution with a concentration of $1*10^9$ cells/ml.
- Prepare tubes for the FACS (mix well & cover the samples).

Tube	[DBCO]	Cells [$1*10^9$] (µl)	DBCO-TAMRA (5mM) (µl)	Contains pAzf
1	0	200	-	Yes
2	30 µM	200	1.21	Yes
3	30 µM	200	1.21	No

- Incubate the samples in a shaking block for 1 hour at 300 rpm and 4°C. Make sure the tubes are in the dark.
- Spin down the cells in the MiniSpin centrifuge for 10 minutes at 13,400 rpm.

[1] http://www.genomics.agilent.com/biocalculators/calcODBacterial.jsp?_requestid=826255

- Discard the supernatant.
- Resuspend with 1 ml ice-cold-PBS-0.5%BSA. (keep the tubes on ice)
- Spin down the cells for 10 minutes at 13,400 rpm.
- Discard the supernatant and put the pellet on ice in the dark until FACS.
- For the FACS: resuspend with 200 µl ice-cold PBS-0.5%BSA.