File name: iGEM2016_01_FACS_comparison_of_cells.docx

iGEM2016 – Microbiology – BMB – SDU

Project type: Plastic **Project title:** Characterizing and optimizing PHB production **Sub project:** 1. Comparison of PHB producing strains	**Creation date:** 2016.08.24 **Written by:** Jakob Rønning **Performed by:** Joel Mario Vej-Nielsen & Jakob Rønning

1. SOPs in use.

SOP0001 – ON culture
SOP0042_v01 Daily use of FACS Aria
SOP0043_v01 Sorting setup FACS Aria II

2. Purpose.

Comparison of different PHB biobricks

3. Overview.

Day	SOPs	Experiments
1	SOP0001	ON culture
2	SOP0042	Sorting setup FACS Aria II
2	SOP0043	Daily use of FACS Aria

4. Materials required.

Materials in use

Name	Components (Concentrations)	Manufacturer / Cat. #	Room	Safety considerations
Appropriate medium ex. LB	1% Tryptone 1% NaCl 0.5% Yeast extract	Oxoid Sigma-Aldrich Merck	Media lab or V18-405-0	
Appropriate antibiotic if needed				
5 ml graduated pipettes		Fisher Scientific / CCI 4487	Micro storage	
Cuvettes		Contact lab-manager	BMB storage	
Blue pipette tips		Contact lab-manager	Micro storage	
Green pipette tips		Contact lab-manager	Micro storage	
Plate Bag		Contact lab-manager	BMB storage	
CST beads	Cytometer Setup and Tracking beads consist of bright (3 µm), mid (3 µm), and dim (2 µm) beads dyed with a mixture of fluorochromes that are excited by the lasers used in BD digital flow cytometers. See paragraph 12. Appendix	BD / 642412	Refrigerator V16-501b-2	
Accudrop beads	This product contains a single population of 6-µm particles. Every particle contains a fluorophore that is excited at 670 nm and emits at 750 nm. The particles are supplied in 1.5 mL of water containing 0.05% Tween® 20 and 2 mM sodium azide. See paragraph 12. Appendix	BD / 345249	Refrigerator V16-501b-2	

PBS	1x Phosphate Buffered Saline (PBS Buffer): 1 L distilled H_2O 8 g NaCl 0.2 g KCl 1.44 g Na_2HPO_4 0.24 g KH_2PO_4	Sigma-Aldrich / 31434N J. T. Baker / 3040-01 MERCK / 1.06580.1000 MERCK / 1.04873.1000	V18-405-0 V18-405-0 V18-405-0 V18-405-0	Autoclave before use
FACS Flow sheet fluid	See paragraph 12. Appendix	BD / 342003	Refrigerator V16-501b-2	
FACS Clean	See paragraph 12. Appendix	BD / 340345	Refrigerator V16-501b-2	
FACS Rinse	See paragraph 12. Appendix	BS / 340346	Refrigerator V16-501b-2	
Deionized H_2O		V18-405-0 or Hallway storage (1. Floor)		
FACS tube		BD / 352054	V16-501b-2	
15 ml tube		Sarstedt	BMB storage	
FACS Flow sheet fluid	See paragraph 12. Appendix	BD / 342003	Refrigerator V16-501b-2	

5. Other

At the link http://2015.igem.org/Team:Stanford-Brown/PHA, "**Using Nile Red to Quantify Plastic Production".** We used their instructions to compare the different PHB biobricks in the FACS.

6. Experiment history.

Date (YY.MM.DD)	SOPs	Alterations to SOPs and remarks to experiments

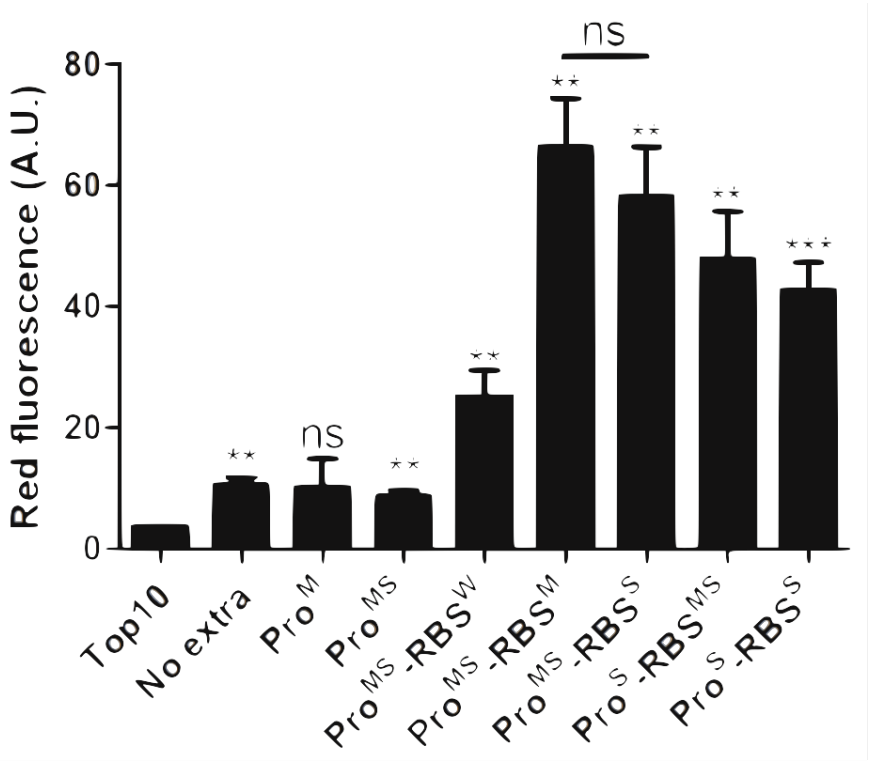
16.09.28	SOP0001	For #8, #9, #53, #90, #91, #92, #93 & #94 .
16.09.29	SOP0042	
16.09.29	SOP0043	

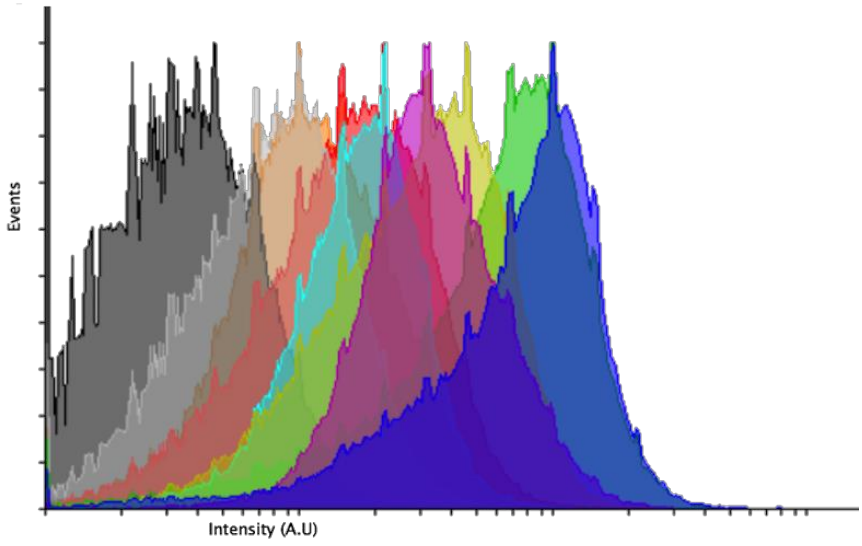
7. Sample specification.

Sample name	Sample content	From	Used for / Saved where
#8	*E. coli* with K2018000		Comparison
#9			Comparison
#53	*E. coli* with K934001		
#90	*E. coli* with K081005		
#91	*E. coli* with K2018033		
#92	*E. coli* with K2018034		
#93	*E. coli* with K2018035		
#94	*E. coli* with K2018036		

8. Remarks on setup.

9. Results and conclusions.





10. Appendixes

Data sheet for CST Beads: **23-9141-01_CS&T Beads_PI_RUO** is found in:

File name: iGEM2016_01_FACS_comparison_of_cells.docx
S:\999 Public\!SOPs and Protecols\FACS\FACS Aria II\SOPs for daily runs\Data sheets

Data sheet for Accudrop Beads: **23-8292-01_AccudropBeads** is found in:

S:\999 Public\!SOPs and Protecols\FACS\FACS Aria II\SOPs for daily runs\Data sheets

Data sheet for: FACS Flow sheet fluid: **342003-MSDS-EUEN-01 FACS Flow** is found in:

S:\999 Public\!SOPs and Protecols\FACS\FACS Aria II\SOPs for daily runs\Data sheets

Data sheet for: FACS Clean: **340345-MSDS-EUEN-00 FACS Clean** is found in:

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Data sheet for: FACS Rinse: **340346-MSDS-EUEN-00 FACS Rinse** is found in:

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