iGEM 2017 – Microbiology – BMB – SDU

Title: Genome exstraction	**Date issued:** 2017.08.09
SOP number: SOP22	**Review date:** 2017.08.09
Version number: 01	**Original by:** MKJ

1. **Purpose**
   Exstract the genome from Geobacter sulfurreducens

2. **Area of application**

3. Apparatus and equipment

Apparatus/equipment	Location (Room number)	Check points	Criteria for approval/rejection
Pipettes (p1000, p20)		●	

4. Materials and reagents – their shelf life and risk labelling

Name	Components (Concentrations)	Manufacturer / Cat. #	Room	Safety considerations
Red pipette tips		Contact lab-manager	Micro storage	
Blue pipette tips		Contact lab-manager	Micro storage	
$MgCl_2$	10 mM		Laboratory (class 1) Chemical room	
$CaCl_2$	5 mM		Laboratory (class 1) Chemical room	
$MgSO_4$	10 mM		Laboratory (class 1) Chemical room	
Sodium citrate	500 mM		Laboratory (class 1) Chemical room	
Chloroform				

5. QC – Quality Control

6. List of other SOPs relevant to this SOP

7. Environmental conditions required

8. Procedure

1.1 - Grow an ONC of the gebactor.

1.2 - spin down and clean in 0,9% NaCl.

1.3 - resuspend in 1 ml lysis-buffer, with 10 µl RNase (25µl/ml).

lysis-buffer :

- 1ml Tris pH 8,0 1M

- 4ml EDTA 0,1 M
- 15 ml H2O

1.4 - Ad 100 µl lysozym (400µg/ml) and incubate at 37 degree in 10 min.

1.5 - ad 50 µl 10% SDS and incubate at 37 degree in 20

1.6 - ad 1 ml phenol and shake for 3 min. then spin at max for 5 min.

1.7 - Transfer the waterfase to a new eppendorftube and do phenol extraction once more.

1.8 - Transfer the waterfase to a new eppendorftube and do chloroform extraction twice.

1.9 - fell with 0,6 volume 96% ethanol 1 hour -> ON at -20 degree

2.0 - Spin down at max 4 degree, 30 min.

2.1 - wash pellet 2x in 70% ethanol and one time in 96% ethanol

2.2 - let the most ethnol dry, and ad 200 µl water. test concentration on nanodrop.

9. Scheme of development

10. Appendices