iGEM2014 – Microbiology – BMB – SDU	
Title: Phusion PCR **SOP number:** 0010_v01 **Version number:** 01	**Date issued:** 2013.06.18 **Review date:** 2013.06.18 **Written by:** Patrick Rosendahl Andreassen

1. Purpose

To amplify DNA with high fidelity DNA polymerase

2. Area of application

This procedure is valid for all genomes

3. Apparatus and equipment

Apparatus/equipment	Location (Room number)	Check points	Criteria for approval/rejection
PCR machine	Laboratory (class 1) - V18-403b-2	●	
Pipettes (p20, p2.5)		●	
PCR tube mini centrifuge	Laboratory (class 1) - V18-403b-2	●	
Gel electrophoresis chamber	Laboratory (class 1) - V18-403b-2	●	
Gel electrophoresis power supply	Laboratory (class 1) - V18-403b-2	●	
Gel electrophoresis comb	Laboratory (class 1) - V18-403b-2	●	
Gel electrophoresis slide	Laboratory (class 1) - V18-403b-2	●	

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

Name	Components	Supplier / Cat. #	Room (hallway storage)	Safety considerations

Purple pipette tips		Contact lab-manager	Micro storage	
Green pipette tips		Contact lab-manager	Micro storage	
PCR tubes		Contact lab-manager	Micro storage	
Distilled water		Contact lab-manager	Micro storage	
5X HF or GC Buffer		Agilent Technologies	Freezer at 1. Floor	
dNTPs (10 mM each dNTP)		Agilent Technologies	Freezer at 1. Floor	
DNA template			Freezer at 1. Floor	
Primer #1 (10μM)			Freezer at 1. Floor	
Primer #2 (10μM)			Freezer at 1. Floor	
Phusion® High-Fidelity DNA Polymerase		New England Biolabs	Freezer at 1. Floor	
PCR/gel purification kit			Micro storage	

5. QC – Quality Control

Analyze the PCR amplification products on an agarose gel□

Check digest with appropriate restriction enzymes and check length of fragments on an agarose gel

6. List of other SOPs relevant to this SOP

7. Environmental conditions required

8. Procedure

1. When <u>not</u> doing a gradient: Prepare a reaction mixture for the appropriate number of samples to be amplified (20ul per reaction). Add in the listed order
    1. Distilled water (dH_2O) → 20.0 µl
    2. 5X HF or GC buffer 4.0 µl
    3. dNTPs (10 mM each dNTP) 0.4 µl
    4. DNA template variable
    5. Primer #1 (10 µM) 1.0 µl
    6. Primer #2 (10 µM) 1.0 µl
    7. Phusion DNA polymerase 0.2 µl

2. When doing a gradient: Prepare a reaction mixture for the appropriate number of samples to be amplified (50ul per reaction). Add in the listed order
    1. Distilled water (dH_2O) → 50.0 µl
    2. 5X HF or GC buffer 10.0 µl
    3. dNTPs (10 mM each dNTP) 1.0 µl
    4. DNA template variable
    5. Primer #1 (10 µM) 2.5 µl
    6. Primer #2 (10 µM) 2.5 µl
    7. Phusion DNA polymerase 0.5 µl
    8. Aliquot 10 µL into 5 PCR tubes
3. Centrifuge PCR tubes in PCR tube mini centrifuge
4. Perform PCR using optimized cycling conditions (see PCR Cycling Parameters).
5. Do the PCR song and dance while sacrificing a latex glove (size optional)
6. Run PCR product(s) on an agarose gel with a DNA ladder
7. Purify DNA using PCR/gel purification kit

Segment	**Step**	**Temperature**	**Duration**
1	Initial denaturation	98°C	2 min
2	25-35 cycles	98°C	5-10 sec
		Primer T_m	10-30 sec
		72 °C	15-30 sec per kb
3	Final extension	72°C	5-10 min
4	Hold	4-10°C	

9. Waste handling

Chemical name	**Concentration**	**Type of waste (C, Z...)**	**Remarks**
One use plastic		GMO waster	Yellow GMO Trash Bin

10. Time consumption

- Total-time 3 hours.
- Hands-on-time 30 min.

11. Scheme of development

Date / Initials	**Version No.**	**Description of changes**
13.06.18 / PRA	01	The SOP has been written
13.06.18 / AK	01	The SOP has been approved

12. **Appendices**