JMJ-Group – Microbiology – BMB – SDU

Title: PCR with MyTaq	**Date issued:** 2013.06.18
SOP number: SOP0011_v01	**Review date:** 2013.06.18
Version number: 01	**Written by:** SIS & TJK

1. Purpose

To amplify wanted fragment of template via MyTaq PCR.

2. Area of application

This procedure is valid for all *E. coli* strains

3. Apparatus and equipment

Apparatus/equipment	Location (Room number)	Check points	Criteria for approval/rejection
PCR machine	Laboratory (class 1) – V18-403b-2	• Check program	Appropriate PCR program
Pipettes (p100,20,10)			
PCR tube rotator	Laboratory (class 1) – V18-403b-2		

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

Name	Components (Concentrations)	Manufacturer/Cat. #	Room	Safety considerations
Water	Demineralised milli-Q autoclaved water	Milli-Q water purification system (Millipore)	RT	
MyTaq™HS Red Mix	http://www.bioline.com/documents/product_inserts/MyTaq%E2%84%A2%20HS%20Red%20Mix.pdf#zoom=130	Bioline	V18-405a-2	
Reverse primer	Made specific to the template	Sigma-Aldrich		
Forward primer	Made specific to the template	Sigma-Aldrich		
PCR tubes		Eppendorf	Micro storage	
1.5 ml tubes		Contact lab-manager	BMB storage	
Green pipette tips		Contact lab-manager	Micro storage	
Purple pipette tips		Contact lab-manager	Micro storage	

5. QC – Quality Control

For more than 2 samples; a premix of primers, MyTaq, primers and water is mixed and aliquoted before adding template.

6. List of other SOPs relevant to this SOP

iGEM2013_SOP007_v01_excision and ligation of PCR products in USER cloning

iGEM2013_SOP008_v01_preparing primers

7. Environmental conditions required

8. Procedure

8.1 Mix primers, water and MyTaq as described under PCR set up; paragraph 12 with the template
8.2 Be sure that all the samples are in the bottom of the PCR tubes by spinning on PCR tube rotator
8.3 Place in PCR machine
8.4 Start the appropriate PCR program for MyTaq or design one yourself, see paragraph 12
8.5 Keep at 4-5 °C until use, if more than 2 days waiting time place in -20°C

9. Waste handling

Chemical name	Concentration	Type of waste (C, Z…)	Remarks
Once used plastic		GMO yellow waste	

10. Time consumption

- Total-time 2 hours
- Hands-on-time 0.5 hour

11. Scheme of development

Date / Initials	Version No.	Description of changes
13.06.18 / SIS & TJK	01	The SOP has been written
13.06.18 / PRA	01	The SOP has been approved

12. Appendixes

PCR set up, 1 sample:

Notes: Smaller volume than recommended by Bioline

Template:	variable
Primers (10 µM):	0.5 µl each
MyTaq HS Red Mix, 2x:	5 µl
Water (sterile):	3,5 µl
Total:	10 µl

PCR cycling conditions:

Step 1: Initial denaturation:	95 °C	2 min
Step 2: Denaturation:	95 °C	15 sec
Step 3: Annealing:	55 °C	15 sec (depending on the primer sequences, 2-5 °C below the lowest Tm of the primers)
Step 4: Extension/Elongation:	72 °C	10-30 sec (30 sec. pr. kilo bp)
Step 5: Repeat step 2-4:	30 times	
Step 6: Extra elongation:	72 °C	2.5 min
Step 7: Keep the samples cold	4 °C	until the samples is removed