iGEM 2013 – SDU	
Title: Colony PCR with MyTaq **SOP number:** SOP0021_v01 **Version number:** 01	**Date issued:** 2012.11.26 **Review date:** 2013.12.03 **Written by:** Tina Kronborg

1. Purpose

Colony PCR with My Taq

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
Heating block	Laboratory (class 1) – V18-404-2 Laboratory (class 2) – V18-501b-2	● Preheat to 96 °C	
PCR machine	Laboratory (class 1) – V18-403b-2	● Check program	Appropriate PCR program
Pipettes (p100,20,10)			
Container for ice			
PCR tube rotator	Laboratory (class 1) – V18-403b-2		

4. Materials and reagents – their shelf life anod 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	
Ice			V18-403a-2	
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

Designing primers:

Generally, the annealing temperature is about 5°C below the lowest melting temperature (T_m) of the pair of primers used, and should be around 55°C so T_m should be around 60°C.

$T_m = 4(G + C)\ °C + 2(A + T)\ °C$

Each primer should be about 20-30 nucleotides.

6. List of other SOPs relevant to this SOP

JMJ_SOP0006_v01_MM_Agarose_gel_DNA

7. Environmental conditions required

When the bacteria samples have been boiled they can be removed from class II and transferred to class I

8. Procedure

1. Add 50 µl sterile water to a 1.5 ml tube – one for each sample
2. Dip a green tips in the colony and transfer it to the eppendorf tube
3. Boil at 96 °C for 5 min in a heating block
4. Or a colony sample can be transferred into a PCR-tube and with open lid be microwaved for 2 min at max effect
5. Place the samples on ice
6. Mix primers, water and MyTaq as described under PCR set up; paragraph 12 with the template
7. Be sure that all the samples are in the bottom of the PCR tubes by spinning on PCR tube rotator
8. Place in PCR machine
9. Start the appropriate PCR program for MyTaq or design one yourself, see paragraph 12
10. 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
Boiled bacteria waste		GMO yellow waste	
Once used plastic		GMO yellow waste	

10. Time consumption

- Total-time 2 hours
- Hands-on-time 1 hour

11. Scheme of development

Date / Initials	Version No.	Description of changes
12.11.26 / TK	01	The SOP has been written
13.05.22 / TK & MM	01	The SOP has been approved

12. Appendixes

PCR set up, 1 sample:

Notes: Smaller volume than recommended by Bioline

Template:	200 ng	Template: 0.5 µl of 50 µl sterile water boiled with some of the bacteria colony or mix directly into the PCR tube with the microwaved colony
Primers (20 pmol):	0.2 µl each	
MyTaq HS Red Mix, 2x:	5 µl	
Water (sterile):	4.1 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:	60 °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