iGEM2013 – Microbiology – BMB – SDU	
Project type: PCR **Project title:** PCR of plasmid pSB1C3 **Sub project:**	**Creation date:** 18/06-13 **Written by:** ASF, MHK **Performed by:** ASF, AK, MHK

1. SOPs in use

SOP0006_v01 - PCR SOP for USER cloning

2. Purpose

To amplify the pSB1C3 plasmid to use in USER cloning.

3. Overview

Day	SOPs	Persons	Experiments
1	SOP0006_v01	ASF, AK, MHK	USER PCR of linearized pSB1C3
2	SOP0006_v01	ASF, AK, MHK	USER PCR of non-linearized pSB1C3. The BioBrick BBa_J04450 was part of the plasmid, but not included in the PCR.
3	SOP0006_v01	ASF	Gradient USER PCR af non-linearized pSB1C3 as above.
4	SOP0006_v01	SIS, MH, KJ	USER PCR of linearized pSB1C3
5	SOP0014_v01 SOP0006_v01 SOP0013_v01	SIS, MH	Gel purification USER PCR on failed purification sample in order to make a new purification. Nano drop on the second purification attempt.
6	SOP0014_v01 SOP0013_v01 SOP0022_v01	SIS	Gel purification Nano drop on the purification Speedy Vac
7	SOP0006_v01	MH, MHK, HWJ	3 PCR with different elongation times (2, 2.5 and 3 min) and a gradrient PCR with temperatures ranging from 50-62^{o}C witn elongation time 2min.

8	SOP0006_v01 SOP0014_v01 SOP0013_v01 SOP0019_v01	MHK, MH, HWJ	USER PCR of pSB1C3 to optimize PCR program. Elongation time and annealing temperature were tested. Mini-prep on ONC with RFP-pSB1C3
9	SOP0006_v01 SOP0022_v01 SOP0014_v01 SOP0006_v01	SIS, TJK, MH	USER PCR of pSB1C3. Speedy Vac on sample green 26. Gel purification. A USER PCR was started and will be analysed tomorrow morning.
10	SOP0014_v01	MH, SIS	Gel purification

4. Materials required

Materials in use

Name	Components (Concentrations)	Manufacturer / Cat. #	Room	Safety considerations
Linearized pSB1C3	25 ng/µL	iGEM	iGEM cupboard	N/A
Primer 006	10 µM	sigma	iGEM freezer	N/A
Primer 007	10 µM	sigma	iGEM freezer	N/A
Transformation efficiency	0.5 pg/µL	iGEM	iGEM cupboard	N/A

5. Other comments

18.06.13

- The BioBrick BBa_J04450 is a RFP Coding Device, and hence the colonies in future experiments will become red if the PCR products contains more than just the backbone of the plasmid.
- The reason for using a different template was that we believe this template contains the full binding sites for the primers.

6. Experiment history

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

17.06.13	SOP0006_v01	The DNA template was linearized pSB1C3. Tm was 56 deg C and 59 deg C, and hence the temperature of segment 2,2 was 51 deg C. Segment 2,3 was 2 minutes since the size of the target was ~2kb.
18.06.13	SOP0006_v01	The DNA template was non-linearized pSB1C3 containing the BioBrick BBa_J04450. Tm was 56 deg C and 59 deg C, and hence the temperature of segment 2,2 was 52 deg C. Segment 2,3 was 2 minutes 10 seconds since the size of the target was ~2kb.
19-06-13		A mastermix of 6 times the volumes of the reagents was made. 50 µL was put in each of 5 PCR-tubes. The temperatures of segment 1 and 2.1 was 98 deg C. The PCR was made with a gradient, the temperatures of the 5 tubes i segment 2.2 was: 44.9, 48.5, 56.1, 61.1 and 65.1. Segment 2.3 ran for 2:10 minutes.
02.07.13	SOP0006_v01	The PCR program was altered from the SOP version to the recommended IGEM procedure: 95 deg 2 min 95 deg 30 sec* 55 deg 30 sec* 68 deg 3 min* 68 deg 10 min *37 runs 50 µL was loaded in each well on the gel.

03.07.13	SOP0014_v01 SOP0006_v01 SOP0013_v01	In the first purification the wash step was accidentally skipped. In order to compensate for the mistake, a USER PCR was made using the flow through as template. The PCR program was altered from the SOP version to the recommended IGEM procedure: 95 deg 2 min 95 deg 30 sec* 55 deg 30 sec* 68 deg 3 min* 68 deg 10 min *37 runs The remaining flow through (unwashed, eluted DNA) was mixed with the first step flow through (capture buffer) and additionally 100 µL capture buffer was added and the purification was started over. To calculate the concentration of DNA in the purification sample, NanoDrop was performed. Overnight PCR using second purification sample 16 as template.
04.07.13	SOP0014_v01 SOP0013_v01 SOP0022_v01	To calculate the concentration of DNA in the purification sample, Nano Drop was performed. The result was very low. In order achieve a higher concentration all three purification pSB1C3 samples (green 22, green 15, green 16) were speedy vaxed.

05.07.13	SOP0006_v01 SOP0014_v01 SOP0013_v01	3 different PCRs with elongation times at 2, 2.5 and 3 minutes were made. Further, a gradient PCR with temperatures ranging from 50-62°C with elongation time 2min was done. All 4 PCR reactions were mixed in 3 master mixes, 1 each prepared by MH, HWJ and MHK. Specific temperatures for the gradient PCR: 49,9°C 50,2°C 50,9°C 52,0°C 53,4°C 54,9°C 56,5°C 58,1°C 59,5°C 60,7°C 61,6°C 62,6 °C A gel purification was done on the PCR products from 1 gel. Nano Drop was used to estimate concentrations.
08.07.13	SOP0006_v01 SOP0022_v01 SOP0014_v01 SOP0006_v01	USER PCR of pSB1C3 with 3 different elongation times (2 min, 2.5 min and 3 min.). Annealing temp.: 61,6 deg. iGEM PCR program Sample green 26 was speedy Vac'ed in order to concentrate the sample. A USER PCR was started and will be analysed tomorrow. Annealing temp. 61,6 deg and elongation time 2,5 min. iGEM PCR program. 4 samples was run for 37 cycles, and 4 samples for 50 cycles.
09.07.13	SOP0014_v01	Gel purification of the bands from 08.07.13.

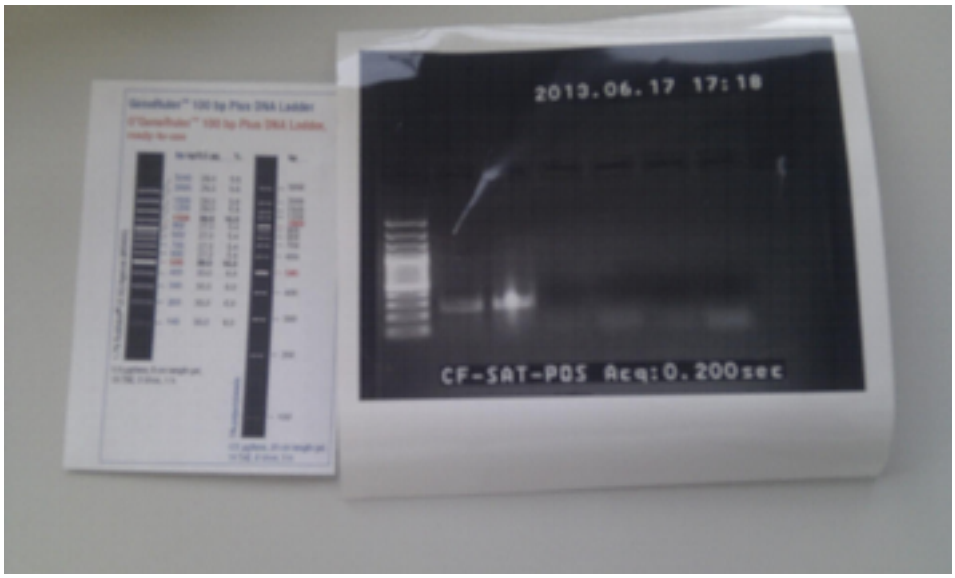
7. Sample specification.

Sample name	Sample content	Description:	Used for / Saved where
Green 15	USER pSB1C3 1	USER PCR product of pSB1C3 1 plasmid purified from gel (2. purification). Concentration: 2.6 ng/µL	Green box in the fridge.
Green 16	USER pSB1C3 2	USER PCR product of pSB1C3 2 plasmid purified from gel (2. purification). Concentration: 2.8 ng/µL	Green box in the fridge.
Green 22	USER pSB1C3	USER PCR product of pSB1C3 2 plasmid purified from gel. concentration: 19,1 ng/µL	Green box in the fridge.
Blue 25	Plasmid pSB1C3 with RFP	Samples from the miniprep of the ONC were poolet. Final concentration: 44.9 ng/µL	Green box in the fridge.
Green 26	USER pSCB1C3	USER PCR product of pSB1C3 plasmid purified from gel Concentration: 22,4 ng/µL	Green box in the fridge.
Green 28	USER pSCB1C3	USER PCR product of pSB1C3 plasmid purified from gel. Concentration: 11,1 ng/µL	Green box in the fridge.
Green 30	USER pSC1C3	USER PCR product of pSB1C3 plasmid purified from gel. Concentration: 15,2 ng/µL	Green box in the fridge.

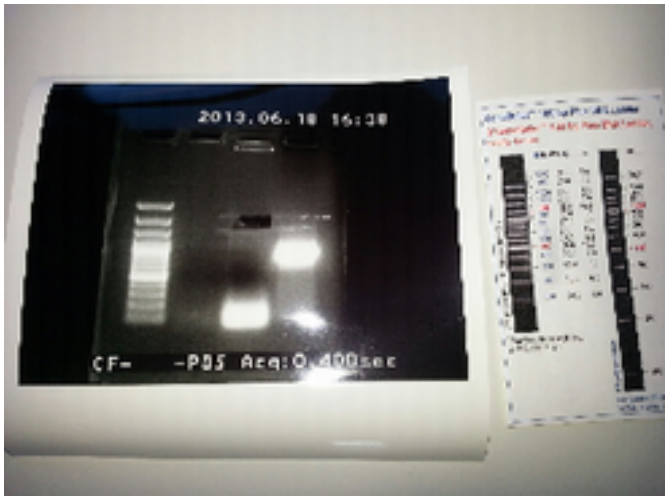
8. Remarks on setup

9. Results and conclusions

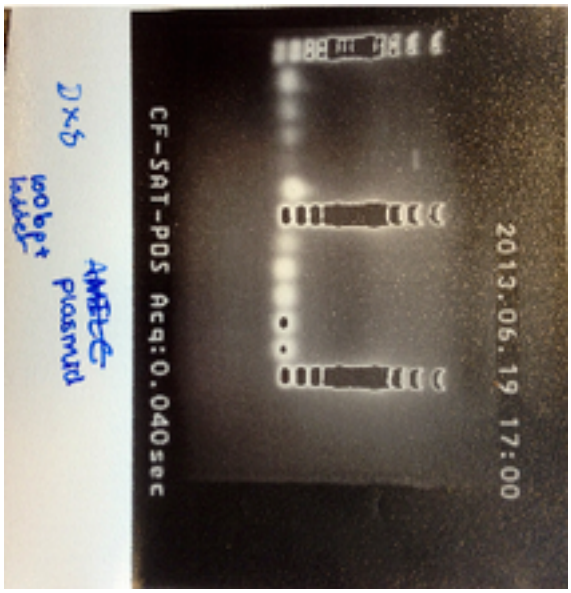
17.06.13 The gel was 1,5% agarose and the light blue (100+bp) ladder was used. The PCR product was loaded in well number 2 (5 µL) and 3 (10µL). The result was approximately 300bp. The expected result was 2070bp. We believe the primers were designed badly since the linearization of the plasmid resulted in the pre- and suffix being cut differently than we expected.



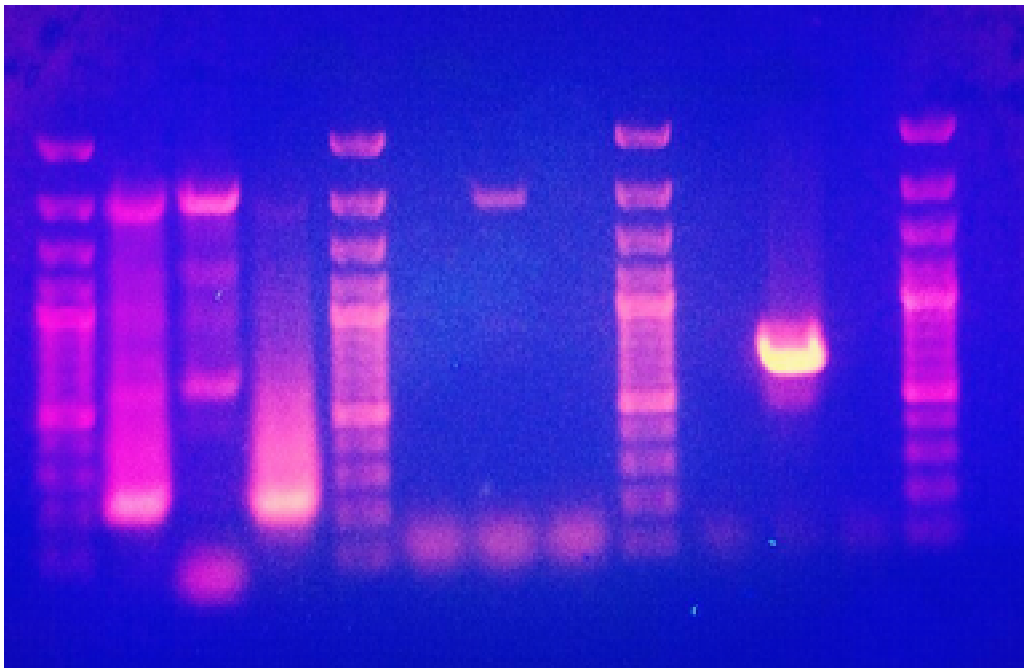
18.06.13 The gel was 1% agarose and the light blue ladder was used. 20µL was loaded in well nr. 2, no band appeared.



19-06-13: 1% agarosegel. 35µL loaded. Ingen resultater med gradient PCR af pSB1C. Ladder 1,12 og 13 er laddere. 8-12 er plasmid. Kun primerdimers.



02.07.13



Gel used: 1% agrose gel. Ladder: 100 bp blue ladder. Load: 50 µL PSB1C3 in wells #2-4 with USER PCR product found in well 7. Sample was cut from gel and placed in eppendorf in the refrigerator.

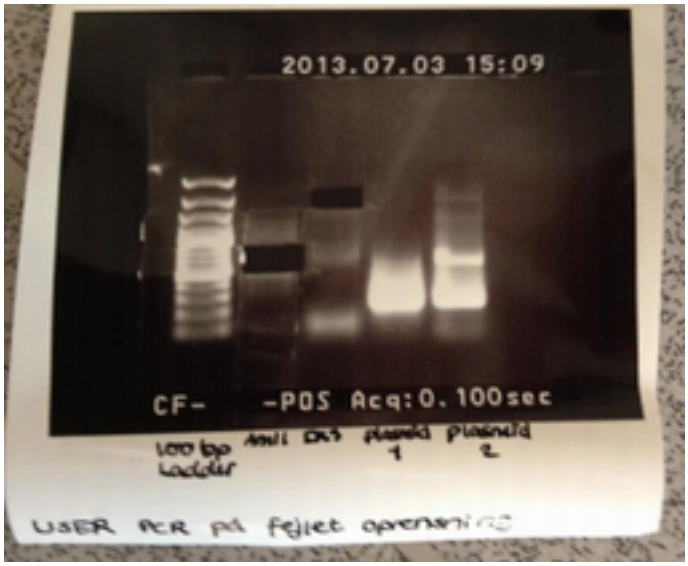
03.07.13

Results for the Nanodrop on the second purification attempt:

	Amil-linker	Dxs	pSB1C3 1	pSB1C3 2
Concentration (ng/µL):	10,8	2,3	2,1	2,7
260/280:	1,93	-5,65	4,72	4,06
260/230:	0,13	0,01	0,03	0,01

Results for the PCR with the unwashed, eluted DNA as template (1. purification):

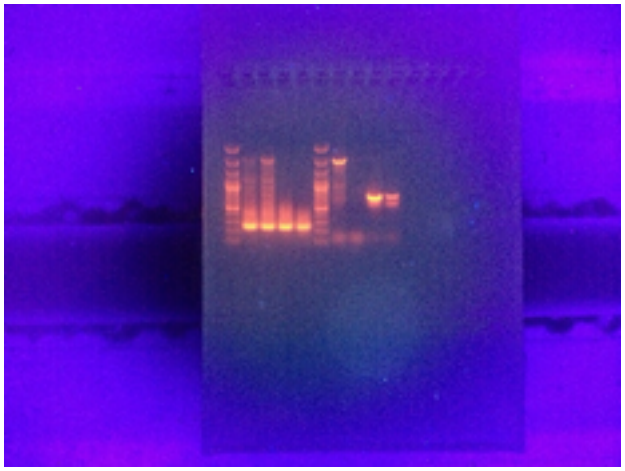
50 µL was loaded in well 4 and 5 on a 1% agarose gel. No band appeared.



04.07.13

Results for PCR with 2. purification sample 16 as template:

50 µL was loaded in well 2-5 on a 1% agarose gel. Only a very small band appeared around 2000 bp length. The band was cut out and purified using the Gel purification kit. The purified sample (green 22) was stored in the green box in the fridge.

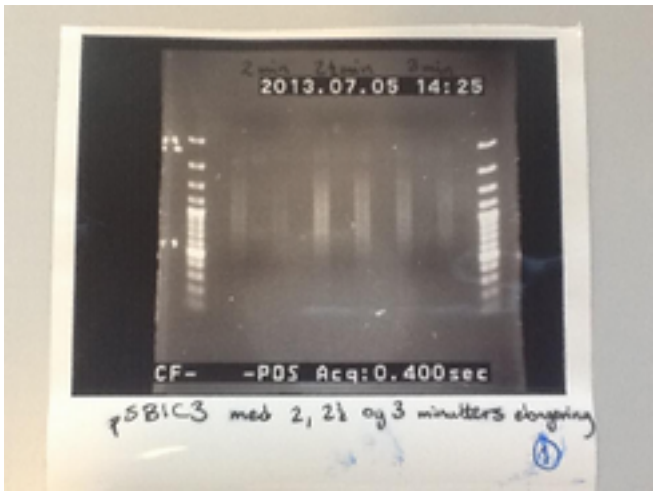


The three purification samples (green 22, green 15 and green 16) was speedy vaxed in order to get higher concentrations. The results:

	Green 22 pSB1C3	Green 15 pSB1C3	Green 16 pSB1C3
Concentration (ng/µL):	19,1	2,6	2,8
260/280:		-74,05	10,0

05.07.13

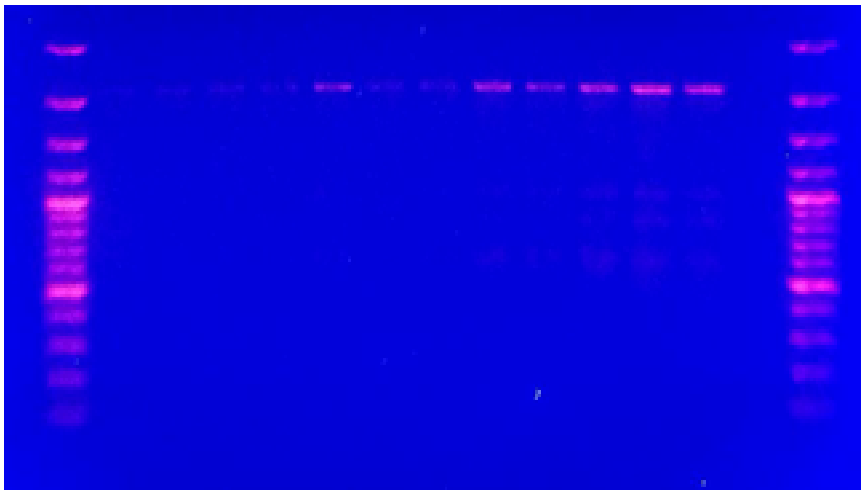
The following pictures of gels refer to the attempted purification of the PCR program for pSB1C3:



Ladder: 100 bp plus. Well 3-5 contain PCR product from elongation time of 2 minutes. Wells 7-9: 2.5 minutes. Wells 11-13: 3 minutes. Wells 1, 6, 10, and 14 are empty.
The bands are unclear. The elongation times will be redone in a PCR with annealing temperature to be determined from gradient PCR.

Ladder: 100 bp plus. Wells 2-13 contain samples corresponding to temperatures as found under experiment history for 05.07.13. The gel was disposed of, due to lacking PCR product.

Ladder: 100 bp plus. Wells 2-13 contain samples corresponding to temperatures as found under experiment history for 05.07.13, with the following exception: Well 14 contains the sample which should have been loaded in well 11, and the following wells (12-13) are displaced. The gel was disposed of due to lacking PCR product.



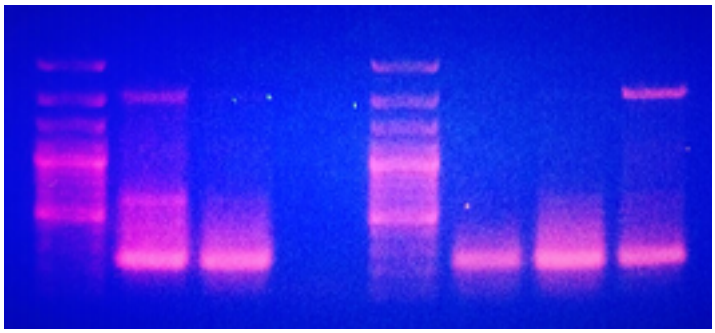
Ladder: 100 bp plus. Wells 2-13 contain samples corresponding to temperatures as found under experiment history for 05.07.13. The bands at 2000 bp were purified and the combined concentrations were found to be 6.6 ng/µL. It has been decided to concentrate the sample in the Speedy Vax on 08.07.13.

08.07.13

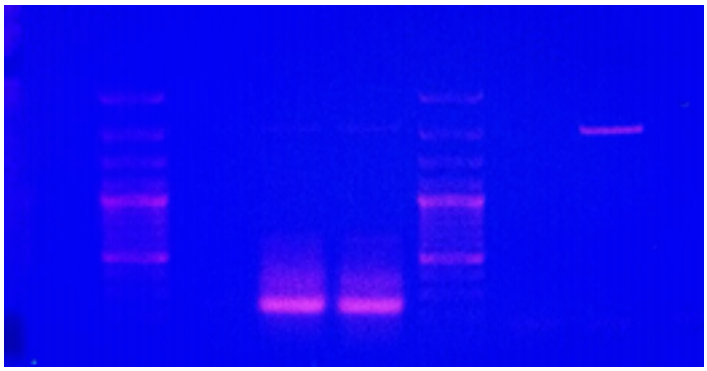
Result for the Speedy Vac: 22,4 ng/µL.

Resultat af USER PCR:

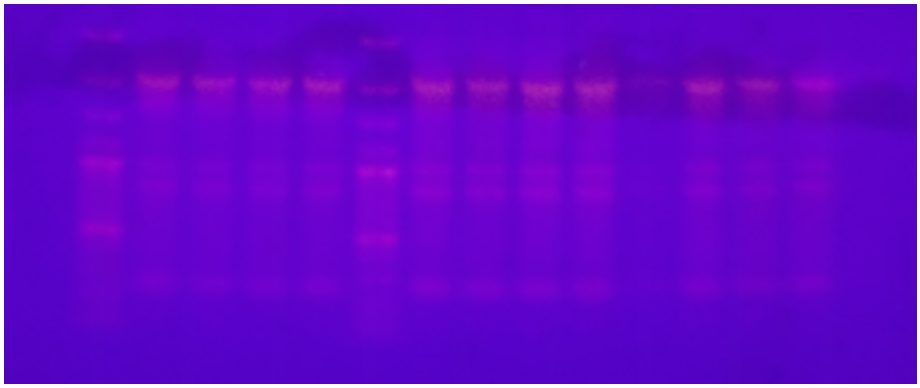
50 µL was loaded in each well. Well 2-5 contains pSB1C3 PCR performed with 2 min elongation time and well 7-9 contains pSB1C3 PCR performed 2,5 min elongation time. A clear band appeared in well 2 and 9. The bands were cut out and purified and the concentration measured. The samples was pooled and stored in the green box in the fridge.



50 µL was loaded in each well. Well 2-5 contains pSB1C3 PCR performed with 3 min elongation time and well 7-9 contains Dxs PCR. No clear band appeared.



09.07.13



Ladder: 100 bp plus. Gel: 1% agarose. Load: app. 50 µL. Wells 2-5 contain PCR product from program with 37 cycles. Wells 7-10 contain PCR product from program with 50 cycles. Wells 11-14 contain remnants of PCR product from program with 37 cycles, which could not fit into wells 2-5. There are bands in all wells. These were cut and purified to a concentration of 15,2 ng/µL after the samples were combined.

10. Appendices