iGEM 2015 – Microbiology – BMB – SDU

Project type: Characterization **Project title:** Characterization of PcstA	**Creation date: 27.08.2015** **Written by:** JSP, TBA, CEM **Performed by:**JSP, TBA, EMT, ADK, CEM

1. SOPs in use

iGEM2013_SOP0021_v01_ Colony PCR with MyTaq
iGEM2015_SOP0017_v01_Fast digest
iGEM 2014 SOP0009 Transformation
iGEM2015_SOP015_v02_ ligation
iGEM2013_SOP0028_v01-Northern blotting

2. Purpose

To better characterize the cAMP-inducable promotor PcstA.

Three experiments are designed to characterize the sensitivity of the promotor;

1. Promotor activity in a WT during growth. Measured in amount of RNA of GFP downstream of PcstA.
2. Promotor activity over time induced by 1 mM cAMP in a ΔcyaA-strain. Measured in amount of RNA oof GFP downstream of PcstA.
3. Promotor activity depending on different concentrations of cAMP in a Δ cyaA-strain. Measured by fluorescence of GFP.

3. Overview

Date (DD.MM.YY)	**Person(s) (initials)**	**Experiments**	**SOPs**

25.08.2015	JSP	Miniprep of PcstA-GFP	Bio-Rad Quantum Prep: plasmid miniprep Kit
26.08.2015	TBA	Transformation of R99 in MG1655, BTH101 (adenylate cyclase deficient) and MG1655 Δ*cyaA* ::kan	iGEM 2014 SOP0009 Transformation
26.07.2015	JSP	Phusion PCR	iGEM2013_SOP0021_v01_ Colony PCR with MyTaq
27.08.2015	JSP	Colony-PCR	iGEM2013_SOP0021_v01_ Colony PCR with MyTaq
28.08.2015	TBA, JSP	Collection of samples for RNA purification	
29.08.2015	TBA	Miniprep of pSB1C3-PcstA Fast Digest of pSB1C3-PcstA and PCR product of GFP-LVA Ligation of aforementioned digestions.	Thermo-Scientific miniprep kit
30.08.2015	EMT TBA, JSP	Trasnformation of ligations RNA purification	iGEM 2014 SOP0009 Transformation
31.08.2015	TBA, JSP TBA	Wash of RNA samples New ligation of GFP-LVA	iGEM2015_SOP015_v02_ ligation
01.09.2015	ADK, TBA	Concentration measurement of RNA samples	iGEM 2014 SOP0009 Transformation

	CEM	Transformation of ligations	
02.09.2015	JSP ADK, TBA JSP JSP	Colony-PCR. Negative results. Test run of RNA-samples on agarose gel Fast Digest of G49 (GFP-LVA) and pSB1C3-PcstA Ligation	iGEM 2013 SOP0021 Colony PCR with My Taq iGEM2015_SOP0017_v01_ Fast digest iGEM2015_SOP015_v02_ ligation
03.09.15	ADK, CEM	Northern blotting	iGEM2013_SOP0028_v01-Northern blotting
04.09.15	ADK	Northern blotting continued	iGEM2013_SOP0028_v01-Northern blotting
05.09.15	ADK	Northern blotting continued	iGEM2013_SOP0028_v01-Northern blotting
15.09.15	ADK	Northern blotting	iGEM2013_SOP0028_v01-Northern blotting
16.09.15	ADK	Northern blotting continued	iGEM2013_SOP0028_v01-Northern blotting
17.09.15	TBA, JSP	Blots scanned and data processed.	

4. Materials required.

Materials in use

Name	Components (Concentrations)	Manufacturer / Cat. #	Room	Safety considerations

5. Experiment history

Date (DD.MM.YY)	Person(s) (initials)	Alterations to SOPs and remarks to experiments	SOPs
25.08.2015	JSP	Miniprep of overnight culture from LB-agar stab from HQ containing PcstA-GFP in pSB1C3-backbone. Stored as R99	Bio-Rad Quantum Prep: plasmid miniprep Kit
26.08.2015	TBA	Transformation of R99 in MG1655, BTH101 (adenylate	iGEM 2014 SOP0009 Transformation

		cyclase deficient) and MG1655 Δ*cyaA* ::kan	
26.07.2015	JSP	Phusion PCR on GFP with LVA tag (BBa_K1135002 from parts registry) with primer 025 and 026. No alterations to SOP. HF buffer used. Annealing temperature set to 55 C.	iGEM2013_SOP0021_v01_ Colony PCR with MyTaq
27.08.2015	JSP	Colony-PCR and streak plate preparation Preparation of Overnight culture from the three different streak plates.	iGEM2013_SOP0021_v01_ Colony PCR with MyTaq
28.08.2015	TBA, JSP	Collection of samples for RNA purification. Promotor activity in a WT during growth: Overnight culture of WT MG1655, BTH101 and MG1655 Δ cyaA::kan were diluted to OD = 0,005 @ 600 nm. Two dilutions of WT was prepared; one with 0,2 % glucose added and one without. Both adenylate cyclase deficient strains were grown in regular LB. For WT + 0,2% glucose samples were collected at OD = 0,14; 0,55; 0,86; 1,17; 1,49; 1,51; 1,56. For WT in LB samples were collected at OD = 0,14; 0,33; 0,52; 0,77; 1,08; 1,69; 1,94; 2,08.	

		For BTH101 and Δ*cyaA* one sample was collected at OD = 0,29 and OD = 0,30, respectively. <u>Promotor activity over time induced by 1 mM cAMP:</u> A 200 mM stock solution of cAMP was prepared. BTH101/pSB1C3-PcstA-GFP and MG1655 Δ*cyaA* ::kan/pSB1C3-PcstA-GFP was diluted to OD = 0,005, and collection of samples began at OD ≃ 0,2 @ 600 nm. A samples was collected from each solution before addition of cAMP. 1 mM cAMP (500 µL of Stock solution) was added. For BTH101 samples were collected at T = 0,0; 45 sek; 1:15; 2:10; 4:10; 8:04; 16:15; 32:12. For Δ*cyaA* samples were collected at T = 0,0; 0,35; 1:12; 2:06; 4:10; 8:14; 16:10; 32:15. All samples where spun down and stored at - 20 degrees	
29.08.2015	TBA	Miniprep of Overnight culture of MG1655/pSB1C3-PcstA. Stored as R109 Fast Digest of R109 using SpeI and PstI. Fast AP added. Left to	iGEM2015_SOP015_v02_ ligation

		incubate for approx. 1,5 h. Stored as Y107 Fast Digest of G48 (PCR of GFP-LVA) with XbaI and PstI. Done according to SOP. Left to incubate at approx. 1,5 h. Stored as Y106 Ligations of Digestions. Done according to SOP and left overnight	
30.08.2015	EMT TBA, JSP	Transformation of ligations RNA purification of samples with phenol and chloroform according to SOP. Samples stored at -20 degree in solution of sodium acetate and ethanol	iGEM 2014 SOP0009 Transformation
31.08.2015	TBA TBA. JSP	New ligation of GFP-LVA and pSB1C3-PcstA. Left overnight Wash of RNA-samples. Samples spun down and washed with 70% ethanol /ice cold) 3 times. Samples left to dry with lid open in 42 degree incubator for ~ 15 min. Samples resuspended in 100 µL water. stored at -80 degrees	iGEM2015_SOP015_v02_ ligation
01.09.2015	CEM	Transformation of Ligations	iGEM 2014 SOP0009 Transformation

	TBA, ADK	Concentration determining of RNA-samples. A 10x dilution was prepated for each sample and the concentration determined on the NanoDrop.	
02.09.2015	JSP JSP TBA, ADK	Colony PCR on transformation of yesterdays ligations. all colonies contained re-ligated plasmid-backbone. New digestion of GFP-LVA (G48) and pSB1C3-PcstA (R109) and ligation. Amount used for digestion: G48: 15 µl (conc. 9,7 ng/µl) R109: 6 µl (conc. 139 ng/µl approx.) G48 was cut with XbaI+PstI and R109 was cut with SpeI+PstI. Stored as Y115 and Y116. Ligation: no alteration to SOP. Test run of RNA samples on agarose gel. 5 µg of RNA from each sample mixed with 3 µL loading dye were prepared and loaded on the gel. sample 8.2 was left out due to extremely low RNA concentration.	iGEM2013_SOP0021_v01_ Colony PCR with MyTaq iGEM2013_SOP0021_v01_ Colony PCR with MyTaq iGEM2015_SOP0017_v01_ Fast digest iGEM2015_SOP015_v02_ ligation
03.09.15	ADK, CEM	Done according to the SOP until point 3 “Hybridizing the membranes”. Couldn't go any	iGEM2013_SOP0028_v01-Northen blotting

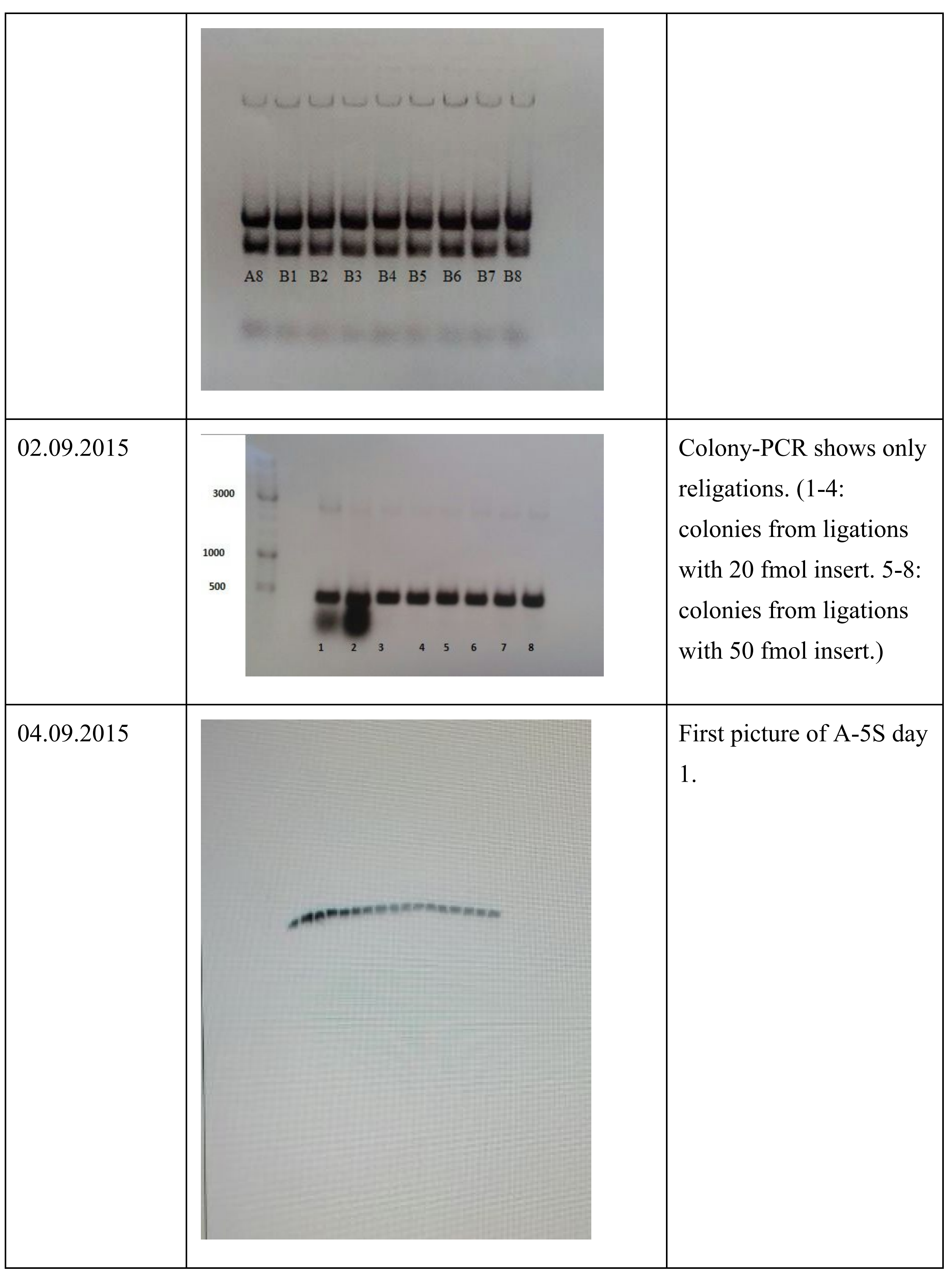
		further tonight because of missing probes, have saved the two membranes in the refrigerator. Loaded 5µg RNA, 3µl loading dye and water to a total volume of 15µl. Run the RNA on a 4,5% polyacryl-amide gel. Had two gels: - Gel A; loading dye - control with ΔcyaA without plasmid - samples with MG1655 ΔcyaA (B1-B8) and BTH101 (A1-A8) (the cAMP experience) - Gel 1; sample 1,1-8,1 and 1,2-8,2 (The OD experience)	
04.09.15	ADK	Cut the two membranes into three: The membrane of gel A: A-5S and A-GFP. The membrane of gel 1 remained at the same membrane: 1-5S-GFP. A-5S got the probe for 5S, A-GFP got the probe for GFP, 1-5S-GFP got the probe for GFP. Probes, prepared by supervisor. The probe for 5S hybridized for approx 4 hours and left in the cassette for approx 4 hours. Scanned the membrane. Transferred the membrane to a clean cassette and left for ON.	iGEM2013_SOP0028_v01-Northern blotting

		The probes for GFP hybridized ON.	
05.09.15	ADK TBA	Washed the A-GFP and 1-5S-GFP free of excess GFP probe. Placed the two membrane in each cassette for 5-6 hours.Scanned the membrane. Transferred the membrane to a clean cassette and left for ON.	iGEM2013_SOP0028_v01-Northern blotting
15.09.15	ADK	Northern blotting of GFP and 5 S of the samples: 1,4 (control), 1.1, 2.1, 3.1, 4.1, 5.1 (WT +glucose) 2.1, 2.2, 2.3, 2.4, 2.5 (WT without glucose) - Done according to the SOP, left with probe ON.	iGEM2013_SOP0028_v01-Northern blotting
16.09.15	ADK	Northern blotting continued - Washed the two membranes according the SOP, and left in the casette ON.	iGEM2013_SOP0028_v01-Northern blotting
17.09.15	TBA, JSP	Cassettes scanned and the data processed. See "Results"!	

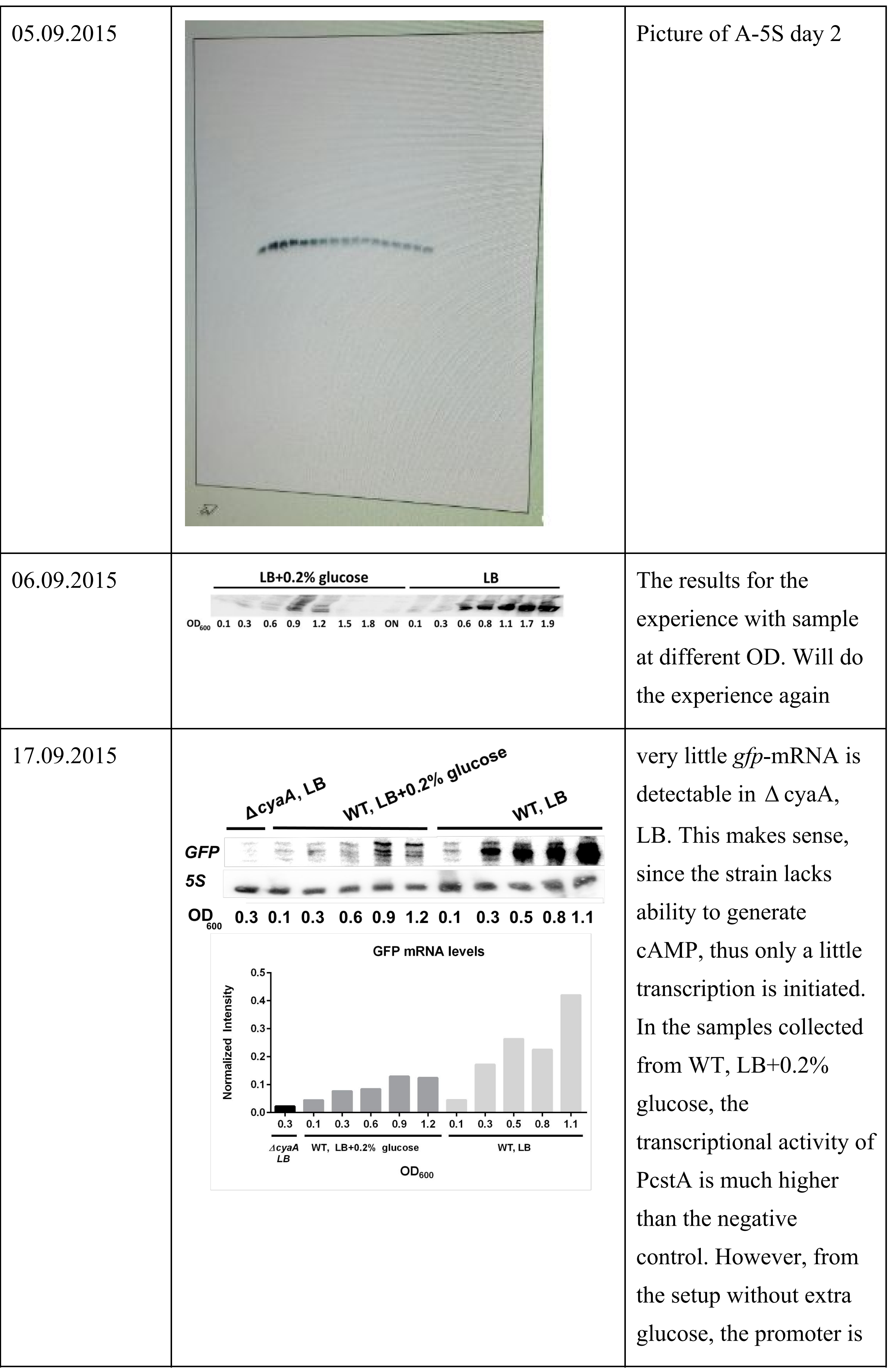
6. Results

Date (DD.MM.YY)	**Picture**	**Comments**
26.08.2105		

27.08.2015		Transformations successful. Colony-PCR. 17-18: PcstA-GFP in MG1655 19-20: PcstA-GFP in BTH101 21-22: PcstA-GFP in Δ cyaA:kan Bands was expected to be approx. 1300. This also the case.
02.09.2015		Test run of RNA samples. samples 8.1, 7.1 and 7.2 left out due to poor concentrations



02.09.2015		Colony-PCR shows only religations. (1-4: colonies from ligations with 20 fmol insert. 5-8: colonies from ligations with 50 fmol insert.)
04.09.2015		First picture of A-5S day 1.



05.09.2015		Picture of A-5S day 2
06.09.2015		The results for the experience with sample at different OD. Will do the experience again
17.09.2015		very little *gfp*-mRNA is detectable in Δ cyaA, LB. This makes sense, since the strain lacks ability to generate cAMP, thus only a little transcription is initiated. In the samples collected from WT, LB+0.2% glucose, the transcriptional activity of PcstA is much higher than the negative control. However, from the setup without extra glucose, the promoter is

		very active. This correlates with the know inverse relationship between glucose and cAMP.

7. Appendices