Author: Please complete your labfolder profile.
Entry : No entry title yet
In Project: Detection-Carotenoids

created: 17.09.2015 20:47
updated: 17.09.2015 21:26

6 JUNE :

Biobricks Arrived : K274100,K274110,K274120,K274200,K274210,K274220

	K274200	K274210	K274220
PROMOTER	-	LacI+pL	pBad+araC
GENE	Rbs+crtE+rbs+crtB+rbs+crtI+rbs+crtY		
BACKBONE	pSB1A2	pSB1A2	pSB2K3
RESISTANCE	Amp	Amp	Kan
REPLICATION ORI	pMB1	pMB1	F1,P1 lytic Ori S
DERIVED FROM	pUC19	pUC19	P1 lytic
COPY NO.	100-300	100-300	
SIZE	6634 bp	6697 bp	10198 bp

	K274100	K274110	K274120
PROMOTER	-	LacI+pL	pBad+araC
GENE	Rbs+crtE+rbs+crtB+rbs+crtI		
BACKBONE	pSB1A2	pSB1A2	pSB1A3
RESISTANCE	Amp	Amp	Kan
REPLICATION ORI	pMB1	pMB1	F1,P1 lytic Ori S
DERIVED FROM	pUC19	pUC19	
COPY NO.	100-300	100-300	
SIZE	5464 bp	5527 bp	6807 bp

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7th June

Primary cultures :

Protocol:

1.Stab the vial with sterilised nichrome loop.

2.Inoculate in LB media (3ml LB media + 3ul antibiotic)

3.Incubate at 37 deg Celsius for 12 hours (200 rpm)

Master Plates

(11 th june)

The protocol is optimised. We did plating 3 times and we did not get

isolated single colony.

Protocol:

1.LB media 998 ul + 1ul antibiotic + 1 ul primary culture

2.Plate 20 ul of above solution

3.Keep the plates at 37 deg Celsius for 12 hours.

• Glycerol stock

It is done two times (7 th and 18 th june)

Protocol :

1. Take 500 ul of primary culture and 500 ul of 80% glycerol in eppendorfs 2. Flash and freeze the eppendorfs and keep it at -80 deg celsius

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10 th JUNE

• Secondary culture 1 % of 110,120,210,220

1. Primary culture 50ul and antibiotic 5 ul in 5ml LB media

2. Added arabinose and IPTG initially

IPTG(in all biobricks) : 1 mM (5ul in 5ml)

Arabinose (in K274120 and K274220) : 1/100 th volume of culture

(50ul)

3. Incubation at 37 deg celsius for 20 hours (200 rpm)

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created: 17.09.2015 21:05
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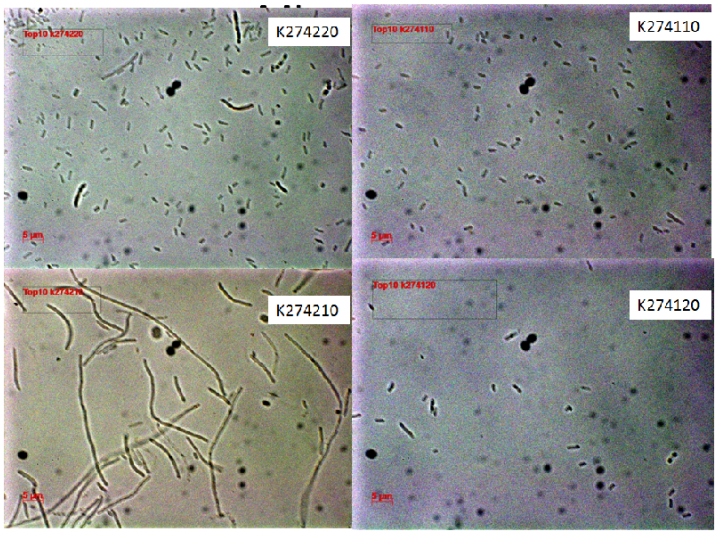
11 June :

MICROSCOPY TOP 10 CELLS :

Cells are washed with PFA and resuspend in PBS.

Observed under apotome microscope 100x magnification.



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created: 17.09.2015 21:06
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• 25 th june

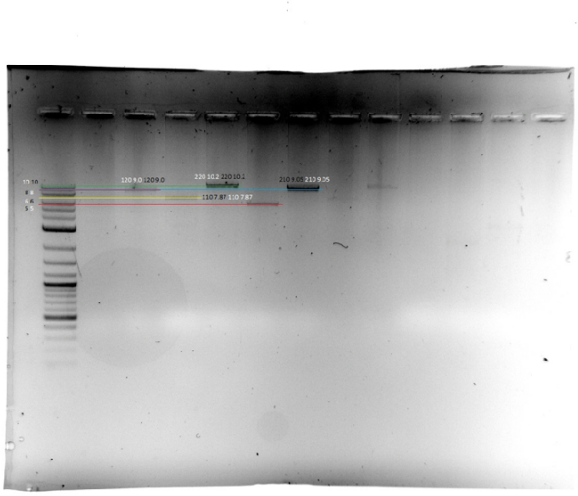
Miniprep by alkaline lysis

Result :

SAMPLE	CONCENTRATION (ng/ul)	A260/A280
110.0	2078.0	1.95
120.0	1058.0	1.98
210.0	1697.0	1.89
220.0	1822.0	1.93

26 JUNE :

Plasmids of k274110,k274120,k274210,k274220 are run on gel . Single cut with EcoR1



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1 JULY :

Isolated plasmids of K274110, K274120, K274210, K274220 are transformed in E.coli Mg1655 cells . Tansformation is done by heat shocking.

Transformed cells are then plated.

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2 July :

Got colonies in each plates.

Primary culture of K274110, K274120, K2274210, K274220

LB 5ml + Antibiotic: 5 ul + Single colony from plate

Then put it for incubation for 20 hours at 37 degree celsius at 200rpm.

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3 July :

Secondary culture (5%)

1. LB : 5ml

2. Primary culture (250 ul)

3. Antibiotic : 5ul

4. IPTG (in all) : 5ul(initially at start of incubation)

5. Arabinose (in k274120,k274220) : 50ul (initially at start of incubation)

6. Incubation for 20 hours at 37 deg celsius .(200 rpm)

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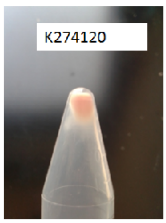
4 JULY :

After 20 hours of induction we got following results in MG1655

- 4th july (e. Coli Mg1655)



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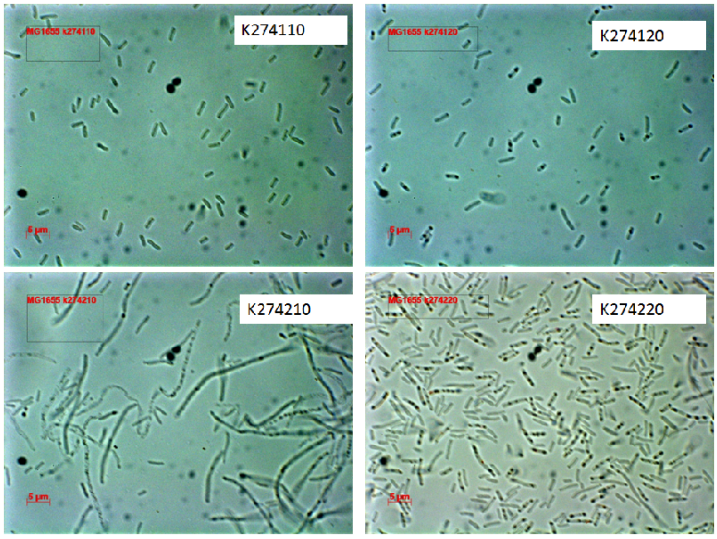
created: 17.09.2015 21:56
updated: 17.09.2015 22:01

7 July

Microscopy :

Cells are washed with PFA and resuspend in PBS and observed under apotome microscope. Magnification 100x



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created: 17.09.2015 22:10
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12 july :

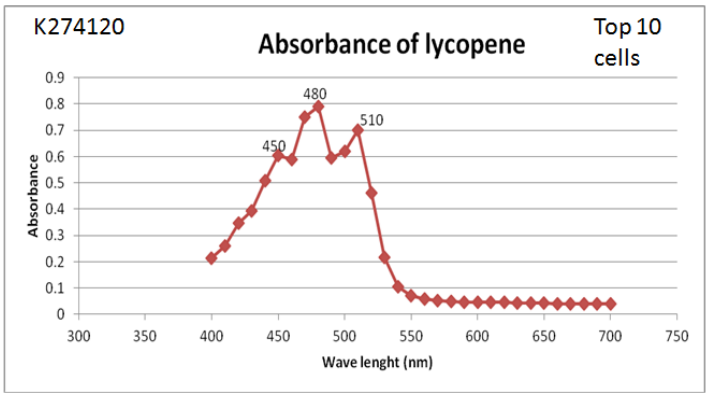
Quantification

Spectroscopy

: After 20 hours of incubation cells are centrifuged for 10 minutes at room temp. at 14000 rpm.

They are re-suspended in 300ul acetone.

Spectroscopy is done for visible wavelenghts.



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created: 17.09.2015 22:24
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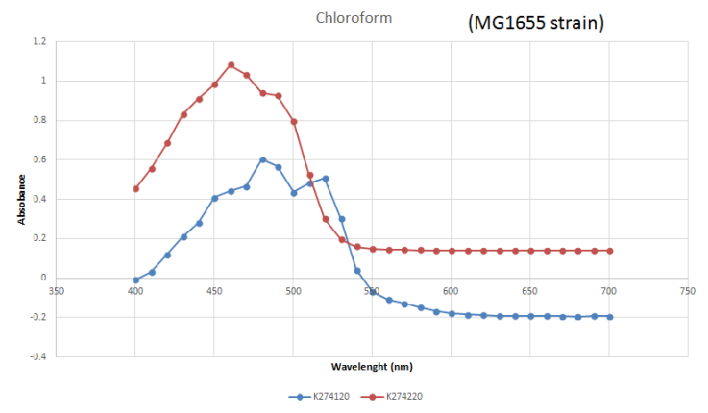
Graphs of K274120 lycopene and K274220 b-carotene in different solvents



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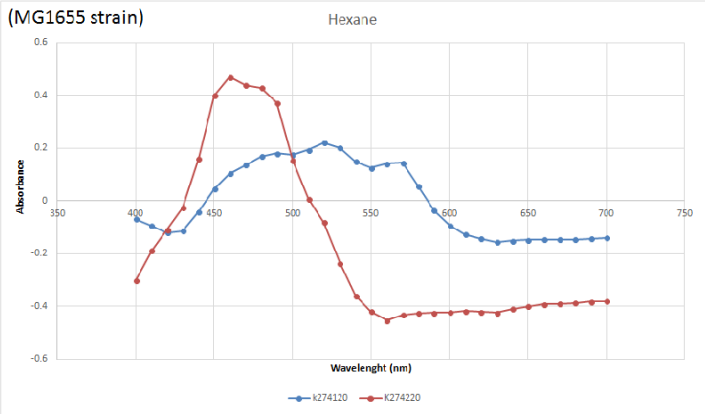
created: 17.09.2015 22:44
updated: 17.09.2015 22:46

IN HEXANE



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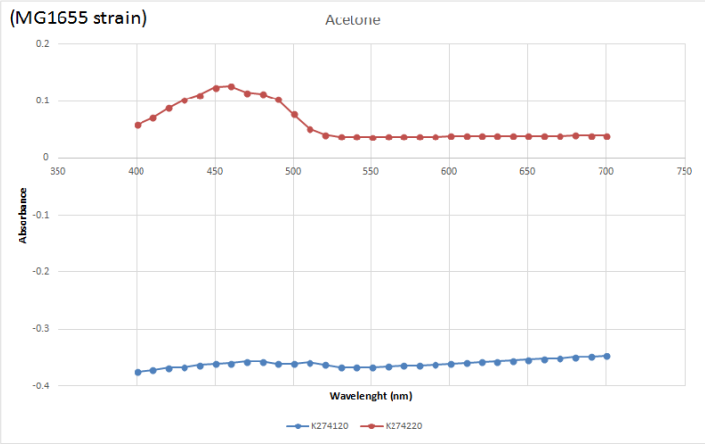
IN ACETONE



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