Protocol promoter characterisation

Characterisation of promoter repression on double repressible promoters for modelling a bistable toggle switch using rhamnose promoter

We want to characterise double repressible promoters using the rhamnose promoter to control the concentration of repressor protein. This because not all repressor proteins used in synthetic biology can be regulated by chemical compounds like IPTG or ATc. With the knowledge of repressor binding strength and promoter strength we can give feedback to our model for designing a bistable toggle switch which can be induced by a repressor protein. The promoter strength and binding strength can be determined by following this protocol.

The box contains the following cultures in LB medium in triplo. The eppendorf tubes can be placed in 37 degrees to grow for a few hours and can be plated on LB agar plates to make a back-up.

Code	Strain	Name part	Backbone	Function	Used for measuring
M1	E. coli DH5α	pRha GFP	pSB3K3	GFP expression regulated by L-rhamnose	Strength pRha
M4	E. coli DH5α	pcI/tet GFP	pSB3K3	GFP expression	Constitutive promoter strength
M5	E. coli DH5α	ptet GFP	pSB3K3	GFP expression	Reference promoter
M8	E. coli DH5α	pRha LacI + pcI/lac GFP	pSB3K3	GFP expression regulated by LacI	Repression by LacI
M9	E. coli DH5α	pcI/lac GFP	pSB3K3	GFP expression	Constitutive promoter strength
M17	E. coli DH5α	pRha cIlambda + pcI/tet GFP	pSB3K3	GFP expression regulated by cI lambda	Repression by cI lambda
M18	E. coli DH5α	pRha cIlambda + pcI/lac GFP	pSB3K3	GFP expression regulated by cI lambda	Repression by cI lambda
Part name	BioBrick Code	Function			
pRha	Bba_K914003	L-rhamnose induced promoter			
GFP	Bba_I13504	Fluorescent protein			
pcI/tet	BBa_K909013	double promoter			
ptet	BBa_R0040	promoter			
LacI	Bba_P0412	Repressor protein			
pcI/lac	BBa_K909012	double promoter			
cIlambda	Bba_P0451	Repressor protein			

Aim:

- Measure constitutive promoter strength of pcI/tet and pcI/lac
- Measure repression in different concentrations of rhamnose
- Measure production rate of GFP in different concentrations of rhamnose

How:

- Fluorescence measurement from plate reader
- 1 day growth to get max GFP
- 1-2 days growth to measure degradation rate of GFP
- Measure absorbance OD600 on time (growth rate)
- Measure fluorescence over time (promoter strength)
- Measure fluorescence in different concentrations rhamnose (effect of repression)

1 L M9 2% glycerol: (all ingredients must be sterilized before adding, 0.2 um filter for filter sterilization)(DH5a strain doesn't grow on medium without thiamine and casamino acids).

- 200 ml 5x M9 salts (autoclave)
- 30 ml 10 mg/ml thiamine HCl (filter sterilized)
- 50 ml 0.4 g/ml glycerol (filter sterilized)
- 1 ml 20 mg/ml kanamycin (filter sterilized)
- 20 ml 10% casamino acids (autoclave)
- 2 ml 1M MgSO4 (filter sterilized)
- 100 ul 1M CaCl2 (filter sterilized)
- Demi water (MQ) to 1 L (autoclave)

Prepare 50 ml each rhamnose or glucose containing M9 media (rhamnose and glucose stocks filter sterilized).

- 0.001 % rhamnose
- 0.01 % rhamnose
- 0.05 % rhamnose
- 0.2% rhamnose
- 0.2 % glucose

Parts used for Protocol for:

- Measuring cIlambda or LacI binding strength to pcI/lac
- -M1, M5, M8, M9, M18
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- Measuring cIlambda binding strentgh to pcI/tet
- -M1, M4, M5, M17

Protocol:

- Take colony/culture from plate/eppendorf tube and inoculate 5 ml M9 medium (2% glycerol) so each part is cultured in triplo (preheated to 37°C) (Colonies are already picked and triplos are supplied in LB medium with 20 ug/ml kanamycin, so inoculate from the tubes).
- Grow overnight at 37°C (16 hours) shaking 150 rpm
- Inoculate in a 96 well plate 200 ul medium containing each concentration of rhamnose and glucose with each culture to a starting OD 0.004. Leave 6 wells containing each concentration rhamnose or glucose medium as blanco.
- Set platereader continuesly shaking at 37 degrees, gain on 70 and measure OD 600 nm and Fluorescence (395 nm excitation, 509 nm emission) every 15 min.
- Run for 24 hours

  Export data to an excel file and send it to michiel.herpers@wur.nl or try to analyse the data yourself if you like to.