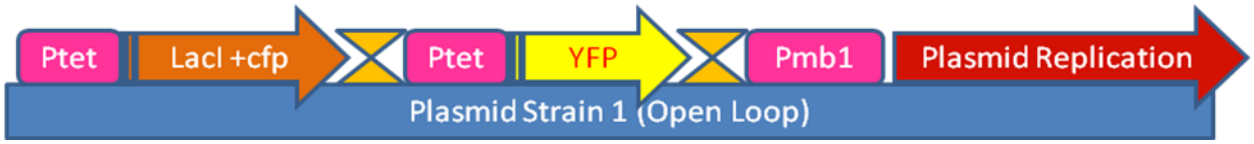
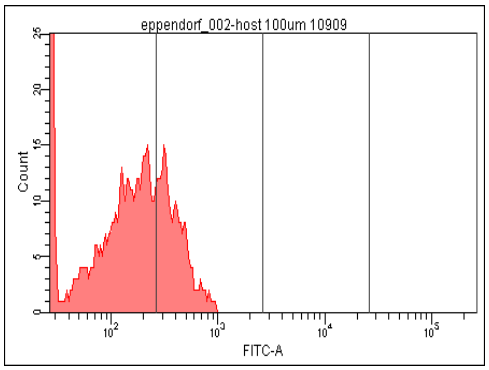
Detailed Experimental studies

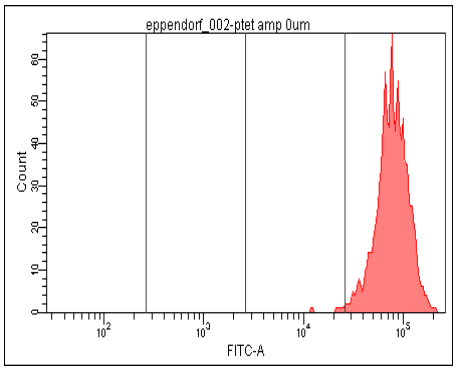
Characterization of copy number by quantifying YFP for all the four strains developed.

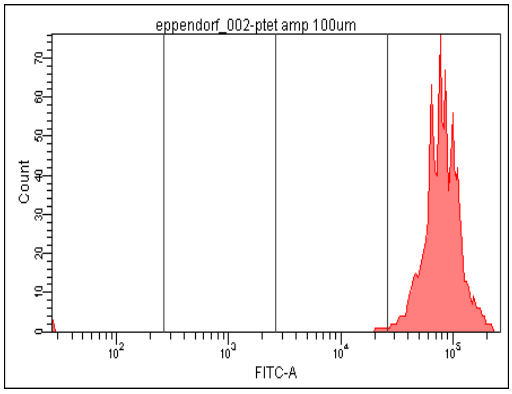
Strain 1 (Open Loop,pTet Amp) with plasmid (BBa_K255004): It has got open loop without any feedback. Here there is constitutive expression of lacI. Here the copy number of the plasmid is fixed.

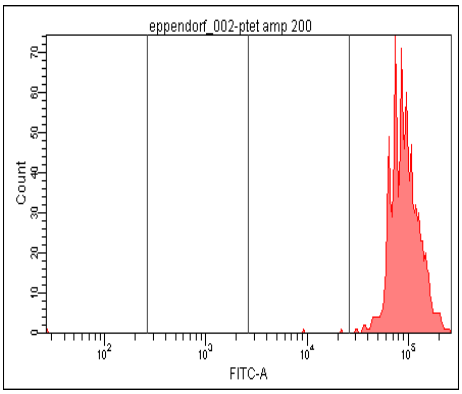


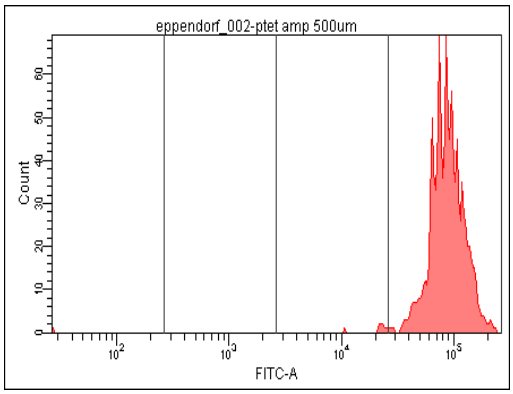
YFP was measured using FACS analysis. Illustrative FACS results are given below: The mean, variance and standard error were noted for difference strains and at different IPTG concentration. The FACS results are shown for varying IPTG concentrations varying from 0um to 500um.





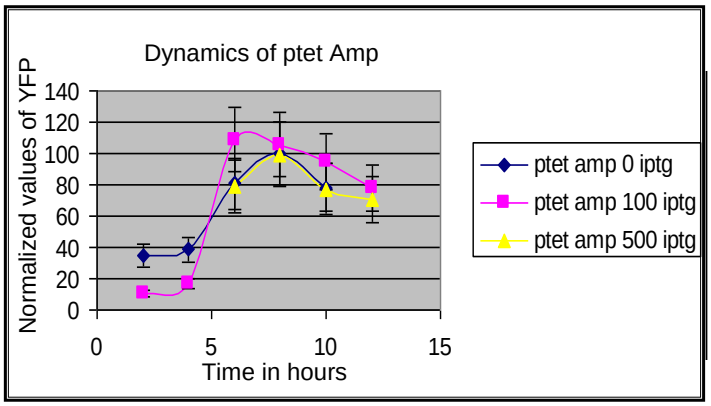






Inference – compared to control which is host the YFP expression was very high and the YFP expression was almost similar at all the Various IPTG conc. Measured.

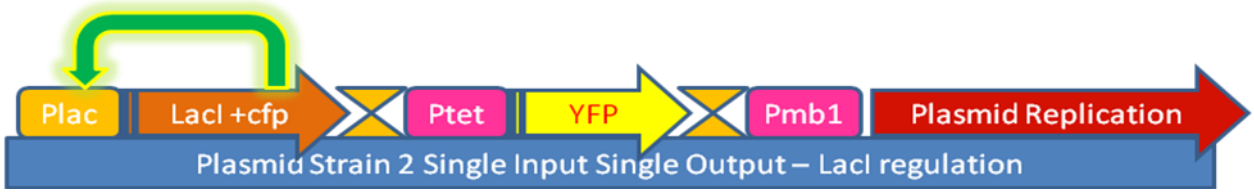
Dynamics of ptet Amp at various time intervals



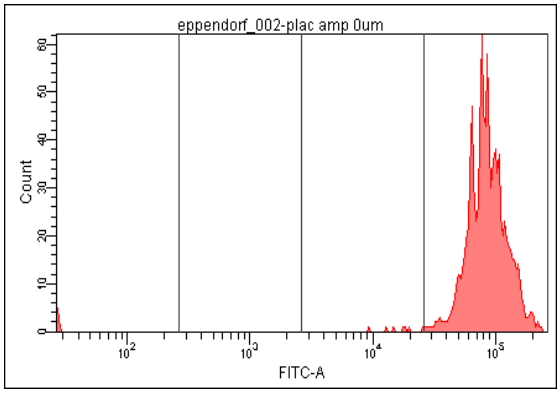
At different time intervals samples were collected and YFP was measured at different time intervals for various concentration of IPTG.

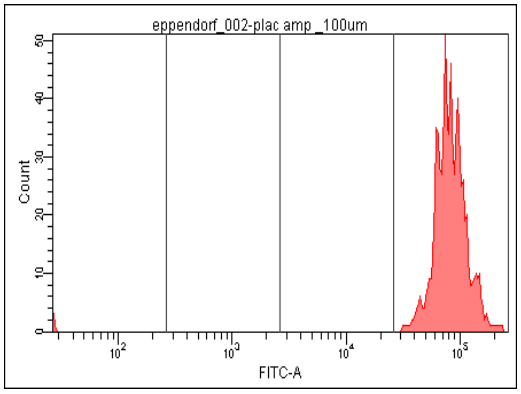
Inference- The YFP expression at all the various concentrations of IPTG maintained nearly a same expression profile**.**

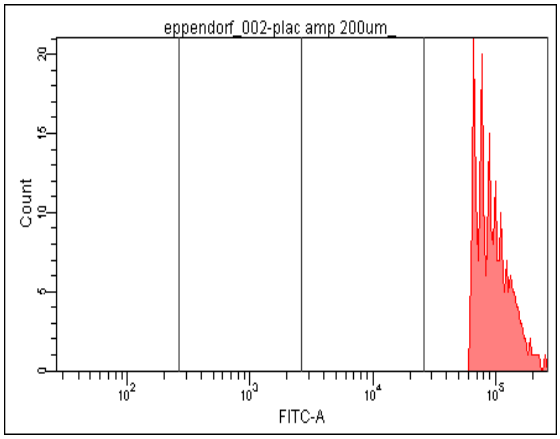
Strain 2 (Single Input Single Output with regulation on LacI [SISO_LacI],plac Amp)) with plasmid (BBa_K255003). It has got a single negative feedback loop.
So the expression of lacI is under regulation. Here also the copy number of the plasmid is fixed.

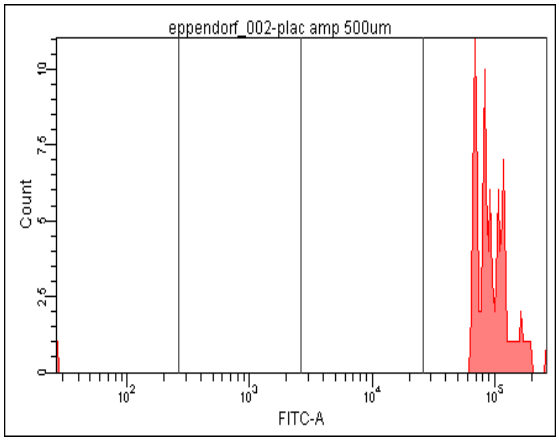


YFP was measured using FACS at different IPTG concentrations. YFP was measured using FACS analysis. Illustrative FACS results are given below:
The mean, variance and standard error were noted for difference strains and at different IPTG concentration. The FACS results are shown for varying IPTG concentrations varying from 0um to 500um.





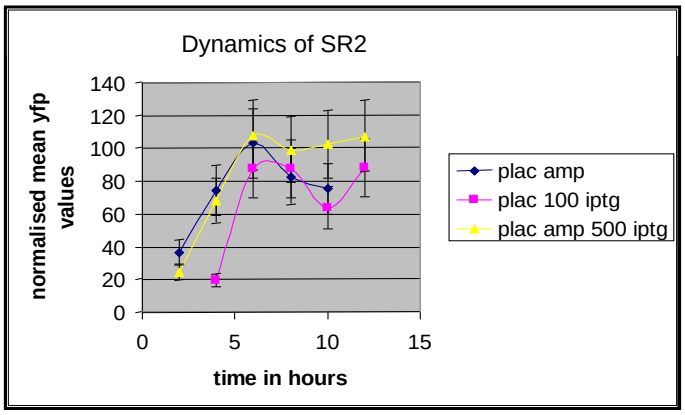




Inference – The YFP expression was found to be nearly same at all the different conc .of IPTG.

Dynamical studies for Strain-2:

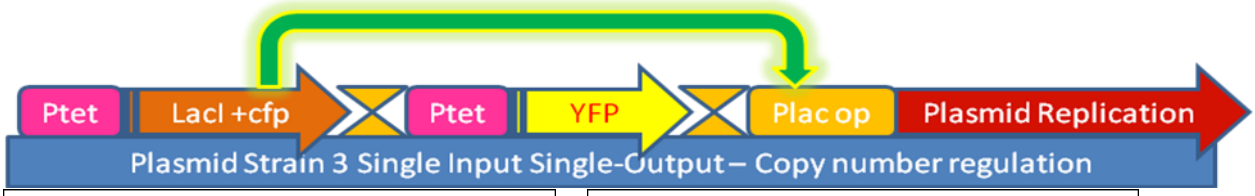
Dynamics of plac Amp varying both time and IPTG

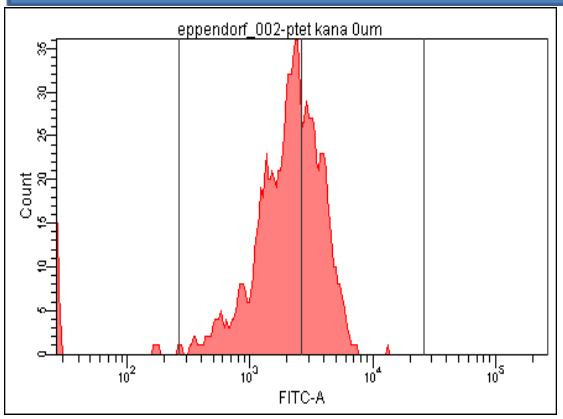


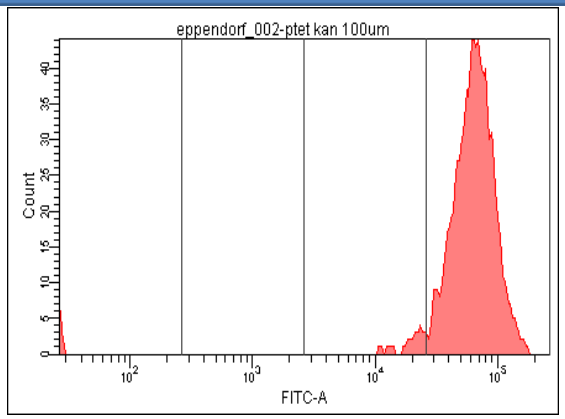
At different time intervals samples were collected and YFP was measured at different time intervals for various concentration of IPTG.

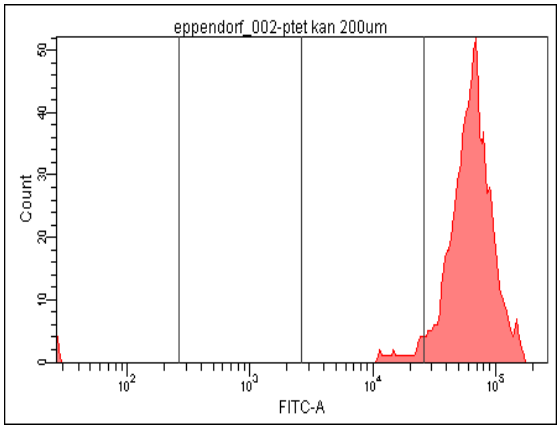
Inference- The YFP expression at various concentrations of IPTG maintained nearly a same expression profile**.**

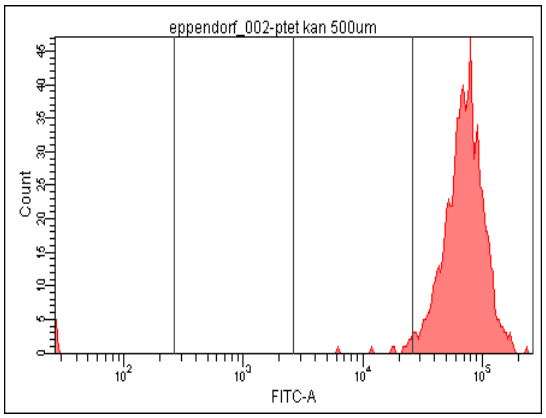
Strain 3 (Single Input Single Output with regulation on copy number [SISO_CN]) with plasmid(BBa_K255002).It has got single negative feedback loop on the plasmid copy number . Here there is no control on the LacI expression.











YFP was measured at different conc of IPTG at steady state.

Inference- The YFP Expression increased around 10 folds from 0 to 100 µM of IPTG Conc and thereafter its value was almost constant to 200 and 500 µM of IPTG conc. After this the expression profile of YFP between 0 and 100 µM was measured. Once can observe that the variability is decreasing as compared to that seen in the open loop.

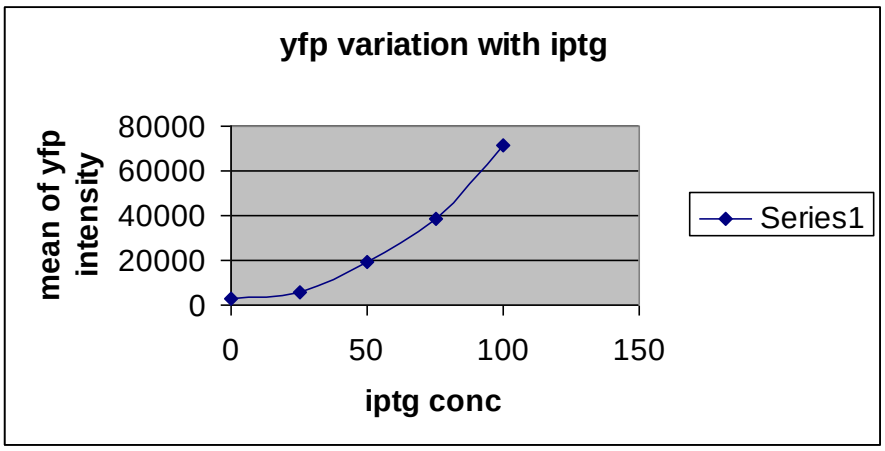


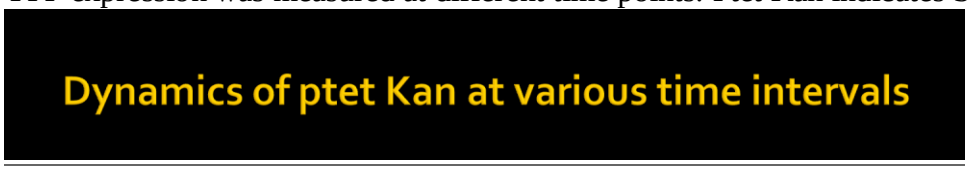
Figure: Variation of YFP expression at various IPTG concentrations in Strain-3, where the copy number is regulated. YPF indicates the status of copy number.

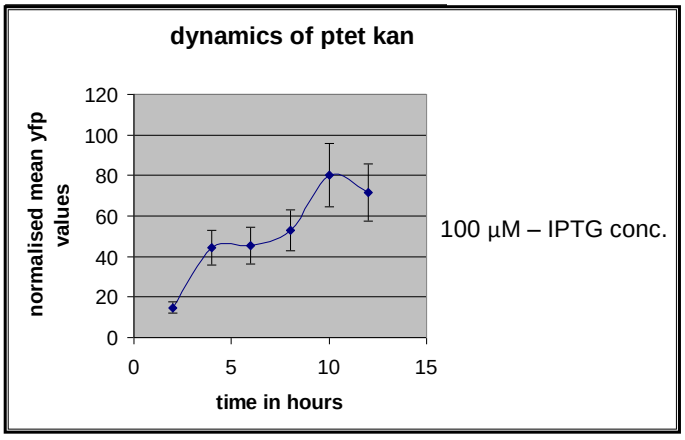
Inference –

The YFP expression continues to increase with the rise in IPTG concentration.

Dynamical study:

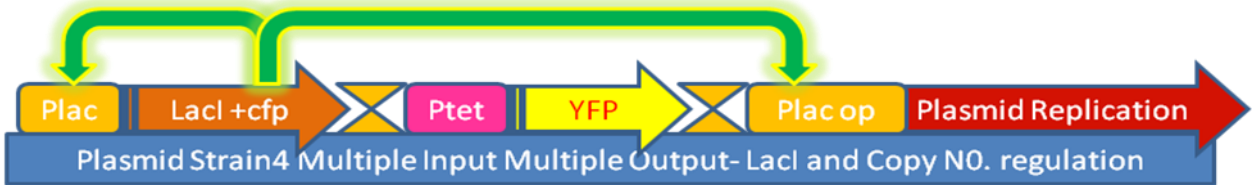
YFP expression was measured at different time points. Ptet Kan indicates Strain – 3.

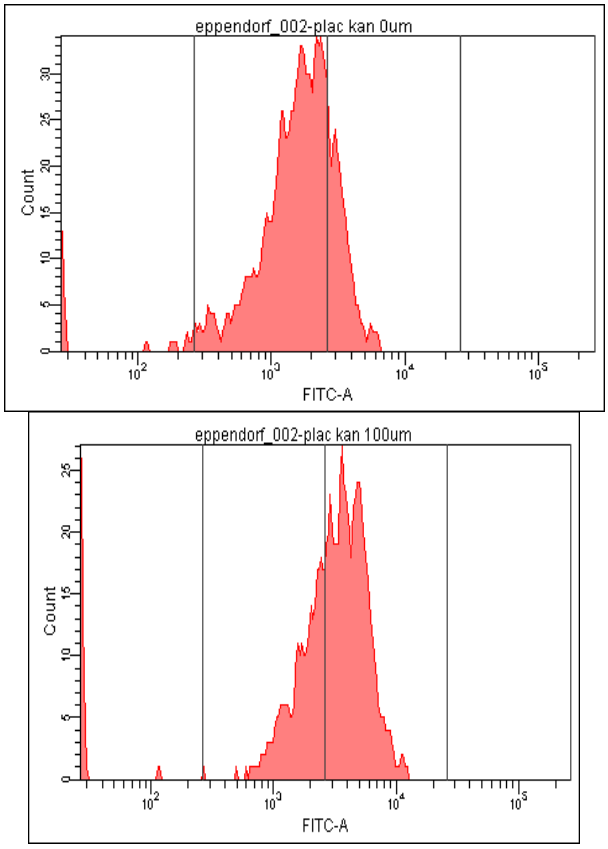


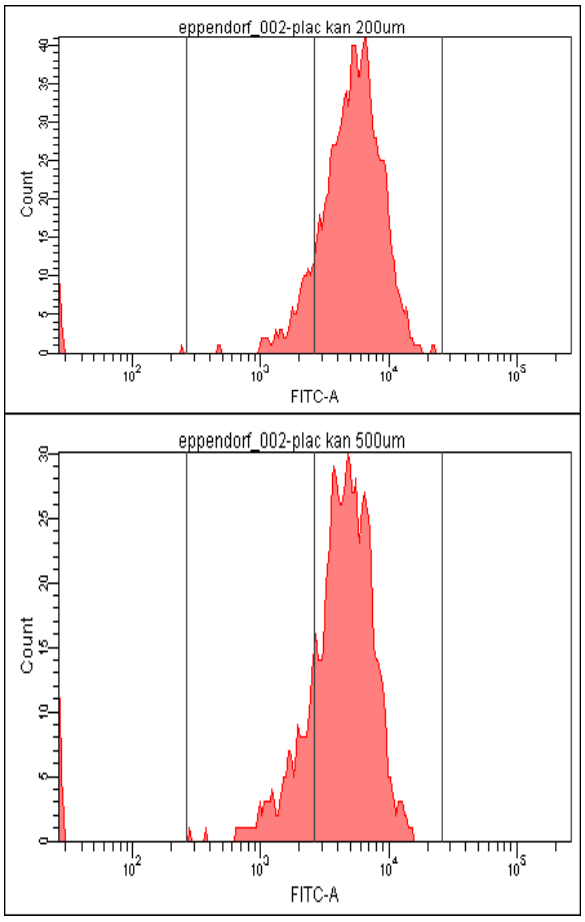


Dynamics of expression to indicate copy number is illustrated above through measurement of YFP for 100 um IPTG. The expression increases in time and saturates in 12 hours. The variance in the measurements increase in time.

Strain 4 (Multiple Input Multiple Output with regulation on copy number and LacI [MIMO]) with plasmid (BBa_K255001). It has dual negative feedback loop one on the plasmid copy number and second on the LacI expression.

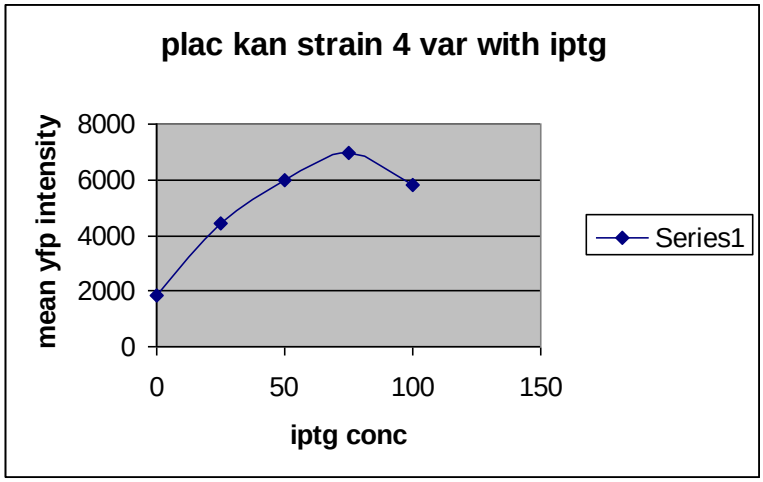






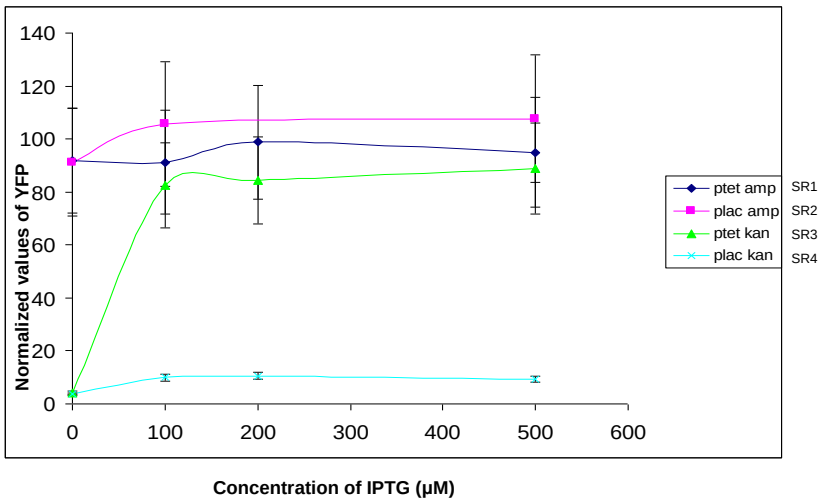
YFP was measured at different conc of IPTG at steady state.

Inference- The YFP Expression increased around 10 folds from 0 to 100 µM of IPTG Conc and thereafter its value was almost constant to 200 and 500 µM of IPTG conc. After this the expression profile of YFP between 0 and 100 µM was measured.



Inference – The YFP expression continues to increase with the rise in IPTG concentration till 75 um IPTG conc . Thereafter at 100um IPTG conc YFP mean value decreases.

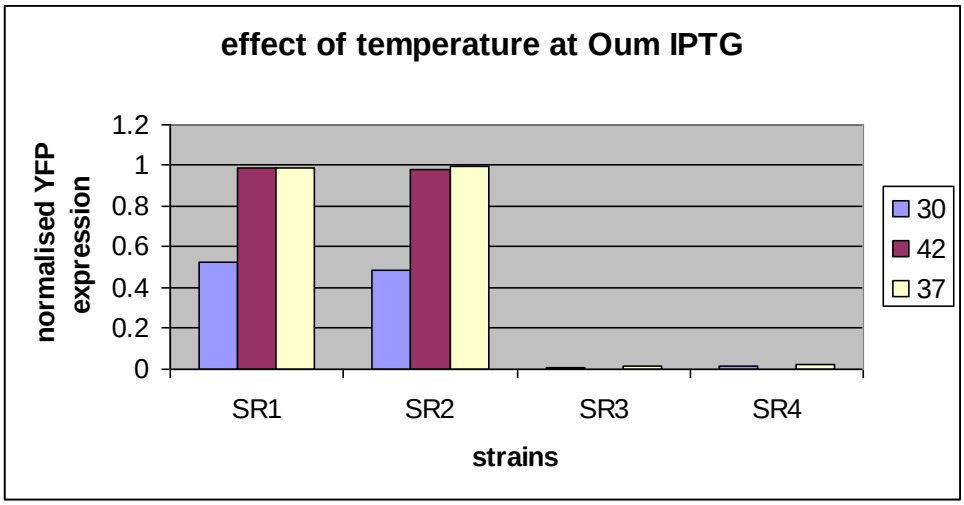
Steady State YFP Profiles for the four strains:



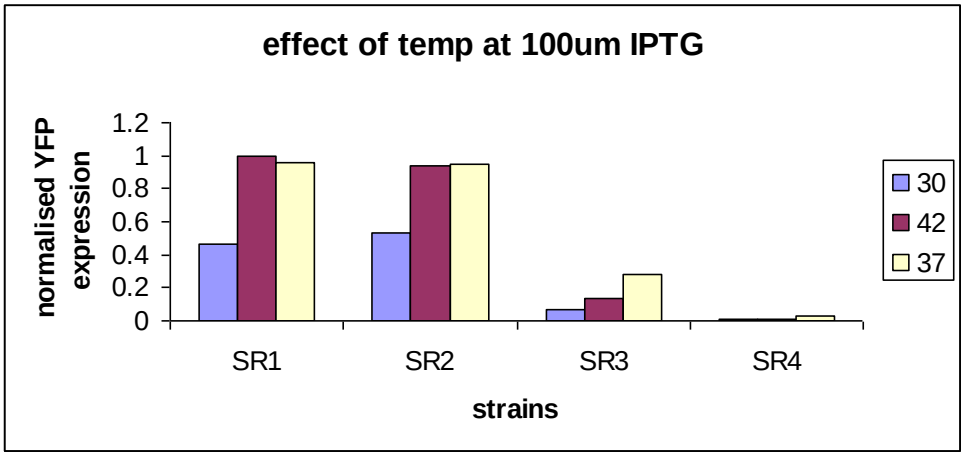
It can be observed in the above strain that Strain-1 (ptetAmp) and Strain-3 (placAMp) did not show any difference in the expression level at different IPTG, as in these two strains the copy number do not change. The YFP profiles changed for the strain-3 and strain-4 where the copy number was regulated. The expression of copy number was controlled in Strain-4.

2. Characterization of YFP to see the effect of temperature on the copy number of plasmid.

effect of temperature on strains			growth on M9 medium
	specific growth rate		
	30	42	37
host	0.2928	0.305	0.4395
strain 1	0.3222	0.2994	0.3965
strain 2	0.3263	0.3033	0.4071
strain 3	0.2875	0.3165	0.3765
strain 4	0.2733	0.2923	0.4065



Inference- The YFP expression of SR1 and SR2 almost remains the same at 42 and 37 degree Celsius but decreases at 30 degree Celsius. In case of SR3 and SR4 YFP expression decreases both at 30 and 42 degree Celsius.



Inference- The YFP expression of SR1 and SR2 almost remains the same at 42 and 37 degree Celsius but decreases at 30 degree Celsius. In case of SR3 and SR4 YFP expression decreases both at 30 and 42 degree Celsius.

3. Growth studies on lactose for host strain (lacI deletion) and host strain transformed with synthetic genetic circuits having multiple feedback loops.

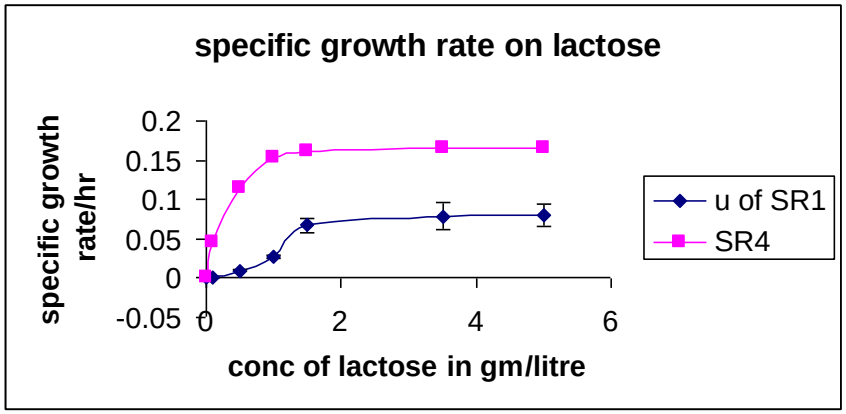


Figure: Specific growth rate for Strain-1 (Open Loop) and for Strain-4 (MIMO). The growth rate on Strain-1 (blue line) was lower and with higher variability, while growth rate on Strain-4 was higher with negligible variance. It was remarkable that Strain-4 demonstrated amazing reproducibility. Also Strain-1 demonstrated considerable growth beyond 1 g/L of lactose, while Strain-4 demonstrated growth even at lower lactose concentrations. The controlled robust synthesis of LacI which further produced commensurate amounts of beta-galactosidase resulted in an optimal phenotype.

Inference- The growth rate on lactose of Strain-1 was lower as compared to Strain-4. Further, the growth of Strain-4 was more sensitive to lower concentration than that observed in Strain-1. The variability in the growth rate was lower for strain-4 indicating that the multiple feedback loop yields robust protein expression which translates to stable growth rates.

Variation of Relative change in OD for Strain-1 and Strain-4 at various lactose concentrations

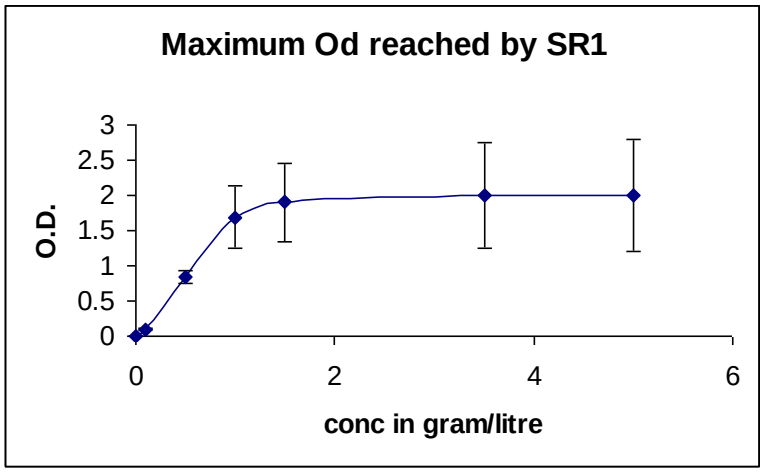


Figure: The relative change in OD was plotted for Strain-1 at different lactose concentration. Relative change in OD is defined as [OD-OD(t-0)]/OD(t-0), where OD is the optical density at some time t and OD(t=0) is the measured OD at time t=0. The relative max OD is reached was 2 indicating lower growth in Strain-1 and further reaching saturation beyond 1.5 g/L of lactose with a high standard deviations.

Inference- The maximum O.D. reached is lower and also the standard deviation is high.

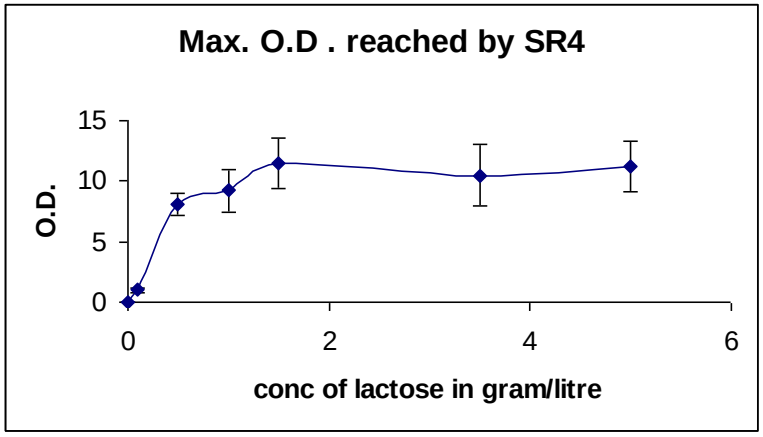
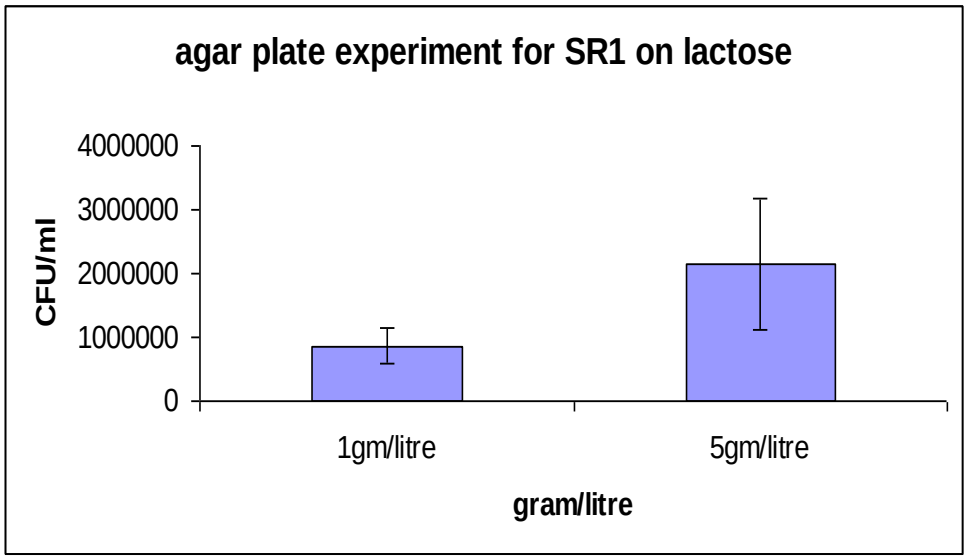


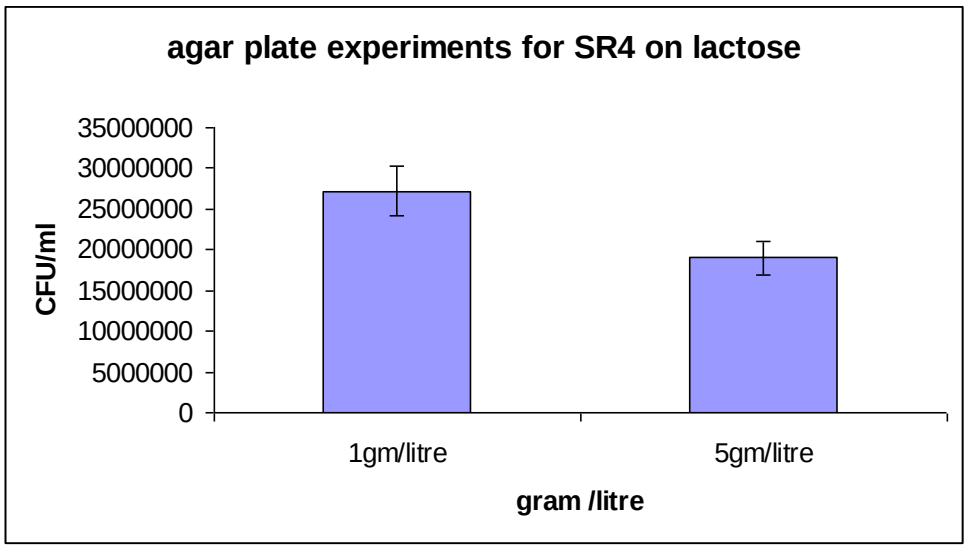
Figure: The relative change in OD was plotted for Strain-4 at different lactose concentration. Relative change in OD is defined as [OD-OD(t-0)]/OD(t-0), where OD is the optical density at some time t and OD(t=0) is the measured OD at time t=0. The relative max OD is reached was10 indicating higher growth in Strain-4 and further reaching saturation beyond 1.5 g/L of lactose with a lower standard deviations.

Inference – The maximum O.D. reached in case of SR4 is more than that of SR1 and also the Standard deviation is less than that of SR1.

AGAR PLATE EXPERIMENT

Several folds dilutions of the original culture was made and was spread on agar plate containing lactose concentrations od 1 g/L and 5 g/L. After around 24 hours the number of colonies on each agar plate with strain-1 and strain-4 was determined. The CFU was calculated for each ml and was plotted against conc. of lactose.



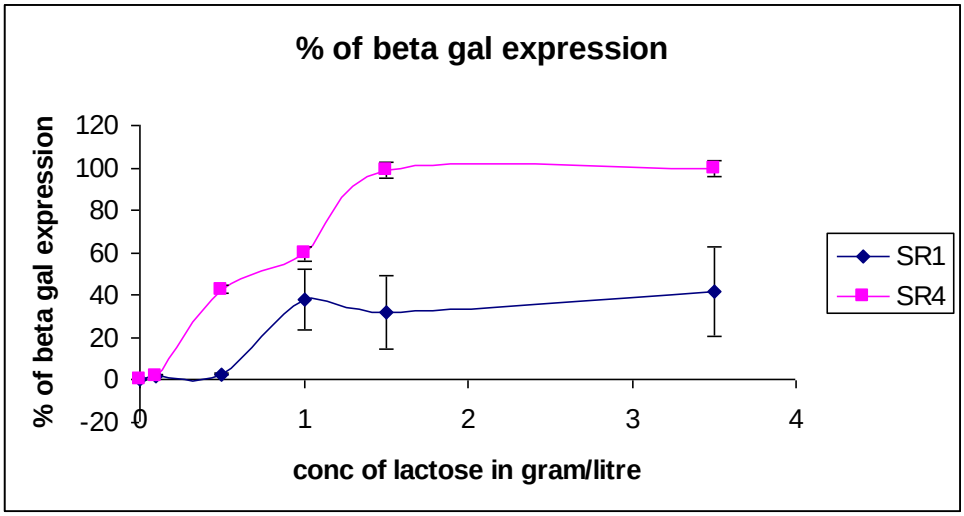


Strain-4 demonstrated higher colony forming unit as compared to strain-1. The increase was about 40%. The interesting fact was that the deviations observed in Strain-4 was minimal as compared to that of Strain-1, reiterating the fact that the noise at the protein level was translated to the phenotypic level.

Inference- The colony forming units (CFU) on lactose for Strain-1 was lower as compared to Strain-4. Further, the growth of Strain-4 was more sensitive to lower concentration than that observed in Strain-1. The variability in the growth rate was lower for strain-4 indicating that the multiple feedback loop yields robust protein expression which translates to stable growth rates.

4. Characterization of β- galactosidase expression from host strain (lacI deletion) and host strain transformed with synthetic genetic circuits having multiple feedback loops.

There was problem in measuring the CFP level due to non avalibilty of specific filter . So in order to cope up with this we went on to measure Beta galactosidase enzyme activity to measure the noise indirectly.



Inference-The β-galactosidase expression of strain-4 was commensurate to the lactose concentration demonstrating that the multiple feedback yields optimal behavior. Strain-1 demonstrated lower β-galactosidase activity due to higher LacI in the system. This added burden in strain-1 (open loop) reduced the growth rate

Conclusion from Experiments.

1. The YFP measurements showed that SR4 have lower variance as compared to all the other strains used in the study.
2. Temperature have profound effect on Copy number of the plasmid as it is evident from the lower YFP expression at 30 degree Celsius as compared to 37 degree Celsius.
3. The specific growth rate also decreased on lower temperature as well as higher temperature.
4. Both agar plate experiments and growth rate measurements demonstrated lower variability for the strain with multiple feedback loops as compared to the open system. The growth experiment also demonstrated that the strain with multiple

feedback loop design could optimally synthesize proteins to the availability of lactose thus minimizing burden of protein synthesis and maximizing growth rate.

5. 4.The β-galactosidase expression of strain-4 was commensurate to the lactose concentration demonstrating that the multiple feedback yields optimal behavior. Strain-1 demonstrated lower β-galactosidase activity due to higher LacI in the system. This added burden in strain-1 (open loop) reduced the growth rate