STUDY OF AN INDUCIBLE LYSIS DEVICE IN *E. COLI*

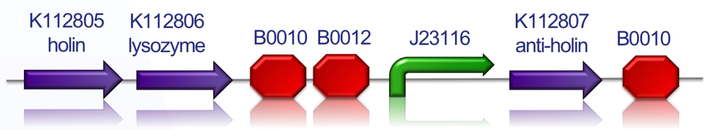
Susanna Zucca[1,2], Lorenzo Pasotti[1,2], Manuel Lupotto[2], Maria Gabriella Cusella De Angelis[2] and Paolo Magni[1,2]

[1]Laboratory for Biomedical Informatics, Department of Computer Engineering and Systems Science, University of Pavia, Italy
[2]Center for Tissue Engineering, University of Pavia, Italy
Primary contact: susanna.zucca@unipv.it

MOTIVATION. In synthetic biological systems, cell lysis can be used to engineer programmed cell death and also to release proteins in extra-cellular environment, when secretion is not feasible. In this work, a promoterless lysis device called BBa_K112808, present in the Registry of Standard Biological Parts, was studied. A $3OC_6$-HSL inducible promoter was cloned upstream of the lysis device and used to characterize it.

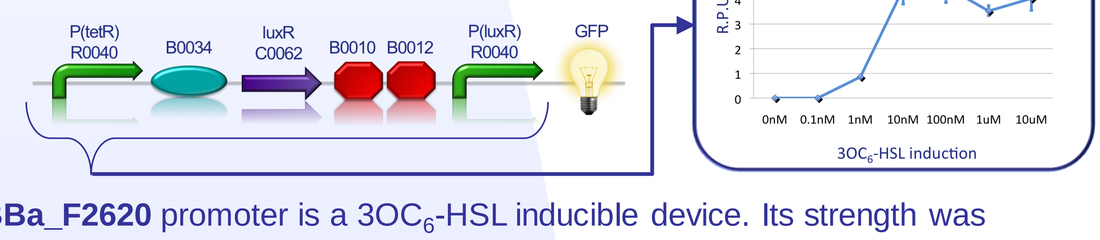
SYSTEM OVERVIEW

Lysis device from enterobacteria phage T4: BBa_K112808

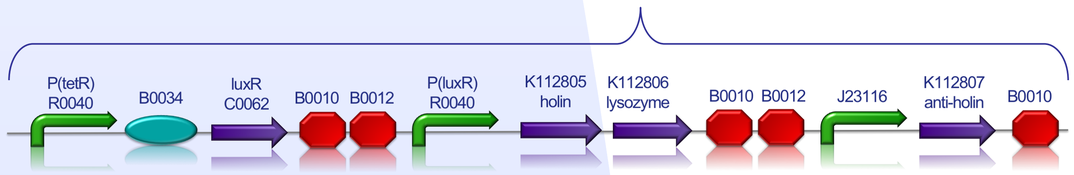


- **holin** forms pores in the inner membrane of bacteria
- **lysozyme** degrades peptidoglycan layer, passing through the pores created by holin, thus performing lysis
- **anti-holin** weakly constitutively expressed prevents the formation of holin multimers due to spurious transcription (uncontrolled lysis)

Lysis device controlled by $3OC_6$-HSL inducible promoter BBa_F2620

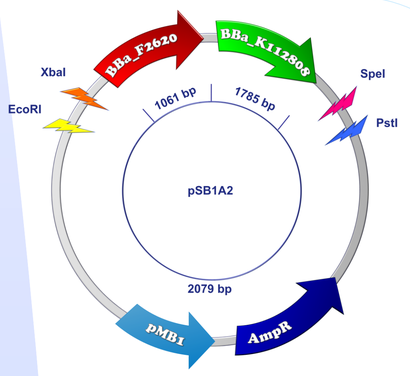


BBa_F2620 promoter is a $3OC_6$-HSL inducible device. Its strength was evaluated in LB medium at different inducer concentrations. BBa_F2620 is used to control cell lysis, obtaining **BBa_K173015** measurement device.

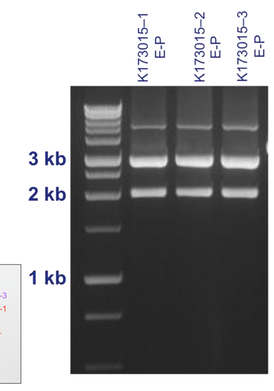


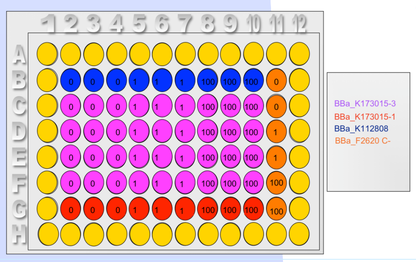
MATERIALS AND METHODS

- BBa_K112808 lysis device was cloned under the control of BBa_F2620 promoter into a high copy number plasmid (pSB1A2), thus obtaining BBa_K173015
- *E. coli* TOP10 strain was used for cloning and testing
- BBa_F2620 expressing GFP (BBa_T9002) and lysis device without promoter (BBa_K112808) were used as negative controls.
- Cell lysis was assayed through $O.D._{600}$ measurement using a microplate reader (TECAN Infinite F200)
- Cultures were assayed in triplicate in a 96-well microplate



Three positive colonies bearing BBa_K173015 were identified with agarose gel elecrophoresis and DNA sequencing.





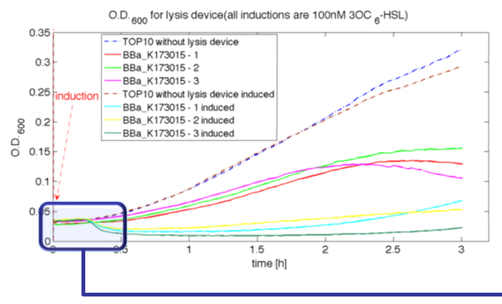
EXPERIMENTAL PROTOCOL

- Cultures were inoculated from glycerol stock (8µl) in 5ml of Luria Broth supplemented with Ampicillin and incubated overnight (ON) at 37°C with orbital shaking (220 rpm)
- Next morning cultures were diluted properly, to have an $O.D._{600}$=0.03, according to (I), where V_k is the volume of culture to keep and dilute in the desired final volume V_f.
- Cultures were tranferred in triplicate in the microplate reader, incubated at 37°C 220 rpm.
- $O.D._{600}$ was measured every 5 minutes.

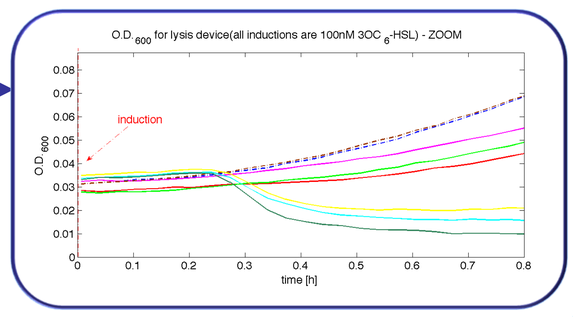
$$(I)\quad V_k = \frac{O.D._{wanted}}{O.D._{measured}} * V_f$$

RESULTS

Lysis assay on three different colonies of BBa_K173015



- The three positive colonies identified by agarose gel electrophoresis and sequencing and a negative control (*E. coli* TOP10 without lysis device) were tested.
- An additional 1:100 dilution and incubation for further 4 h after ON growth was performed.
- Uninduced cultures bearing the lysis device showed a slower growth than the control.
- All colonies 1, 2 and 3 showed $O.D._{600}$ decreasing after about 15 minutes from induction.
- After about 1-2 hours $O.D._{600}$ increased again.



This experiment was repeated 3 times, but for one of them no lysis was observed.

Lysis test on BBa_K173015 with serial inductions

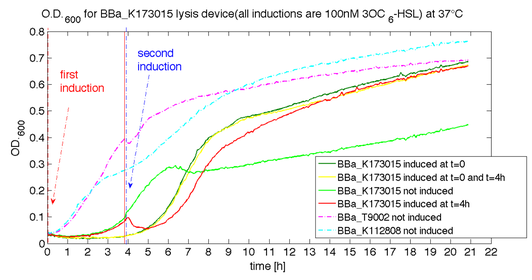
- Cultures were induced at different time points to check their stability over time.

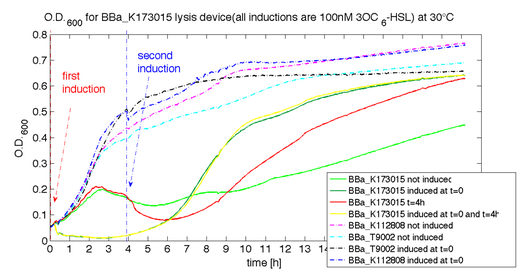
Time of induction:

only at t=0 h | only at t=4 h | both at t=0h and at t=4 h

- In the Registry it is reported that BBa_F2620 promoter has a weaker leakage activity at 30°C than at 37°C.

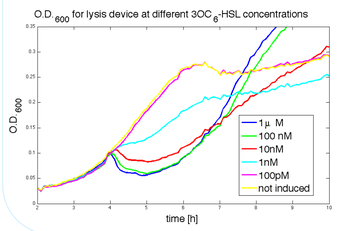
The experiment was repeated at 30°C to have a tighter control on lysis.



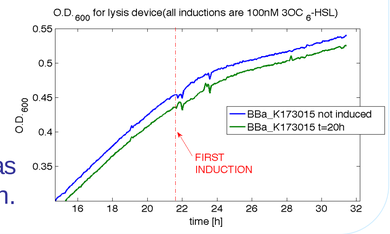


- Uninduced BBa_K173015 grew slower than control at both 37°C and 30°C.
- Lysis occurred for induced cultures (t=0 h) bearing the lysis device. A more evident $O.D._{600}$ decreasing was observed in the first 2 hours at 30°C rather than at 37°C.
- Lysis occurred for the cultures bearing the device induced only at t=4 h, but cultures induced at both t=0 h and t=4 h did not show any additional lysis.
- All the lysed cultures showed an $O.D._{600}$ increasing after 1-2 hours from lysis beginning and their growth re-started with a rate comparable to the controls and higher than uninduced cultures.

Lysis test on BBa_K173015 in different growth phases



- In log-phase the entity of lysis was dependent on the inducer concentration.
- In saturation phase no lysis was observed after induction.



CONCLUSIONS

- A lysis device based on three genes from enterobacteria phage T4 was characterized in a high-copy plasmid using $3OC_6$-HSL molecule to induce holin and lysozyme gene expression.
- Cells bearing the uninduced device grew more slowly than controls, probably due to a leaky expression of the lysis genes.
- Cells bearing the induced device showed lysis after 15 min from induction, but after about 2 h they began to grow again, demonstrating a positive selection of mutant cells and thus the genetic instability of the lysis device.
- Cell lysis occurred in exponential bacterial growth phase and its entity was dependent on the inducer concentration, but lysis did not occur in saturation phase.

REFERENCES

[1] M. Morita et al., *Programmed Escherichia coli cell lysis by expression of cloned T4 phage lysis genes*. Biotechnology progress, 17(3):573–576, 2001.
[2] http://partsregistry.org/Part:BBa_K112808
[3] http://2009.igem.org/Team:UNIPV-Pavia/Parts_Characterization
[4] A. Labno et al., *Refinement and standardization of synthetic biological parts and devices*, Nature Biotechnology, 2008 July; 26(6), 787-93.