VALIDATION OF A STANDARD APPROACH FOR QUANTITATIVE CHARACTERIZATION OF PROMOTER STRENGTH

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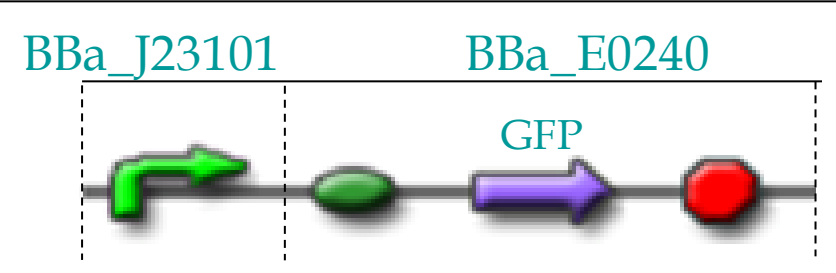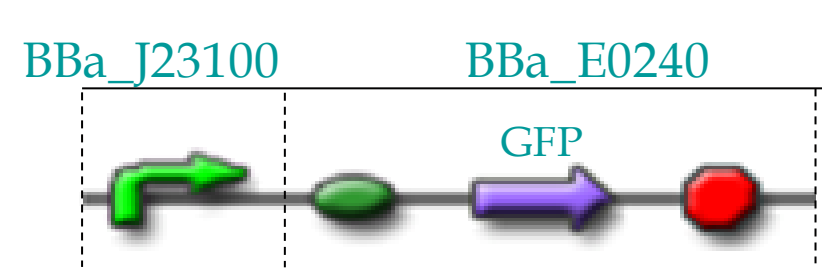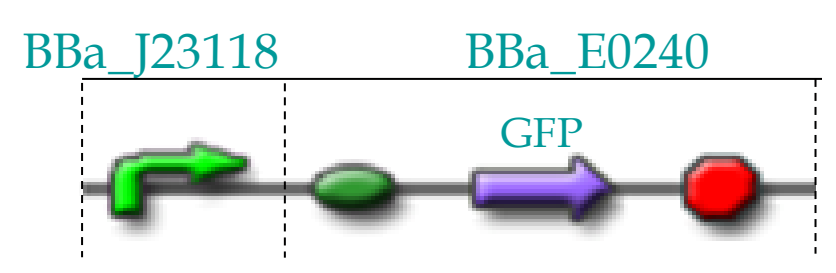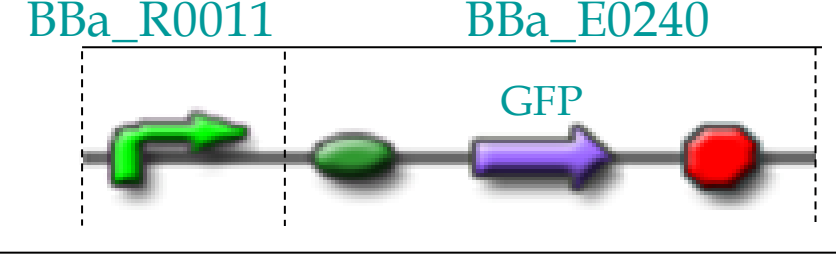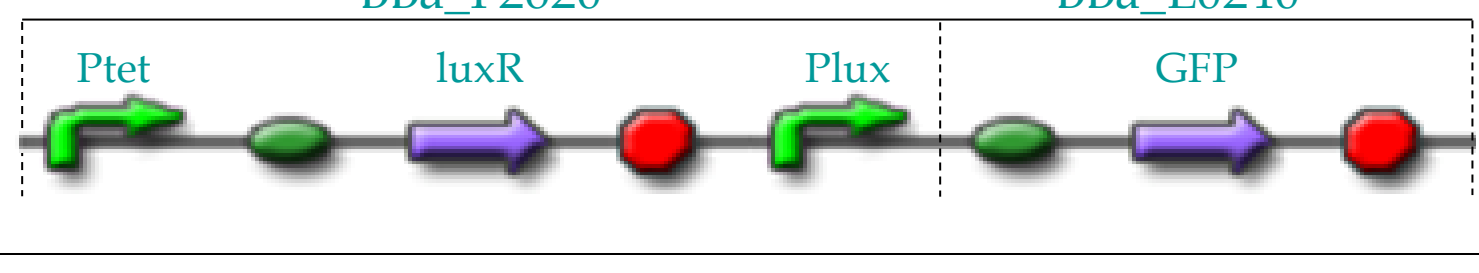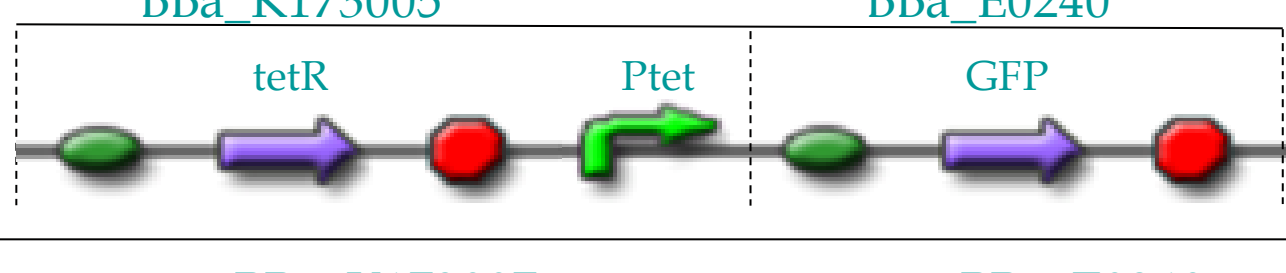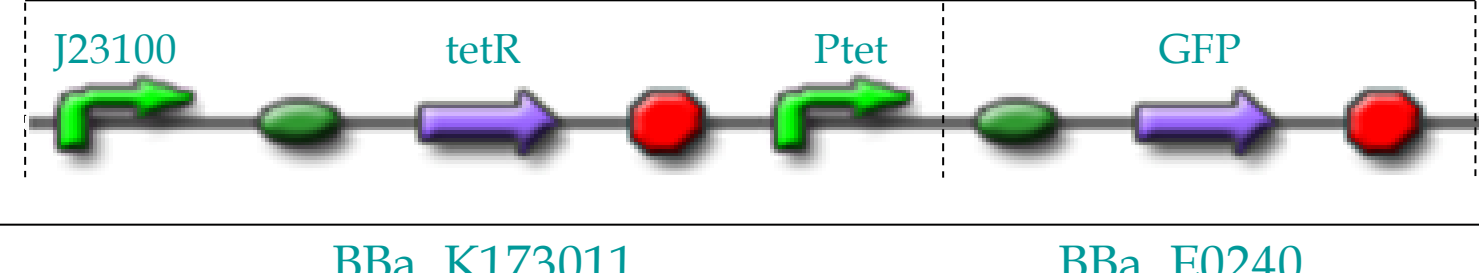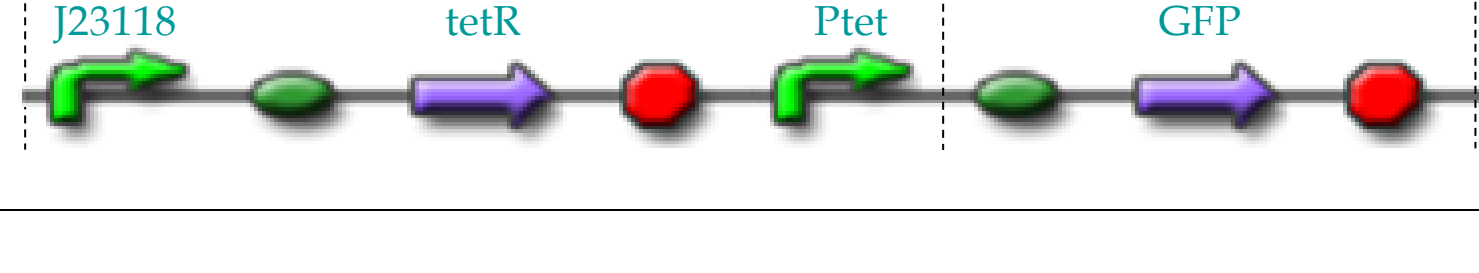[2]Center for Tissue Engineering, University of Pavia, Italy
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MOTIVATION. The rational design of synthetic biological systems can be performed only using a set of well-characterized biological parts. For this reason, standard measurement methodologies must be developed to allow an easy and robust quantitative characterization of parts by independent labs. In this work, the Relative Promoter Units (RPUs) method [2] was validated and applied to measure the strength of eight promoters from the Registry of Standard Biological Parts. The RPU approach allows the measurement of a promoter of interest using BBa_J23101 constitutive promoter as a standard reference.

PROMOTER MEASUREMENT PARTS. To apply the RPU method, each promoter or inducible device has been ligated with BBa_E0240 containing GFP, using BioBrick Standard Assembly.

Part of interest	Assembled part for measurement	Description
BBa_J23101	BBa_J23101 — BBa_E0240 (GFP)	Constitutive promoter from J.C. Anderson collection. Standard reference for RPU method.
BBa_J23100	BBa_J23100 — BBa_E0240 (GFP)	Strong constitutive promoter from J.C. Anderson promoter collection.
BBa_J23118	BBa_J23118 — BBa_E0240 (GFP)	Medium-strength constitutive promoter from J.C. Anderson promoter collection.
BBa_R0011	BBa_R0011 — BBa_E0240 (GFP)	Hybrid Plac consisting of the lambdaphage P_λ promoter with the cI binding sites replaced with lacO1.
BBa_F2620	BBa_F2620 (Ptet, luxR, Plux) — BBa_E0240 (GFP)	$3OC_6HSL$ inducible device with Ptet driving the expression of LuxR transcription factor.
BBa_K173005	BBa_K173005 (tetR, Ptet) — BBa_E0240 (GFP)	Ptet repressible promoter without TetR repressor: it is expected to be a constitutively active device.
BBa_K173007	BBa_K173007 (J23100, tetR, Ptet) — BBa_E0240 (GFP)	Ptet repressible promoter with J23100 strong promoter driving the expression of tetR.
BBa_K173011	BBa_K173011 (J23118, tetR, Ptet) — BBa_E0240 (GFP)	Ptet repressible promoter with J23118 medium-strength promoter driving the expression of tetR.

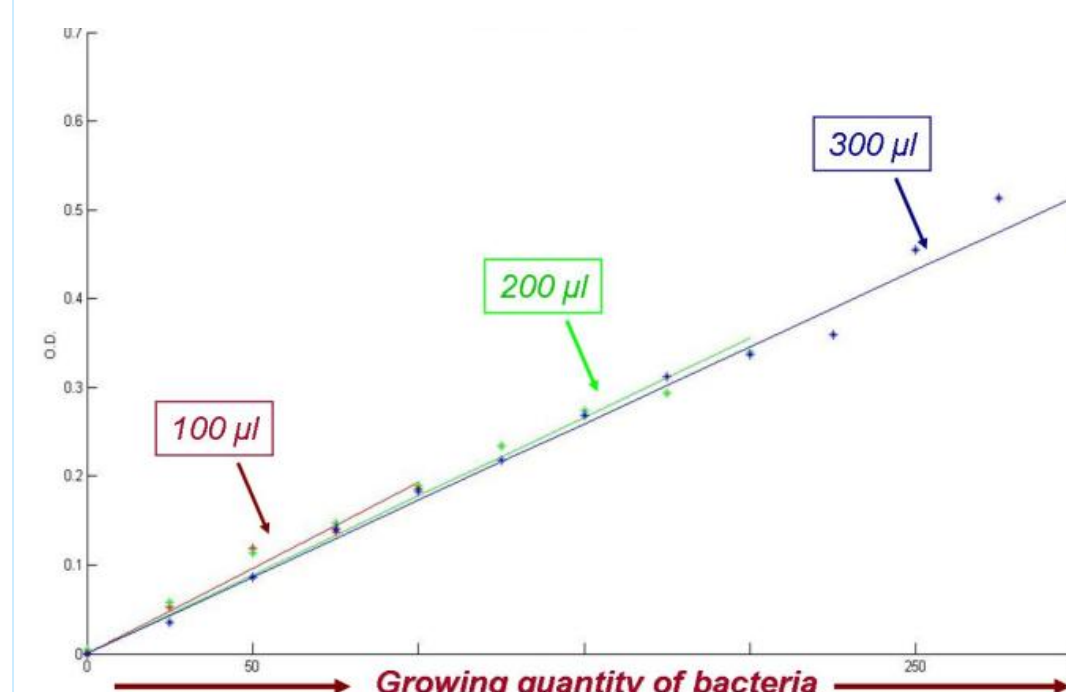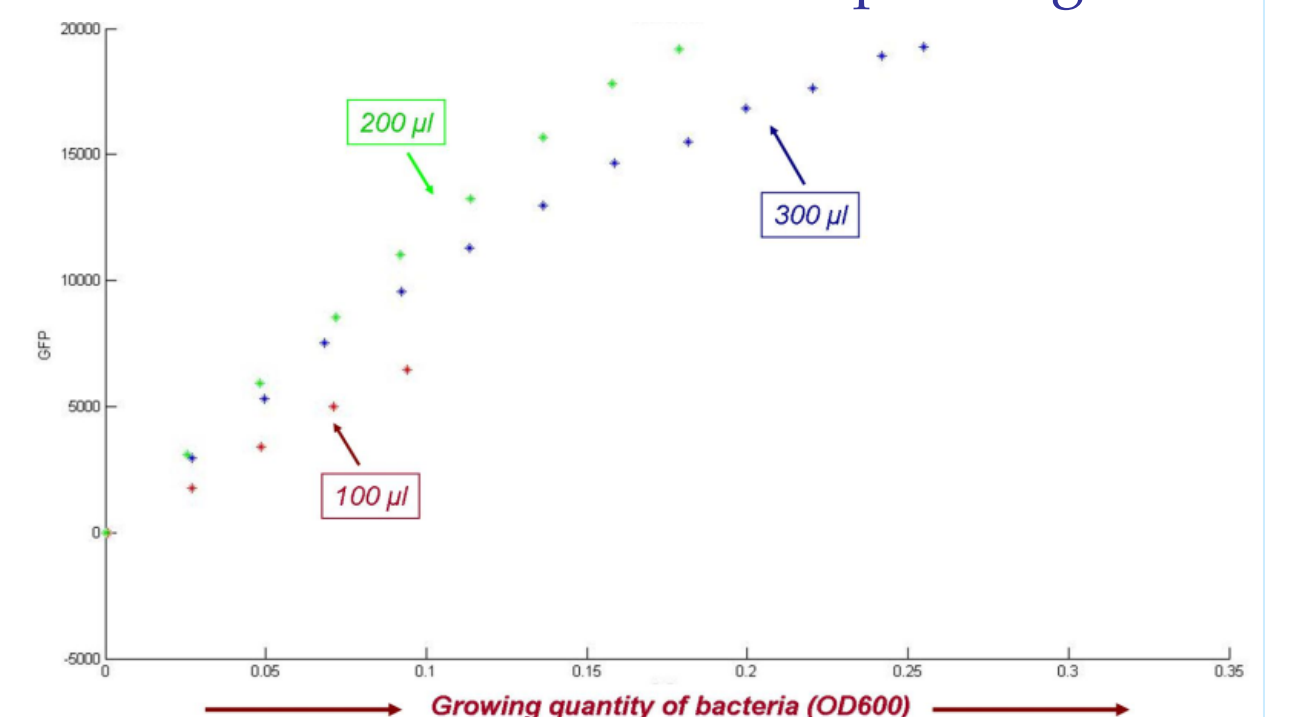**MEASUREMENT SYSTEM.** TECAN Infinite F200.

OVERVIEW

- multiwell microplate reader
- temperature/shaking control
- absorbance measurements (OD)
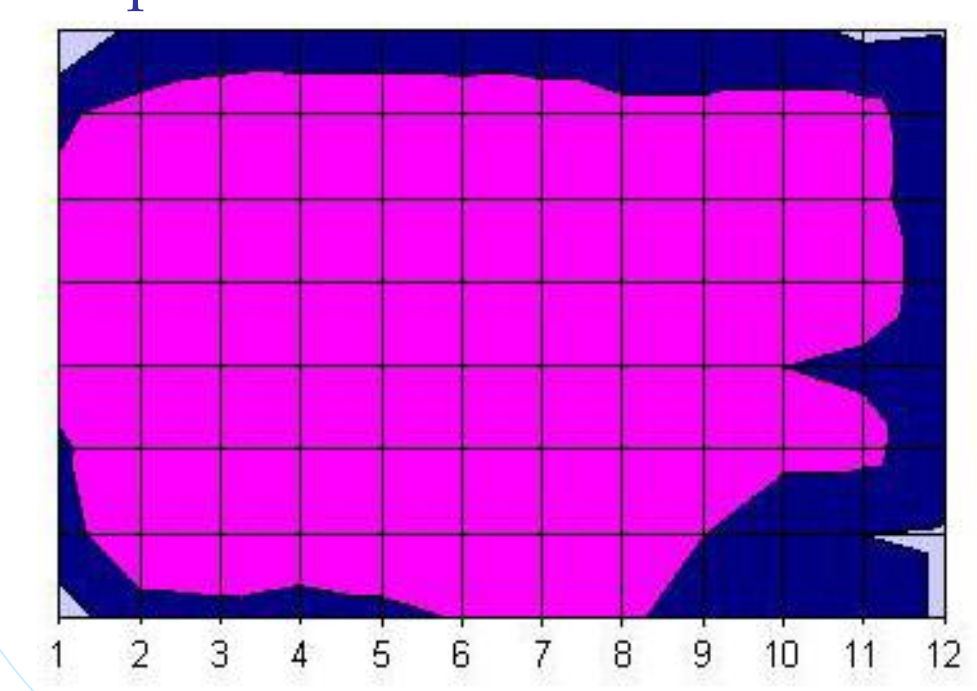- fluorescence measurements (F)

EXPERIMENTAL SETUP

Absorbance linearity validation: serial dilutions of a bacterial culture
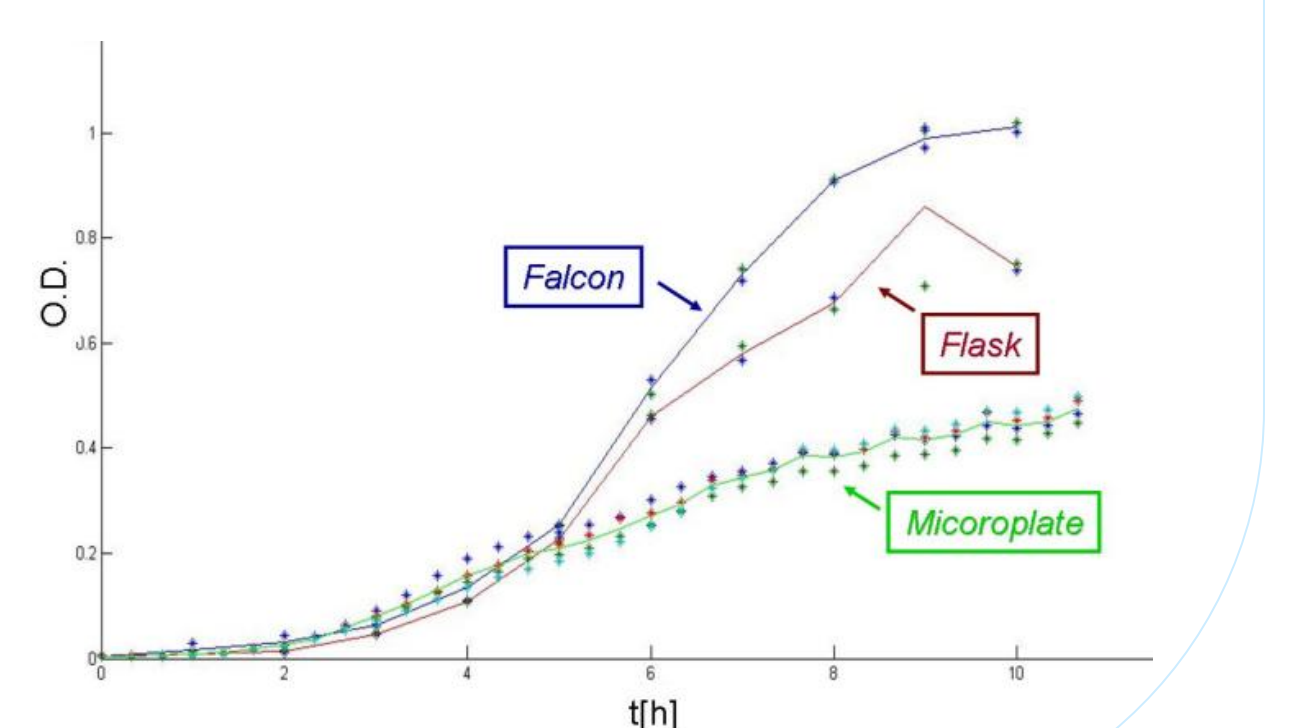
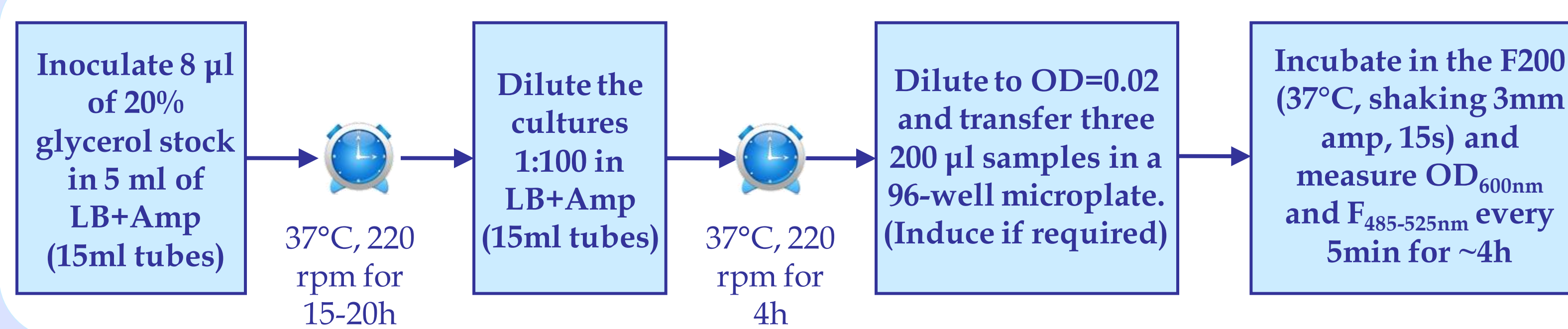Fluorescence linearity validation: serial dilutions of a culture expressing GFP

Water evaporation in long term experiments: frame effect definition

Characterization of bacterial growth in different environments

EXPERIMENTAL PROCEDURE. This protocol was followed for i) the measurement part of interest, ii) J23101-E0240 (standard reference) and iii) B0032 (non-fluorescent culture).

Inoculate 8 µl of 20% glycerol stock in 5 ml of LB+Amp (15ml tubes) → 37°C, 220 rpm for 15-20h → Dilute the cultures 1:100 in LB+Amp (15ml tubes) → 37°C, 220 rpm for 4h → Dilute to OD=0.02 and transfer three 200 µl samples in a 96-well microplate. (Induce if required) → Incubate in the F200 (37°C, shaking 3mm amp, 15s) and measure OD_{600nm} and $F_{485-525nm}$ every 5min for ~4h

RELATIVE PROMOTER UNITS. Data were processed according to the RPU approach, under the steady-state hypothesis [2]:

$$R.P.U._\phi = \frac{mean(\frac{dF_\phi}{dt} \cdot \frac{1}{OD_{600,\phi}})}{mean(\frac{dF_{J23101}}{dt} \cdot \frac{1}{OD_{600,J23101}})}$$

(RPU $\propto$ Promoter transcriptional rate)

where:
- Φ is the promoter of interest
- J23101 is the standard reference
- F and OD are the blanked time series of the measurement parts during the bacterial log-phase

RESULTS.

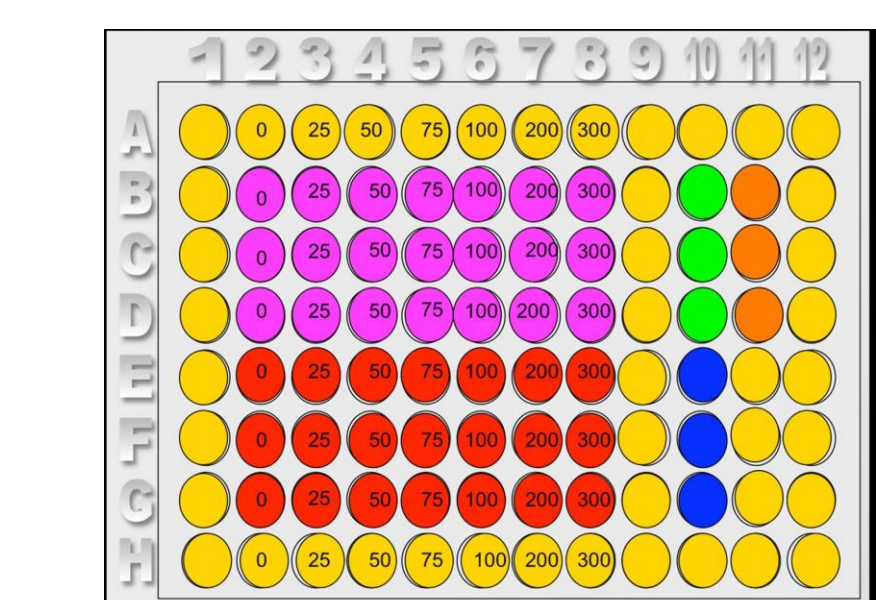Example of data acquisition and processing: BBa_K173011 inducible device
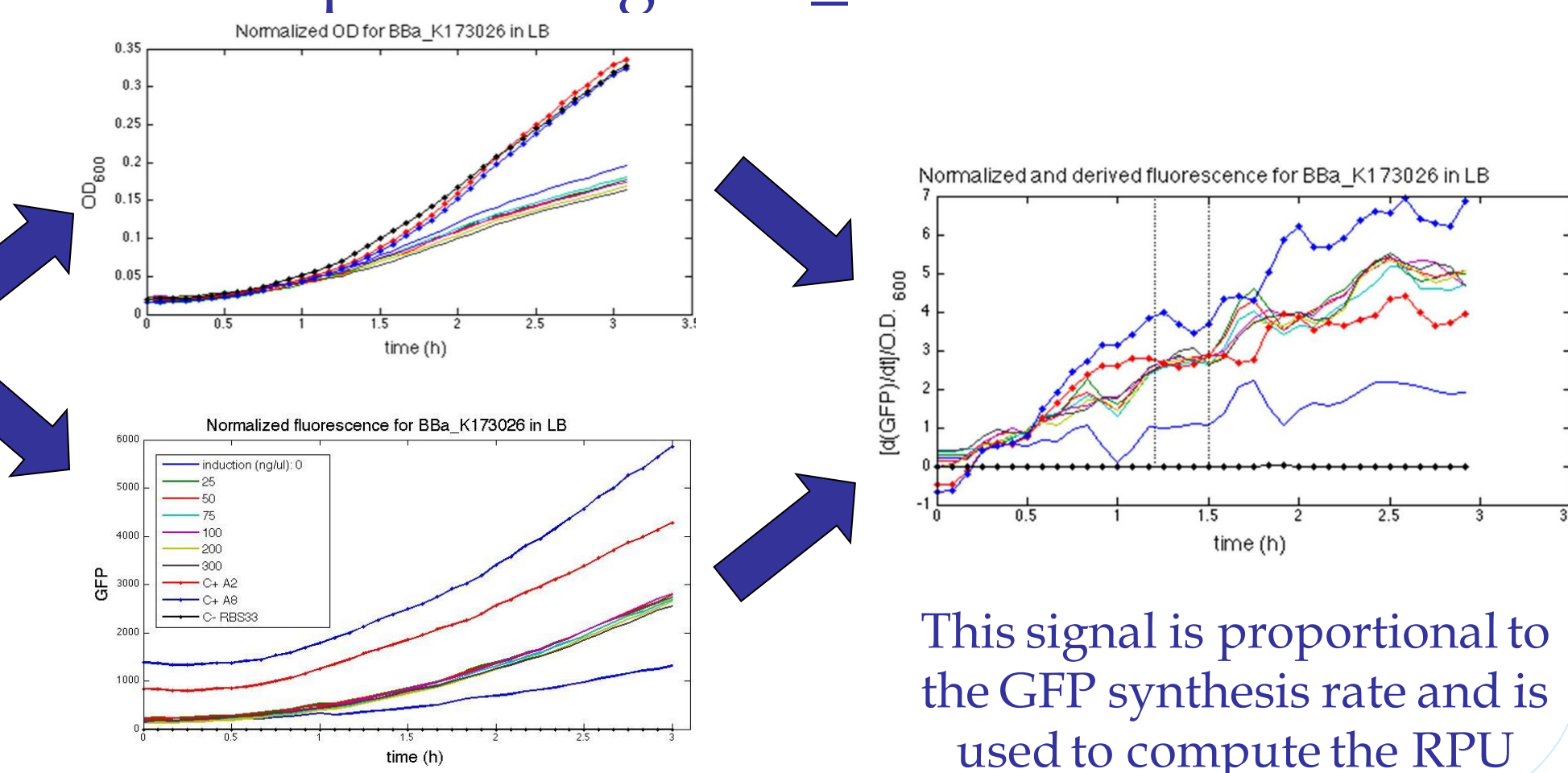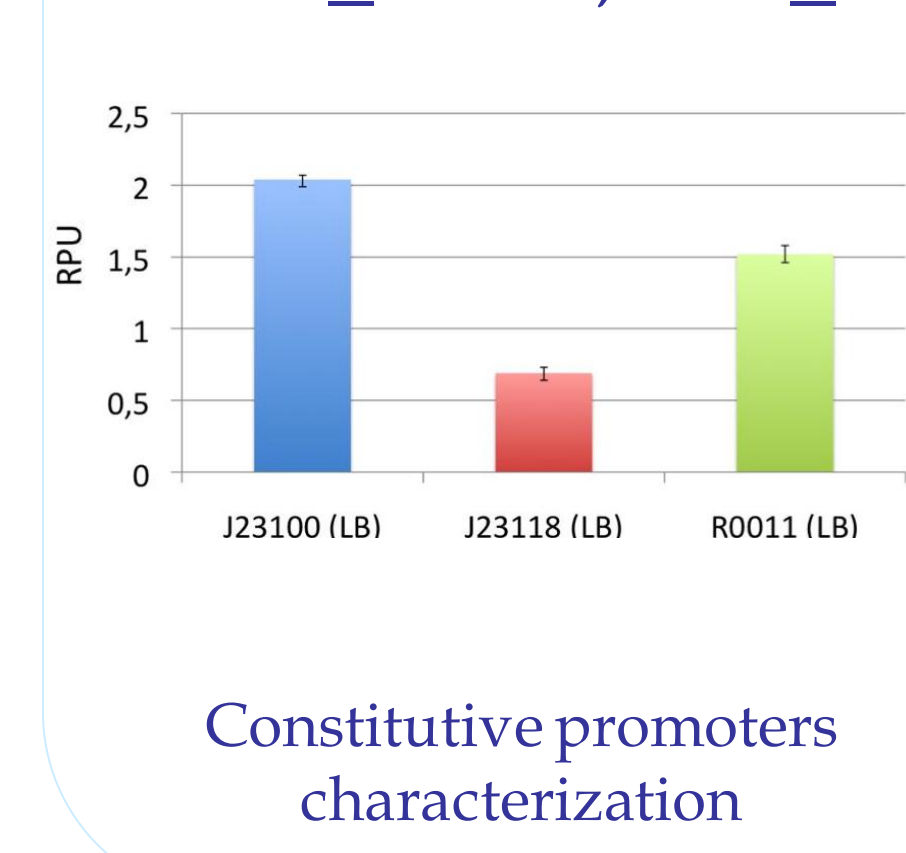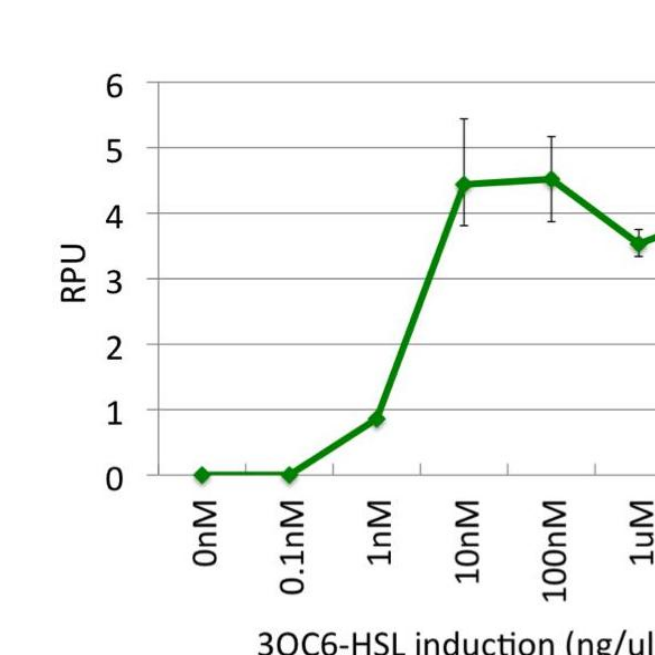

Plate definition: part of interest, standard reference, non-fluorescent culture and media

This signal is proportional to the GFP synthesis rate and is used to compute the RPU
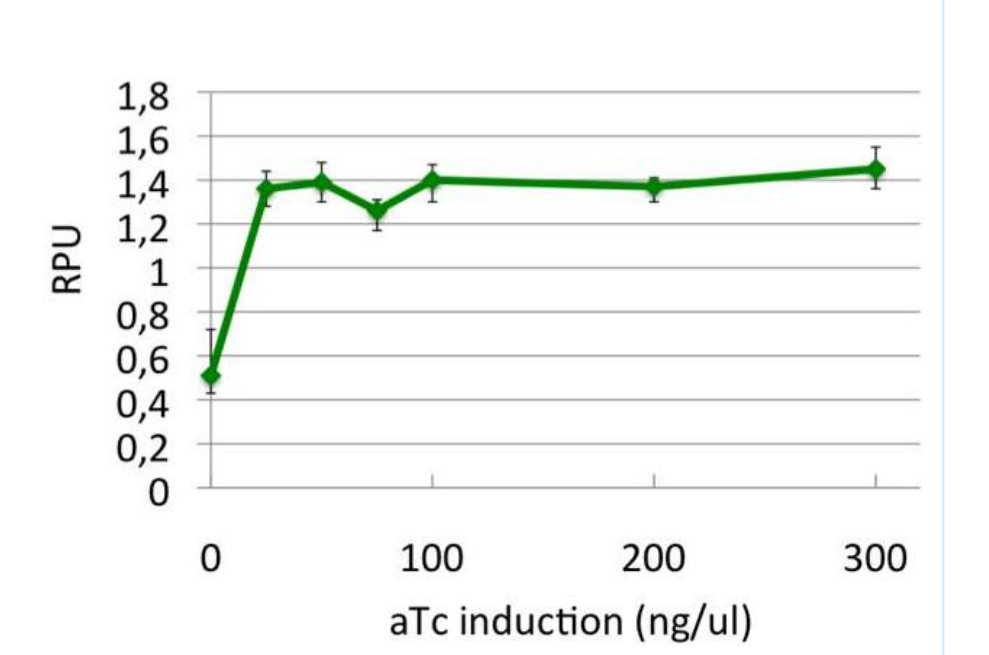
RPU estimation for: BBa_J23100, BBa_J23118, BBa_R0011, BBa_F2620, BBa_K173011

Constitutive promoters characterization

BBa_F2620: $3OC_6HSL$ inducible device characterization

BBa_K173011: aTc inducible device characterization

CONCLUSIONS

- The relative strength of four constitutive promoters and three inducible devices from the Registry was estimated using the RPU standard method.
- RPU values of BBa_R0011 and BBa_K173005 were in full agreement with those reported in the literature [2].
- GFP synthesis rate was not always found to be constant over time during the log-phase.
- Sometimes RPU values are highly sensitive to the log-phase boundaries that some times are hard to determine.

REFERENCES

[1] R. Shetty et al., *Engineering BioBrick vectors from BioBrick parts*. Journal of Biological Engineering, 2008 Apr 14;2:5.
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[3] J.R. Kelly PhD thesis, *Tools and reference standards supporting the engineering and evolution of synthetic biological systems*. MIT PhD, 2008.
[4] A. Labno et al., *Refinement and standardization of synthetic biological parts and devices*, Nature Biotechnology, 2008 July; 26(6), 787-93.