BBF RFC 78: Novel Normalization Standard using Fluorescence

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1. Purpose

The Biotec_Dresden Team 2010 developed an approach where two fluorescent proteins are simultaneously expressed. The fact that one reporter, in our case RFP, is constitutively expressed allows to monitor cell growth. Secondly, an inducible promoter drives the expression of the second reporter, YFP in the case of the part tested. The constitutively expressed reporter protein (R1) serves as normalization factor for the inducible reporter (R2) by simple division.

2. Relation to other BBF RFCs

BioBrick Assembly Standards
1.12 BBF RFC 11: BioBrick™ assembly standard modifications
1.15 BBF RFC14: Protein domain fusions in BB-2 assembly
1.26 BBF RFC 25: Fusion Protein (Freiburg) BioBrick™ assembly standard
1.27 BBF RFC 26: In-Fusion BioBrick™ Assembly

BioBrick Determination of Promoter Activity
1.20 BBF RFC 19: Measuring the Activity of BioBrick™ Promoters Using an In Vivo Reference Standard

3. Copyright Notice

4. Background

Performing kinetic measurements involving fluorescence in living cultures like bacteria requires the monitoring of growth generally performed using optical density (OD) measurements. These help to normalize the obtained fluorescent data and gain the actual values of fluorescence containing information about the actual cell number contributing to the overall fluorescence. To date this procedure is routinely followed.

5. Novel Approach

Instead of using OD measurements for normalization of fluorescent data R1 is introduced to the analyzed DNA construct containing inducible R2. Tests showed that this normalization standard is comparable and even more advanced than the routinely performed OD procedure.

6. Standard Procedure

- Assemble R1 with the target construct using any of the mentioned BioBrick standard assemblies (Chapter 2) resulting in the following properties:
    - R1 MUST be constitutively expressed
    - Both fluorescent reporters MUST NOT have an overlapping emission spectra
    - Both fluorescent reporters SHOULD NOT have an overlapping emission spectra
    - R2 SHOULD only be expressed upon desired induction
    - The complete construct MUST NOT include a negative feedback loop
    - R2 MUST NOT express a pigment
    - Promoter activity SHOULD be defined

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References

http://2010.igem.org/Team:BIOTEC_Dresden/Results