Beta-Glucuronidase (GusA) reporter enzyme assay for *Escherichia coli*

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Abstract

The beta-Glucuronidase (GusA) is a long-known reporter enzyme for many different species [1]. The *E. coli gusA* gene is often used in plant research because plants lack an endogenous *gusA* gene. In *E. coli*, the transcript of the *gusA* gene is more stable than that of the highly used reporter gene beta-galactosidase (*lacZ*) [2]. The GusA activity can be determined using the artificial substrate p-nitrophenyl-β-D-glucopyranosid (pNPG). pNPG is converted to glucoronic acid and para-nitrophenol (pNP), which can be quantified spectrometrically at 405 nm. To avoid background, it is best to use an *E. coli* strain with a deletion of the chromosomal *gusA* gene, which is available e.g. at the Keio collection [3]. The *gusA* gene can be used for transcriptional fusions, e.g. to characterize promoters, and also for translational fusions, e.g. to study translational regulation. The assay was adapted to the microtiter plate format to enable the parallel handling of a large number of samples. The “procedure” (see below) describes an application with the *gusA* gene in a translational fusion with the gene of interest cloned under the control of the inducible arabinose promoter P_{BAD}.

Reagents

- 20% (w/v) arabinose
- 1M Tris-HCl pH 7.2
- Sodiumphopsphate buffer: 50mM Na_2HPO_4, 50mM NaH_2PO_4
- GusA lysis buffer: 10mM sodiumphosphate buffer, 10mM KCl, 1mM $MgSo_4$
- 2x β-glucuronidase buffer: 10mM sodiumphosphate buffer, 20mM β-mercaptoethanol, 0.2% (v/v) triton-X-100
- pNPG: 8mg/mL in 0.1M Tris-HCl pH7.2 (prepared freshly)

Equipment

- Microtiter plate (MTP) photometer
- 96 well microtiter plates
- Pierce^{TM} BCA Assay Kit (Thermo Scientific)

Procedure

Cell culture and harvesting

1. Inoculate 50 mL medium in 100 ml Erlenmeyer flasks with plasmid-containing overnight cultures to an inital OD_{600} of 0.05. At least three biological replicates of each sample should be used and a negative control (culture with an empty vector) should be added. When the cultures reach an OD_{600} of about 0.6, induce the p_{BAD} promotor with a final concentration of 0.2% (w/v) arabinose.

2. Incubate the cultures for at least 30 minutes to allow expression of the *gusA* fusion gene. Harvest 20mL for the reporter enzyme assay by centrifugation at 4000rpm for 10 minutes at 4°C. If a transcript analysis is also desired, further 8mL culture can be harvested in parallel (4000rpm, 10 minutes, 4°C). The pellets can be stored at -80°C for later RNA Isolation (compare the Method "Northern blot analysis").

3. Resuspend the pellets for the reporter enzyme assay in 1mL GusA lysis buffer. Lyse the cells vial shaking with glass beads (500 mg/reaction tube) in a FastPrep™ (MP Biomedicals; 3x 45 seconds at 6.5 intensity with 20 seconds pauses on ice in between).

4. Sediment glass beads and cell debris by centrifugation (13000 rpm, 10 minutes, 4°C). Transfer the cleared lysate to a new reaction tube.

Determination of the specific GusA reporter enzyme activity

5. Start the MTP photometer and equilibrate it at 37°C for at least 30 minutes.

6. For every reaction pipette 120µL of suitable dilutions of the lysate into a well of a microtiter plate. We recommend 1:2; 1:10 and 1:100 dilutions and technical duplicates for every sample.

7. Add 150µL of 2x β-glucuronidase buffer and 30µL pNPG, and immediately transfer the MTP to the photometer and start the measurement.

8. The change of absorption is measured at 405nm for 30 minutes at 20 second intervals using a MTP photometer with shaking of the plate bevor every measurement.

9. For the analysis of the volume activity only dilutions can be used that exhibit a linear increase of

absorption for at least 3 minutes. The photometer software is used to calculate the change in extinction per minute (ΔE/min) for all samples.

10. The extinction change in the negative control is subtracted from the extinction changes of all samples to yield the GusA-specific extinction changes.

11. Calculate the GlpD volume activity using the following formula with the ΔE/min:

$$\text{Volume activity [U/mL]} = \Delta E * \text{min}^{-1} * \varepsilon^{-1} * d^{-1} * D$$

U: Enzyme activity (µmol x min^{-1}) 1U=16.67nkat

ΔE/min: Change in extinction at 405 nm (min^{-1})

ε: Specific extinction coefficient for pNP (18.1 mM^{-1} x cm^{-1})

d: thickness of the cuvette [cm] (300 µl in a 96 well MTP: 0,95 cm)

D: Dilution of the sample

12. To quantify the protein concentration, the PIERCE™ BCA (Bicinchoninic acid) protein assay kit is used with suitable dilutions of the lysates according to the instructions of the manufacturer. The assay has been adapted to 96 well MTPs to enable the parallel processing of many samples. Bovine serum albumin (BSA) is used to generate a standard curve, using concentrations form 0 mg/ml to 2 mg/ml. The colorimetric biuret-reaction results in the formation of a blue color, which can be quantified photometrically at 562 nm. The BSA standard curve is used to calculate the protein concentrations of all samples (mg/ml).

13. The specific GusA activity [U/mg] is calculated by dividing the volume activity [U/ml] with the protein concentration [mg/ml].

References

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Translational coupling via termination-reinitiation in archaea and bacteria

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