A protein-based biosensor for mRNA

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- **Single strand binding protein (SSB) is a suitable biosensor that is able to bind and quantify mRNA**
- **The fluorescent MDCC-SSB biosensor is compatible with high throughput and unpurified mRNA**
- **The CLARIOstar® microplate reader detects increase in fluorescence of MDCC-SSB bound to RNA**

Introduction

Transcription, catalysed by RNA polymerases, is one of the most fundamental processes in living cells (1). Characterizing the enzymatic process or RNA generation for use in CRISPR requires quantifying RNA transcripts. This typically requires purification of the nucleic acids leading to experimental inaccuracies and loss of product. Synthetic fluorescent dyes are available but are relatively expensive. Here, we describe the use of a fluorescent biosensor based upon the single-stranded binding (SSB) protein (2), which has previously been established as an ssDNA biosensor (2). The protein is easily expressed in E. coli and can be used without prior purification of the RNA, thereby providing a low cost, easy to use alternative for measuring mRNA.

Assay Principle

SSB binds to 65-70 base lengths of ssDNA as a tetrameric protein. The binding site length represents the limiting resolution of the biosensor.

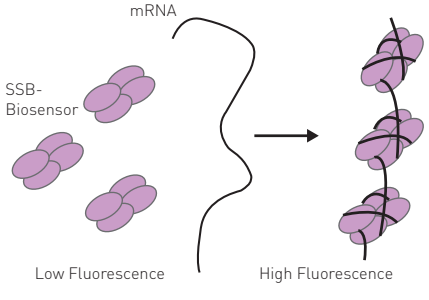

Fig. 1: The MDCC-SSB (red) tetramer binds to the mRNA transcript. This alters the environment of the MDCC leading to increase fluorescence emission compared to the apo-MDDC-SSB.

Materials & Methods

- Black 96-well microplate from Nunc, USA. #7605
- CLARIOstar® BMG LABTECH, Germany.
- MDCC from Invitrogen, UK. # D10253
- *E.Coli* SSB G26C, Addgene, USA. Plasmid #78203.
  $ssRNA_{70}$, Eurofins, UK
  HiScribe T7 RNA Synthesis Kit (NEB)
  All other reagents supplied by Sigma Aldrich, UK

Experimental Procedure

Purification and Labelling of SSB with MDCC

SSB protein expression, purification and labelling with MDCC was performed as described by Dillingham et al (1) and Cook et al (2).

Titration of Oligonucleotides to MDCC-SSB (calibration)

All reactions were performed at 25 °C in a buffer containing 50 mM Tris.HCl pH 7.5, 3 mM $MgCl_2$ and 100 mM NaCl with 200 nM MDCC-SSB in a final volume of 100 µL. SSB is a tetrameric protein therefore biosensor concentration is 50 nM. For the measurements, dispense 100 µl MDCC-SSB to the 10-20 wells. Serial dilute 100 µl of 2 µM $ssRNA_{70}$. Fluorescence change is then calculated relative to an MDCC-SSB only control. Measurements were done on a CLARIOstar microplate reader with the settings indicated below.

In vitro Transcription and mRNA quantification

In vitro transcription was performed with the HiScribe T7 RNA Synthesis kit. The control template generates a 2225 base run-off transcript. Reactions were performed at 30 °C for 20 120 min. mRNA was purified using RNeasy® kit. Half of the sample was used for RT-qPCR quantification with the QuantiFast SYBR Green qPCR kit. The remaining sample was transferred to a microplate and mixed with MDCC-SSB with the assay conditions outlined above.

Instrument Settings

Optic Settings	Fluorescence spectra, Top optic	
	Monochromator settings	Ex. 400-10->440-10/470-16 Em. 430-10/455-10->550-10
	Gain	Adjusted prior to measurement
	Focus Height	
General settings	Number of flashes	40 per well
	Settling time	0.1 s

Results & Discussion

The MDCC-SSB fluorescence emission increases 2.1 fold when binding to $ssRNA_{70}$ (Fig. 2).
Fluorescence of the MDCC-SSB depends on the concentration of $ssRNA_{70}$ and becomes saturated once all biosensor is bound to substrate (Fig. 3). The MDCC-SSB biosensor (200 nM) detects mRNA in a linear range over two orders of magnitude, 5-500 nM. Within this linear range the biosensor can be calibrated in terms of concentration of SSB binding sites.

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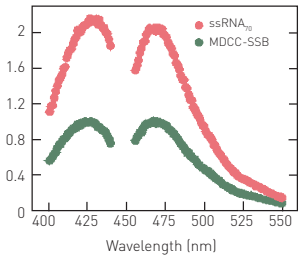

Fig. 2: Fluorescence excitation and emission spectra for 200 nM MDCC-SSB measured in the apo-state (green) and in the presence of 1 µM ssRNA70 (red).

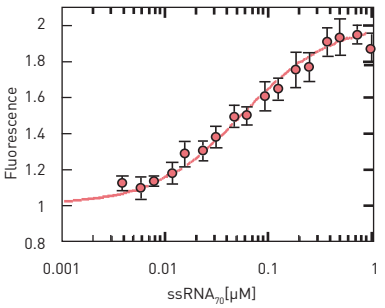

Fig. 3: Fluorescence intensity of MDCC-SSB increases with $ssRNA_{70}$ concentration.

In vitro transcription by T7 polymerase driven from a T7 promoter resulted in a 2225 nucleotide run-off transcript. Transcription assays of different reaction duration (20-120 min) yielded different amounts of mRNA products. After product purification, MDCC-SSB was added to each mRNA sample and MDCC-SB binding to the transcripts was recorded (Fig. 4). The linear response in fluorescence intensity versus amount of mRNA indicates that the biosensor detects differences in transcript yield.

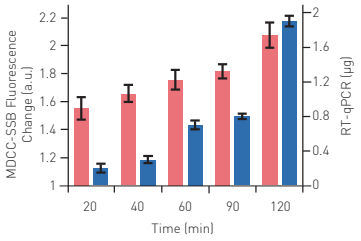

Fig. 4: Fluorescence intensity of MDCC-SSB upon binding to mRNA following *in vitro* transcription (red). Reactions were prematurely terminated up to 120 min. Performance of the biosensor was compared to quantification by RT-qPCR (blue).

To increase versatility of the biosensor, we tested if mRNA purification is required. We found that MDCC-SSB binds to both purified and non-purified mRNA (Fig. 5). The latter prevents loss of product through the isolation steps. Performing the titration in Fig. 3 enables the fluorescence signal to be calibrated in terms of SSB binding sites therefore the biosensor can generate quantitative data. Calibrations should be performed under the same experimental conditions as the transcription reactions.

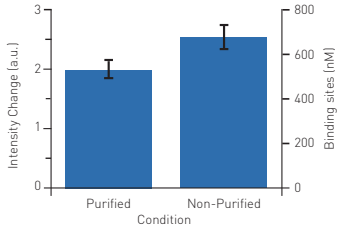

Fig. 5: MDCC-SSB binds purified mRNA or mRNA in transcription reactions. The fluorescence signal was calibrated using the titration in Fig. 3 to give concentration of MDCC-SSB binding sites (70 bases).

Conclusion

The MDCC-SSB biosensor is a low cost, fast, easy to use and reliable tool for measuring mRNA from *in vitro* transcription assays. The tool does not require RNA purification. Moreover, the biosensor can be used in both qualitative and quantitative assays. With this tool faster identification of transcription components will lead to a better understanding of the complex nature of transcription. The CLARIOstar assisted in assay development and reliably quantifies mRNA with MDCC-SSB.

References

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2. Cook, A. *et al.* (2018) Biochemical and biophysical research communications. DOI: 10.1016/j.bbrc.2018.01.147
3. Dillingham, M. *et al.* (2008). Biophysical Journal, 95, 3330-3339. DOI: 10.1529/biophysj.108.133512