Profiling bacterial kinase activity using a genetic circuit

van der Helm, Eric; Bech, Rasmus; Lehning, Christina Eva; Vazquez-Uribe, Ruben; Sommer, Morten Otto Alexander

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Phosphorylation is a post-translational modification that regulates the activity of several key proteins in bacteria and eukaryotes. Accordingly, a variety of tools has been developed to measure kinase activity. To couple phosphorylation to an in vivo fluorescent readout we used the *Bacillus subtilis* kinase PtkA, transmembrane activator TkmA and the repressor FatR to construct a genetic circuit in *E. coli*. By tuning the repressor and kinase expression level at the same time, we were able to show a 4.2-fold increase in signal upon kinase induction. We furthermore validated that the previously reported FatR Y45E mutation results in a functional kinase. The strains containing a functional fatR gene (green shades), (+/+), show a 3.57±0.13 and 4.17±0.09-fold increase in the GFP/mCherry ratio upon kinase induction. The strains containing the fatR promoter driving fatR transcription results in more transcriptional repression of the kinase plasmid. Increasing amounts of benzoate induces the expression of FatR, which attenuates the DNA binding of FatR to the operator site in front of gfp. Derepression of the operator site leads to expression of GFP and thus an increase in GFP/mCherry ratio. Different promoter strengths are used to drive the transcription of the fatR promoter. mCherry fluorescence is used to normalize the fluorescent signal of GFP.

**Figure 1a:** General concept of kinase activity measurement through GFP fluorescence. The repressor binds the operator site upstream of gfp, thereby preventing transcription. The kinase phosphorylates the repressor, which attenuates binding of the repressor to the operator site, leading to transcription of gfp and thus fluorescence.

**Figure 1b:** An abstraction of the genetic circuit where the first inverter gate represents the phosphorylation of FatR by PtkA-TkmA on the post-translational level. The second inverter gate represents the transcriptional regulation by the binding of FatR to the operator upstream of gfp.

**Figure 2:** Outline of the implementation with FatR as repressor and PtkA as kinase. The kinase PtkA and kinase-activator TkmA expression is induced by the benzoate/XylS system. The PtkA-TkmA complex can phosphorylate the repressor FatR, which attenuates the DNA binding of FatR to the operator site in front of gfp. Derepression of the operator site leads to expression of GFP and thus an increase in GFP/mCherry ratio. Different promoter strengths are used to drive the transcription of the fatR repressor. mCherry fluorescence is used to normalize the fluorescent signal of GFP.

**Figure 3a:** Raw FACS counts of 5 different strains with various fatR (repressor) genotypes under conditions where the kinase operon is not expressed. These strains each display a different fadR activity, which is reflected in the signal in the GFP channel (FITC-A). A strong promoter driving fatR results in a low GFP signal (green), whereas the strain containing no fatR shows a high GFP signal (red). Only one biological replicate of each strain is shown for clarity. This is the data used to calculate the values in Figure 3b.

**Figure 3b:** GFP/mCherry ratio measured of 5 different strains with various fatR (repressor) genotypes (based on data from Figure 3a). The two negative control reporters fatR Y45E and ΔfatR show an expected high ratio of GFP/mCherry. A stronger promoter driving fatR transcription results in more repression of GFP and thus a lower GFP/mCherry ratio as expected.

**Figure 3c:** Circuit response of *E. coli* containing both the platform plasmid and the kinase plasmid. Increasing amounts of benzoate induces the expression of the kinase PtkA and activation domain TkmA. Error bars represent SD of 3 biological replicates.

**Table 1:** Derouiche A. et al. *Nucleic Acids Res* 2013, 41:3971–81

**Figure 4:** Nanopore sequencing works by threading a DNA molecule through a nanopore (green) in a membrane, thereby creating a transient ionic current that is measured by an external circuit. The ionic current generated by threading DNA through the pore can be used to measure the sequence of the DNA. The nanopore sequence is then converted into a nucleotide sequence by downstream analysis. The nanopore sequencing method produces a mean sequence length of 3,086 reads using consensus calling of error correction, followed by polishing, showing a high sequence identity agreement between the Sanger and nanopore sequencing methods.

**Figure 5:** Histogram of the 2D nanopore sequencing data showing the length distribution. The 2D read length is distributed as a mean of 1587 bp. A mean sequence length of 1370 bp is observed for library B (orange) with a mean read length of 1370 bp. The 2D read length distribution is overlaid with the DNA input sample intensity showing an expected high agreement between the Sanger and nanopore sequencing methods.

**Figure 6:** The 2D read length distribution is overlaid with the DNA input sample intensity showing an expected high agreement between the Sanger and nanopore sequencing methods. The 2D read length distribution is overlaid with the DNA input sample intensity showing an expected high agreement between the Sanger and nanopore sequencing methods.