

Supplementary Material

Directed evolution of cellobiose dehydrogenase on the surface of yeast cells using resazurin based fluorescent assay

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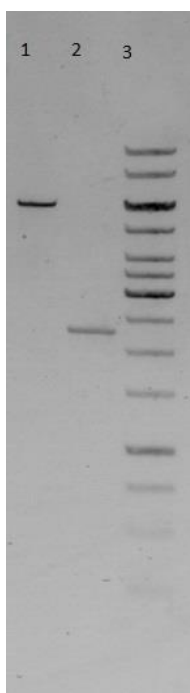


Figure S1. DNA electrophoresis on 0.6% agarose gel. Samples: (1) Empty pCTCON2 vector, (2) wtCDH PCR for pCTCON2, and (3) Molecular markers.

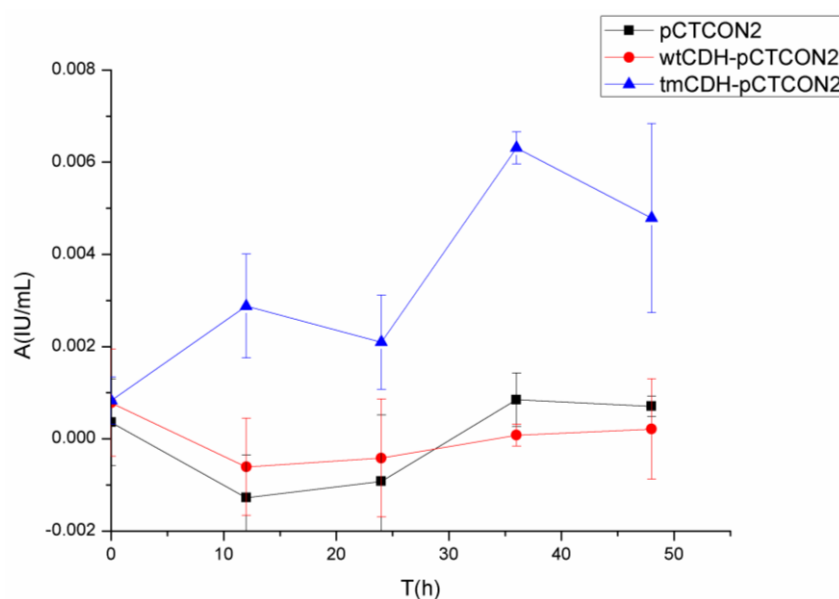


Figure S2. Fermentation graph after galactose induction at 25 °C. Samples were EBY100 cells transformed with empty pCTCON2 vector, cells transformed with wtCDH-pCTCON2 and cells transformed with tmCDH-pCTCON2. Activity was measured with DCIP (0.3 mM), lactose (30 mM) in sodium acetate buffer (0.1 M, pH 4.5). All measurements were done in triplicate.

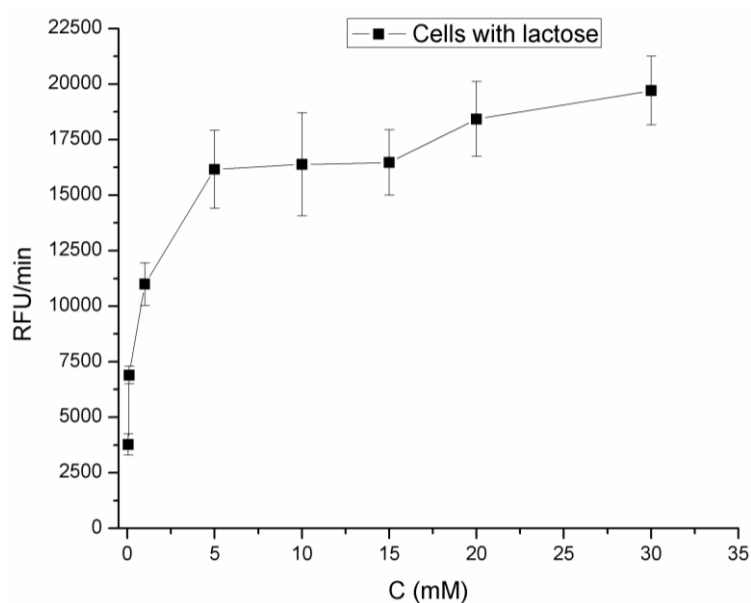


Figure S3. Dependence of fluorescence slope in time with lactose concentration. Resazurin (0.1 mM), sodium phosphate (0.1 M; pH 6), EBY100 cells with tmCDH (0.1 OD_{600nm}) with range of lactose concentration (0.05–30 mM), assay measured at ex/em 540/590 nm. All measurements were done in triplicate.

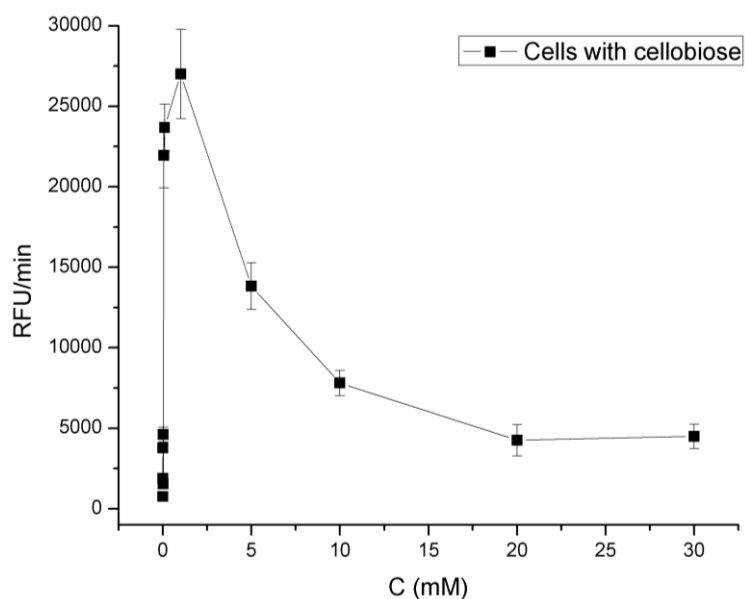


Figure S4. Dependence of fluorescence slope in time with cellobiose concentration. Resazurin (0.1 mM), sodium phosphate (0.1 M; pH 6), EBY100 cells with tmCDH (0.1 OD_{600nm}) with range of cellobiose concentration (0.001–30 mM), assay measured at ex/em 540/590 nm. All measurements were done in triplicate.

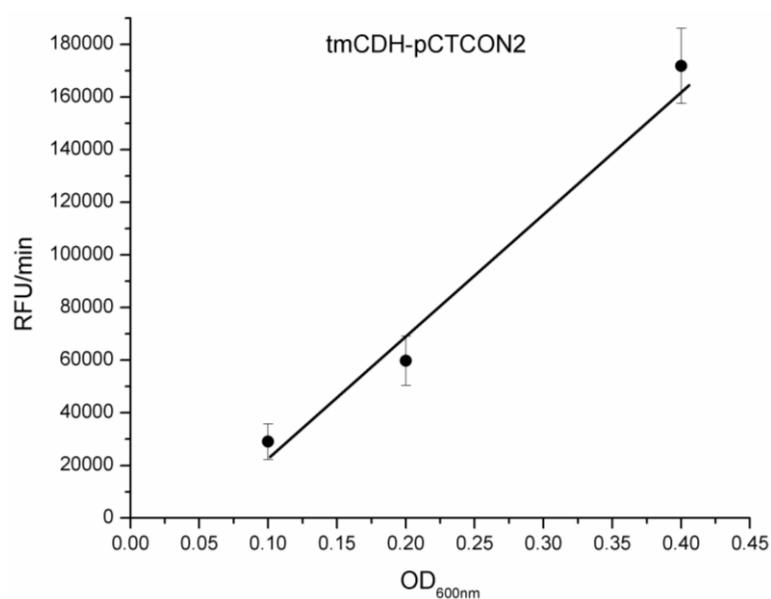


Figure S5. Dependence of fluorescence signal (RFU/min) on OD at 600 nm of yeast cells expressing cellobiose dehydrogenase. Relative fluorescence units (RFU) at excitation/emission (ex/em) wavelengths of 540/590 nm. All measurements were done in triplicate.

Table S1. Cellobiose dehydrogenase synthetic gene sequence (U46081.1).

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