

## Electronic Supplementary Information

### Efficient bioconversion of crude glycerol from biodiesel to optically pure D-lactate by metabolically engineered *Escherichia coli*

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**Table S1. Composition of crude glycerol after autoclaving<sup>a</sup>**

Composition	Concentration (g/L)	% (w/w)
Glycerol	778.24	63.51
Methanol	ND	ND
Water	285.83	24.22
Fatty acids	23.61	1.96
Ash	32.27	2.81
Others	55.73	8.51
Total	1175.68	100

The pH was 6.38 and the density was 1.18 g/mL.

<sup>a</sup> Data are means of three independent repeats, errors were 5% or less.

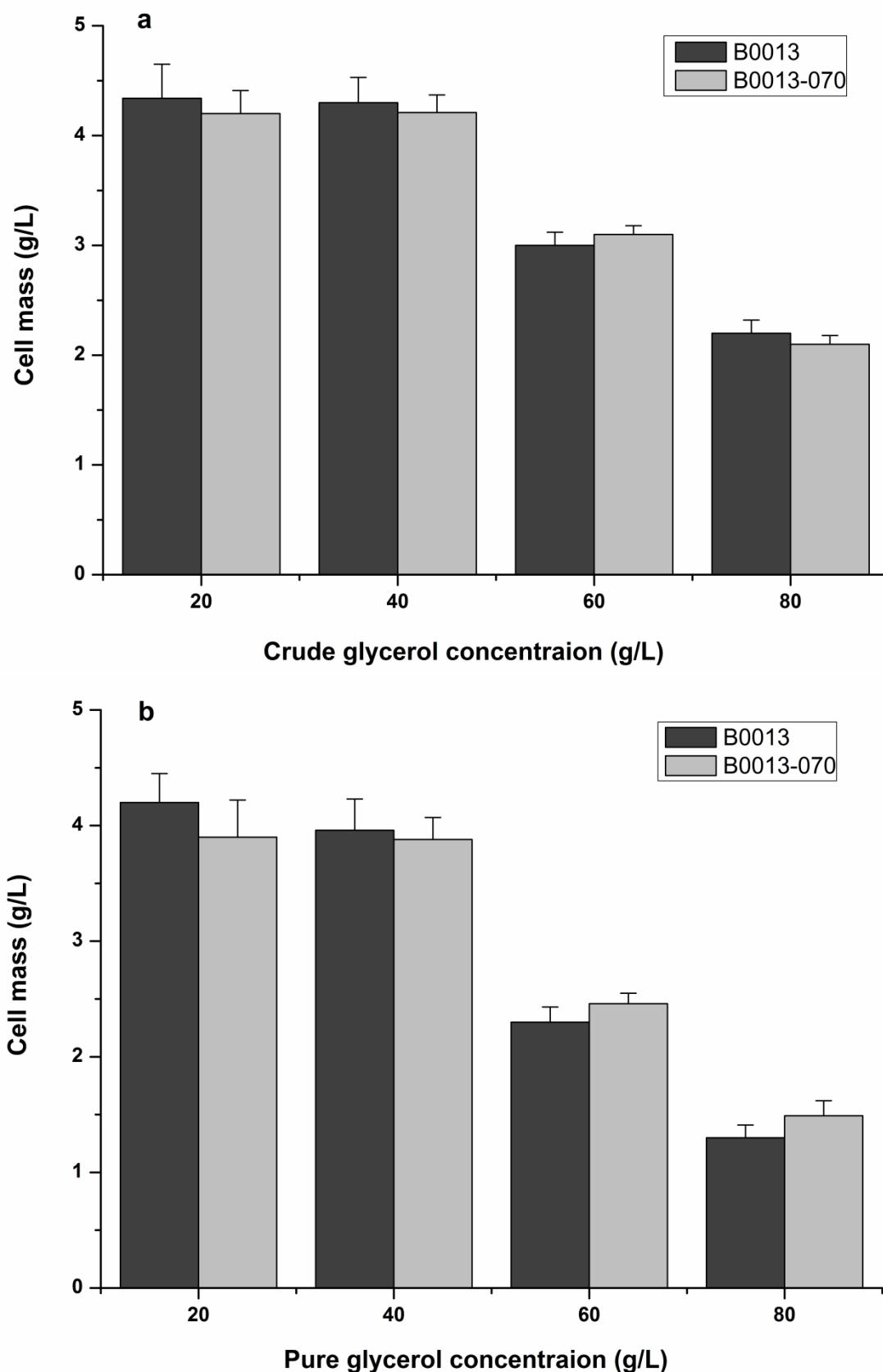
<sup>b</sup> Not detected

**Figure S1** Effect of concentration of crude glycerol on the growth of the wild type and engineered *E. coli* strains. Pre-cultured cells were inoculated into defined media containing the indicated concentration of crude glycerol (a) (Concentration of crude glycerol indicates the final glycerol content in the medium containing crude glycerol which has 63.5% of glycerol) or pure glycerol (b) and incubated for 24 h under aerobic conditions at 200 rpm. OD values were obtained and cell dry weight was calculated. Error bars represent standard deviations for triplicate measurements.

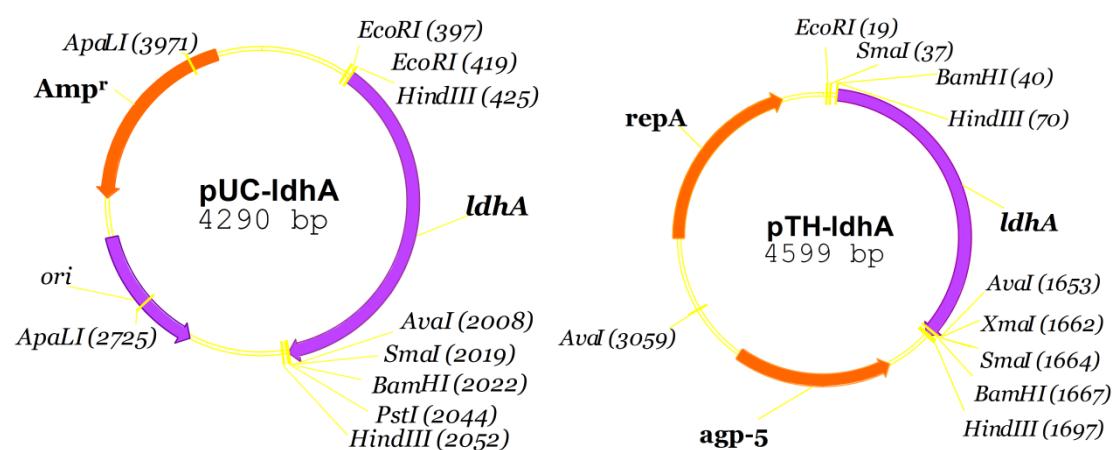
**Figure S2** Maps of constructed polycistronic plasmids pUCldhA and pTHldhA.

**Figure S3** Functional characterization of constructs during overexpression of D-lactate dehydrogenase. All activities were measured as described in the Materials and Methods, and error bars represent standard deviations for triplicate measurements. Reported values are from shake flask cultures grown for 16 h.

**Figure S1**



**Figure S2**



**Figure S3**

