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Improvement of isobutanol production by a genetically modified *Escherichia coli* ΔldhA

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Biofuels synthesized from renewable resources are of increasing interest because of global energy and environmental problems. Compared to ethanol, isobutanol offers many advantages as a substitute for gasoline due to higher energy content and higher hydrophobicity. *Escherichia coli* is a well-characterized microorganism and its physiological regulation is well studied. However, it does not produce isobutanol as a fermentation product. We are engineering a synthetic pathway in *E. coli* to produce isobutanol. Isobutanol is produced from pyruvate through valine biosynthesis. Therefore we want to delete ldhA (lactate dehydrogenase A) that contribute lactate formation. This deletion will increase the level of pyruvate available for the valin biosynthesis. One strategy for knocking out a special gene is homologous recombination. In this way left and right homologous arms at both side of the gene in *E. coli* genome, were amplified by designed primers including restriction sites at their 5' ends. Left arm primers contain *BamHI* and EcoRI recognition sites and Right arm primers have *HindIII* and pstI. The PCR products were cloned separately by TA cloning kit and were confirmed by sequencing. pTZ plasmid containing arms were digested with respective restriction enzymes and the digested arms were cloned in MCS of pUC19 vector one after each other. In next step the kanamycin resistance gene was amplified with the primer which contain XbaI recognition site and it was inserted between left and right arms. When three fragments had been inserted the construct prepared, it was sent to *E. coli* cell and after recombination, kanamycin resistance gene stand instead of gene. Finally this strain could use as a strain which increases isobutanol production in *E. coli*.

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