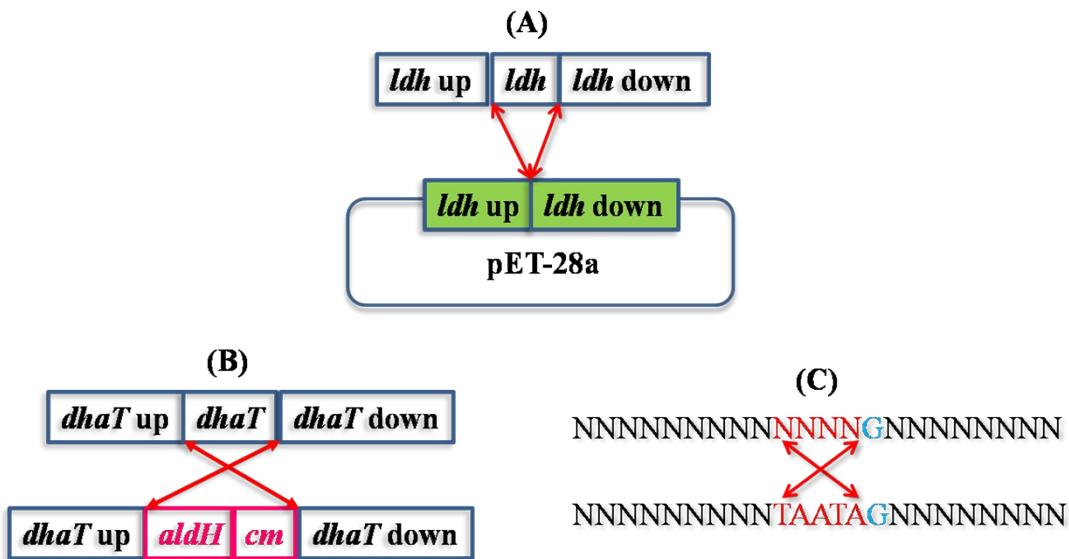
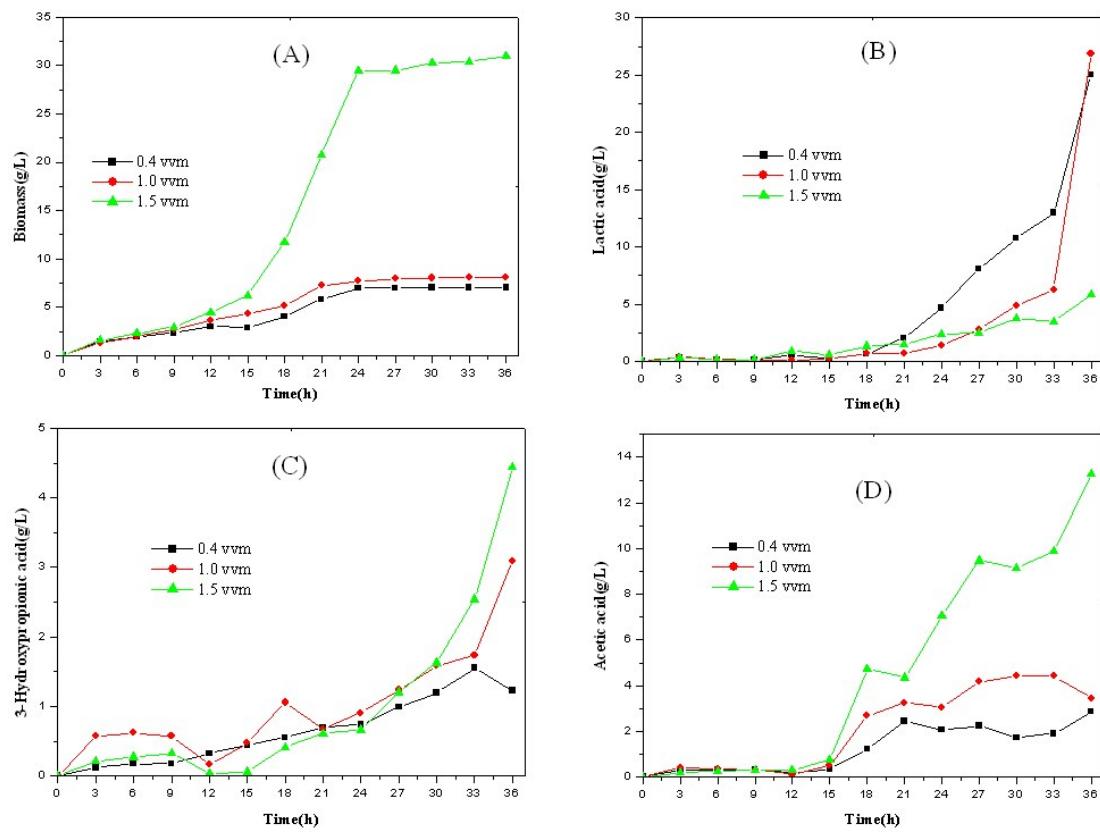


Supplementary Fig. 1 Oligo-mediated homologous recombination to modify the enzymes associated with glycerol metabolism. 3-HPA: 3-hydroxypropionaldehyde; 3-HP: 3-hydroxypropionic acid; 1,3-PDO: 1,3-propanediol; DHAP: dihydroxy acetone phosphate; 2,3-BDO: 2,3-butanediol; PEP: phosphoenolpyruvic acid; AHL: acyl-homoserine lactone; SAH: S-Adenosyl-L-homocysteine; AI-2: autoinducer 2; SRH: S-ribosyl-L-homocysteine.



Supplementary Fig. 2. Diagram of gene knock-out and replacement in *K. pneumoniae*. **(A)** The *ldh* gene was deleted by RecA homologous recombination. “*ldh* up” and “*ldh* down” respectively indicate upstream and downstream homologous arms of 1000 bp each. **(B)** The *K. pneumoniae* *dhaT* gene was replaced by the *aldH* gene from *E. coli* through RecA homologous recombination. “*dhaT* up” and “*dhaT* down” respectively indicate upstream and downstream homologous arms of 25 bp each. “*cm*” indicates chloramphenicol resistance gene. **(C)** Red homologous recombination with oligos carrying wrong nucleotides.



Supplementary Fig. 3 Bioreactor cultivation of the recombinant strain Kp3 under different conditions. Agitations were set at 200 rpm, 300 rpm and 400 rpm with air supply at 0.4, 1.0 or 1.5 vvm.

Supplementary Table 1. Primers used in this study.

Primer	Sequence (5'-3')
<i>BamH</i> -Red-F	CGCGGATCCACGCTTTATCGCAACTCTCT (for PCR amplification of Red recombinases)
<i>Hind</i> -Red-R	CCCAAGCTTCATGCCATTGCTCCCCAAATAC
<i>BamH</i> -aldH-F	CGCGGATCCATGAATTTCATCATCTGGC (for PCR amplification of <i>aldH</i> gene)
<i>Sac</i> -aldH-R	TCCGAGCTCTCAGGCCTCCAGGCTTATCCA
<i>Sac</i> -cm-F	TCCGAGCTCCAATAAACCGGTAAACCAGCA (for amplification of chloromycetin resistance gene)
<i>Hind</i> -cm-R	CCCAAGCTTAGCTGATAGAACAGAAGCC
AT-F	ATAACCTGAAGCGAGAAGGTATATTATGAATTTCATCATC
AT-R	GACATGACGGCCCCCTCGTTAACAGAGCTGATAGAACAGAAG
<i>BamH</i> -llddU-F	CGCGGATCCCAGCAGATTGGCGTCTCCGATATTC (for PCR amplification of the upstream homologous arm of <i>lldd</i> gene)
<i>EcoR</i> -llddU-R	CCGGAATTCCACGAGTTCTCCCTGGAATTTCAT
<i>EcoR</i> -llddD-F	CCGGAATTCTCTCCACGTTCCCTACGCCGC (for PCR amplification of the downstream homologous arm of <i>lldd</i> gene)
<i>Hind</i> -llddD-R	CCCAAGCTTTGGTGAACGGCGGTGATTGAGGA
<i>BamH</i> -anti- <i>ldh</i> -F	CGCGGATCCCTGAAAGATCGCGGCT (for PCR amplification of antisense module of <i>ldh</i> gene)
<i>BamH</i> -anti- <i>ldh</i> -R	CGCGGATCCAGTTGCTGATGAACCCATAT
<i>EcoR</i> -anti- <i>poxB</i> -F	CCGGAATTCACATCCCATCCAGCGAAAT (for PCR amplification of antisense module of <i>poxB</i> gene)
<i>Hind</i> -anti- <i>poxB</i> -R	CCGGAATTCAATAAGCTGCCAGCT
<i>EcoR</i> -and- <i>poxB</i> -R	CCGGAATTCAATAAGCTGCCAGCT
<i>Sac</i> -pk-F	TCCGAGCTCCGTTATTTGTCGCCCGCCAT
<i>Hind</i> -aldH-R	CCCAAGCTTCAGGCCTCCAGGCTTATCCAGAT

pET-F	AGATCTCGATCCCGGAAATTAAATACGAC (for colony PCR)
T7-ter	CAAAAAACCCCTCAAGACCCGTTAGAGGC
Below are oligos	
M-02059	TTCGGCCTCGCACCTTAATGAGCTGTAATAGAGCTGATGCCCTCAGCAGTCAGGTC
M-01632	ACCATCTCCACCCCGGAGGCCAGCGACTAATAGATGTCGATATCGCGGTGATCACCGTC
M-02143	GATGCCCATGGCGATGCCGTGGCGTAATAGGCCACCAGGATGTACGCCGCCGC
M-04368	CTGACGGCGCTACAGCTGGCGCAAATAATAATTTCGCCCCGGAGCAGGCCATGG
M-00764	TTGAAGTATGCAAAGACGGAGAGAAGCTAATAGCTTTAATTTTATGATTAGCGCG
M-03613	ATGCAGCTAACATTACAGGACACAACATAATTACGCCTGCGATGCGGAATTCGTT
M-01740	GCTGCCACACTGTTACTCTTACACCATAATAGATACCGCGCTCATCTGCCACGGCG
M-04320	GGCGCGGCGCGTCGATTGCCGTTGTAATAGGGCTGCCATTATCGTGTGGTT
M-04011	CAGGGCACTACCAGCTCCGGCGTTAATAGATCATGATGCCAATATCGTCAGCGTC
M-00904	ATTAACGGTTGTTGACTGTCATCGCTAATAGTCCGGTGCTGCCATGCCGCCAC
M-01695	GCACTGGGCCTGGCGCCGCTGGATTAATAGATCAACAACAACGAAGTGGAAAGCCTG
M-02688	AGAGATCATTGCCAACTACCACGCGAATAATAGGACGCGGAAGTGGTGCTGGAGG
M-03018	CTTATGCGCGATCATCTGAACGGGAATAATAGGAAATTATCGACATTCGCCAATGGG
M-02792	AGCCCCTCGCGCTCGGCAGTCGCCTAATAGGCGAACGTCTGCCGTGACCGGGGTG
M-0030	GGCGGCCTGCTCTCCAATATCCGGAGTAATAGGCTGGCGCTACCGCCCTGGAGAGCC
M-03828	TTCAACGAGCAGGTGCGCTATGCCCTGGTAATAGCGCGCTGCAGGATCTAAAGACCG
M-01662	TTCGATGAGCTGCAGGGATGAAGCGCTAATAGCGTTAGCGTGGCATGGCAGGCGC
M-03762	CCGCCAGGCTCCGGCAAATCACCACCTAATAGCAGCGATGTACGTAACAGATGCCA
M-02580	TCATTGACCTTACCCAGGATCTGGAGTAATAGGACCGGCATACGGACGCGCGCTTG
M-03260	ATGATGAAAGAGGGGCGCGGGCGAGCTAATAGCGGGTAACCTCTGTTAGCAAAA
M-00395	CGCTACACGCCGCCGTTCTGGCGTTAATAGCGGCTGGCTGTTGACCACCCAGGTAT
M-02893	ATTGCAACCTGGTGACGGCGTTTATAATAGCCTGGACAAGTTCAATTTGACCGA
M-03830	CGTAGCCTGCTGAAACGAAGCAAATTAAATAGTCCGGATTATCAGCCTGTTCCGCG
M-04434	GCGCATGCTGAAAAGCTTGCACAGTAATAGCGTACGCCTGACTCCACAGCGTCTGG
M-04462	CGGCCGATCTTGATATGCCATGGATTAATAGCTACGTCTCGCAGCAAACCTCTCCC
M-04463	TATGAGAGTAAAGGATTGATCCACAGTTAATAGGAATGCCGGCAATCAGCGCGCTACC

M-02788	CCGCTGTGGCTGGGCATCGATTGGGCTAATAGCGACGTGGTGTGATGGTGGTTGACC
M-04285	GTGAACGTTGAGAACGAGCTGGATATTAAATAGGCTTTCGCGCTTATGGCAGGAA
M-04527	AAGGTGGCAGCCTGCGCTACGCTGCTGTAATAGAGCCACCTCTCTGTATTACTGGGAAG
M-03602	ATGAATGAAGCGCGCAGAAAACCTCTAATAGCCTGAAGAACAGCCGAAGATTA
M-00254	TTTGCCGGGCTGCTGGTGGTATGATTAAATAGTATGCTGGCCGAAATGGCGGTGCCA
M-00986	AAACTGGGTTGGTCCCAGTATCACGACTAATAGTTCTACGGTAACGGTTCCAGAACCA
M-00051	GGCAACTACCCGGACAACGCCGTCTTTAATAGCAACGTCGATATTAACCAGGGAAATA

The underlined sequences indicate restriction sites. F, forward; R, reverse.