Supplementary Information

Rapid Screening of Point Mutations by Mismatch Amplification Mutation Assay PCR

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Supplementary Methods

Construction of plasmids and plasmid library.

For the construction of plasmid pFST-proB-proB^{G149K}, the fragment obtained by annealing primer sgRNA-proB-F/R was digested by *Eco*R I/*Hin*d III and cloned into pFST to obtain plasmid pFST-proB. The primers proB^{G149K}-F1/R1 and proB^{G149K}-F2/R2 were amplified by *C. glutamicum* ATCC 13032 genome to obtain fragments proB^{G149K}-1 and proB^{G149K}-2, respectively. The primer pFST-vector-F/R was amplified with pFST-proB as the template to obtain the fragment pFST-proB-vector; Finally, the three fragments were ligated by the ClonExpress MultiS One Step Cloning Kit to obtain plasmid pFST-proB-proB^{G149K}.

For the construction of plasmid pFST-gRNA1, the fragment obtained by annealing primer sgRNA-zwf-1-F/ sgRNA-proB-R was digested by *Eco*R I/*Hin*d III and cloned into pFST to obtain plasmid pFST-gRNA1.

For the construction of plasmid pFST-gRNA2, the fragment obtained by annealing primer sgRNA-zwf-2-F/ sgRNA-proB-R was digested by *Eco*R I/*Hin*d III and cloned into pFST to obtain plasmid pFST-gRNA2.

For the construction of plasmid pFST-gRNA4, the fragment obtained by annealing primer sgRNA-gnd-F/ sgRNA-proB-R was digested by *Eco*R I/*Hin*d III and cloned into pFST to obtain plasmid pFST-gRNA4.

For the construction of plasmid library pJYS3_0, the primer pJYS3_0-F/R was used to whole plasmid PCR using plasmid pJYS3_crtYf as template, and the PCR product was digested by *Dpn* I, and then purified. Then, the purified fragment was ligated by the ClonExpress II One Step Cloning Kit to obtain plasmid pJYS3_0.

For the construction of plasmid library pJYS3_gRNA3, the primer crRNA-zwf-F/R was used to whole plasmid PCR using plasmid pJYS3_crtYf as template, and the PCR product was digested by *Dpn* I, and then purified. Then, the purified fragment was ligated by the ClonExpress II One Step Cloning Kit to obtain plasmid pJYS3_gRNA3.

For the construction of plasmid library pJYS3_gRNA5, the primer crRNA-gnd-F/R was used to whole plasmid PCR using plasmid pJYS3_crtYf as template, and the PCR product was digested by *Dpn* I, and then purified. Then, the purified fragment was ligated by the ClonExpress II One Step Cloning Kit to obtain plasmid pJYS3_gRNA5.

For the construction of plasmid library pJYS3_odhARBS-LIB, the primer crRNA-odhARBS-F/R was used to whole plasmid PCR using plasmid pJYS3_crtYf as template, and the PCR product was digested by *Dpn* I, and then purified. Then, the purified fragment was ligated by the ClonExpress II One Step Cloning Kit to obtain plasmid pJYS3_odhA$_{RBS}$. The primer odhA$_{RBS}$-F/R was amplified by P12 genome to obtain the fragment odhA$_{RBS}$; The primer pJYS3-vector-F/R was amplified by using pJYS3-odhA$_{RBS}$ as a template to obtain a fragment pJYS3-odhA$_{RBS}$-vector; Subsequently, the two fragments were ligated by the ClonExpress II One Step Cloning Kit to obtain plasmid pJYS3_odhA$_{RBS}$-HD$_{odhA}$. Then, the whole plasmid PCR was carried out by primer odhA$_{RBS}$-LIB-F/R with plasmid pJYS3_odhA$_{RBS}$-HD$_{odhA}$ as template, and the PCR product was digested by *Dpn* I, and then purified. Finally, the purified fragment was ligated by the ClonExpress II One Step Cloning Kit and transformed into *E. coli* JM109 competent, and the plasmid library pJYS3_odhA$_{RBS}$-LIB was obtained.

For the construction of plasmid library pK18-Zwf^{A243T}, the upstream and downstream gene fragments of *zwf* were amplified from genomic DNA by primers Zwf^{A243T}-up-F/R and Zwf^{A243T}-down-F/R respectively, and then the two fragments were fused by overlapping extension PCR to obtain DNA

fragment Zwf^{A243T}-UD. Subsequently, Zwf^{A243T}-UD was inserted into *Hin*d III/*Xba* I-digested pK18*mobsacB* to obtain pK18-Zwf^{A243T}.

For the construction of plasmid library pK18-Gnd^{S361F}, the upstream and downstream gene fragments of *gnd* were amplified from genomic DNA by primers Gnd^{S361F}-up-F/R and Gnd^{S361F}-down-F/R respectively, and then the two fragments were fused by overlapping extension PCR to obtain DNA fragment Gnd^{S361F} -UD. Subsequently, Zwf^{A243T}-UD was inserted into *Xba* I/*Bam*H I-digested pK18*mobsacB* to obtain pK18-Zwf^{A243T}. DNA sequencing was used to verify the plasmids.

Supplementary Figures

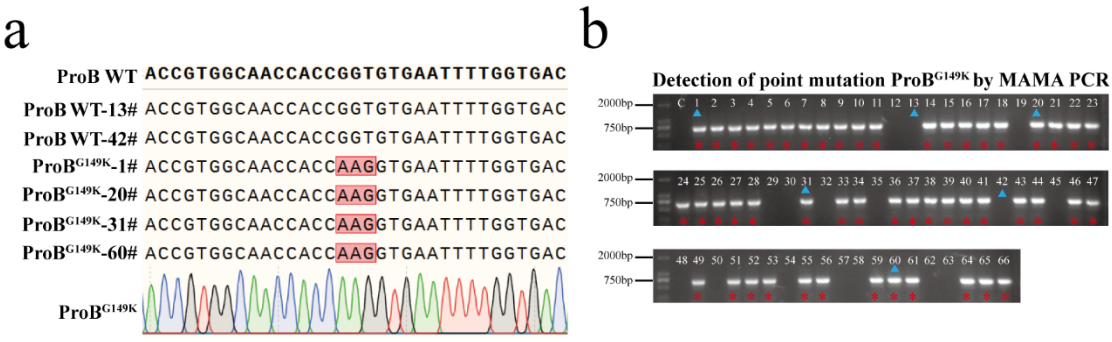
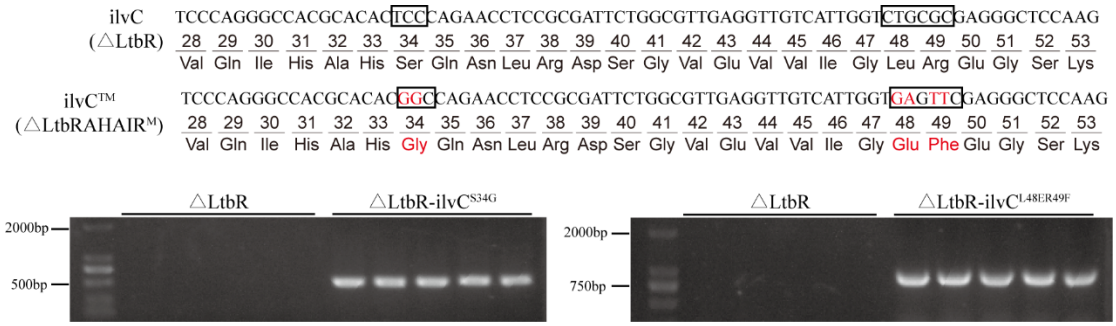


Figure S1. **Detection of point mutation ProB^{G149K} by MAMA PCR.** (**a**) Sequencing results of *proB* point mutation transformants edited by CRISPR-Cas9 system. Six transformants were randomly selected from 66 transformants for sequencing. GGT encodes glycine and AAG encodes lysine. (**b**)The mutation of ProB^{G149K} in 66 transformants was detected by MAMA PCR. Primers 15-AGG-F-1 (MAMA primer) and 17-AGG-R were used to detect ProB^{G149K} point mutation. The annealing temperature of primers is 70℃, and other PCR parameters are as described in Materials and Methods. * represent that the mutation was identified in the recombinant by MAMA PCR; ▲ represents the transformant selected for sequencing.



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80 **Figure S2**. **Detection of point mutation *ilvC*™ by MAMA PCR.** △LtbR and △LtbRAHAIR^{M} are

81 leucine-producing bacteria, which are preserved in the laboratory(Wang et al. 2019). The extracted △

82 LtbR and △LtbRAHAIRM genomes were used as templates for PCR amplification. Primers ilvC^{S34G}-

83 V-F (MAMA primer) and ilvC^{S34G}-V-R were used to detect ilvC^{S34G} point mutation. The annealing

84 temperature of primers is 66℃, and other PCR parameters are as described in Materials and Methods.

85 Primers ilvC^{L48ER49F}-V-F (MAMA primer) and ilvC^{L48ER49F}-V-R were used to detect ilvC^{L48ER49F} point

86 mutation. The annealing temperature of primers is 60.9℃, and other PCR parameters are as described

87 in Materials and Methods.

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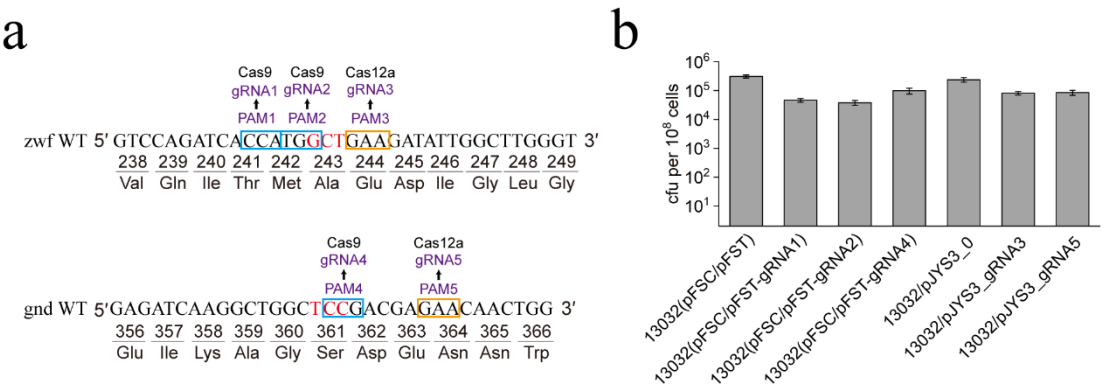
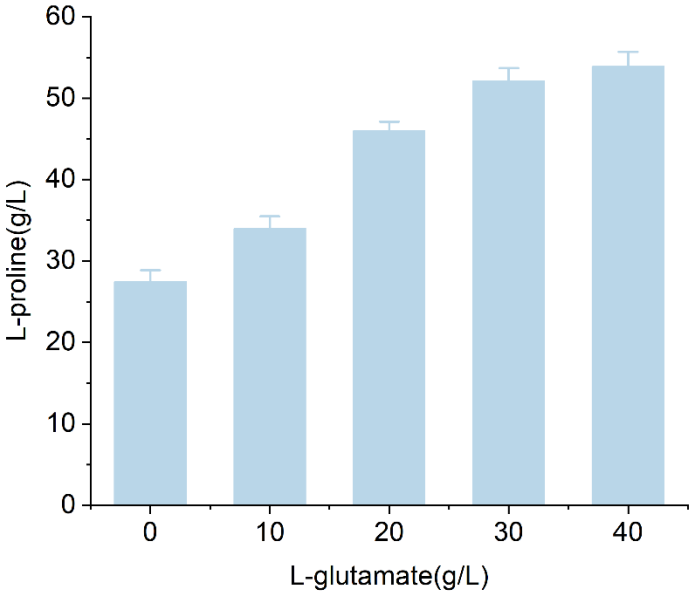


Figure S3. Activity determination of gRNA (guide RNA) at the mutation site of *zwf* and *gnd*. (a) PAM distribution of CRISPR-Cas9/Cas12a system near mutation *zwf* and *gnd*. The marked base in red corresponds to the amino acid residue to be mutated; gRNA1, gRNA 2 and gRNA 4 are sgRNA of Cas9 targeting PAM1, PAM2 and PAM4; gRNA 3 and gRNA 5 are crRNA of Cas12a targeting PAM 3 and PAM 5. **(b)** The activity of gRNA was detected in *Corynebacterium glutamicum* ATCC 13032 based on CRISPR-Cas9 and CRISPR-Cas12a. There are few transformants corresponding to gRNA, which indicates that gRNA activity is high, on the contrary, it indicates low activity. Error bars indicate standard deviations from three parallel experiments.



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104 **Figure S4**. **Effect of L-glutamate addition on L-proline fermentation in P12 strain.** Error bars

105 indicate standard deviations from three parallel experiments.

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Supplementary Tables

Table S1. Sequencing results of *proB* site-directed mutation transformants edited by CRISPR-Cas9 system

Strain	Sequence(5'→3')[a]	Strain	Sequence(5'→3')[a]
13032	GCAACCACC**GGT**GTGAATTTT	**34#**	GCAACCACC**AAG**GTGAATTTT
1#	GCAACCACC**AAG**GTGAATTTT	**35#**	GCAACCACC**GGT**GTGAATTTT
2#	GCAACCACC**AAG**GTGAATTTT	**36#**	GCAACCACC**AAG**GTGAATTTT
3#	GCAACCACC**AAG**GTGAATTTT	**37#**	GCAACCACC**AAG**GTGAATTTT
4#	GCAACCACC**AAG**GTGAATTTT	**38#**	GCAACCACC**AAG**GTGAATTTT
5#	GCAACCACC**AAG**GTGAATTTT	**39#**	GCAACCACC**AAG**GTGAATTTT
6#	GCAACCACC**AAG**GTGAATTTT	**40#**	GCAACCACC**AAG**GTGAATTTT
7#	GCAACCACC**AAG**GTGAATTTT	**41#**	GCAACCACC**AAG**GTGAATTTT
8#	GCAACCACC**AAG**GTGAATTTT	**42#**	GCAACCACC**GGT**GTGAATTTT
9#	GCAACCACC**AAG**GTGAATTTT	**43#**	GCAACCACC**AAG**GTGAATTTT
10#	GCAACCACC**AAG**GTGAATTTT	**44#**	GCAACCACC**AAG**GTGAATTTT
11#	GCAACCACC**AAG**GTGAATTTT	**45#**	GCAACCACC**GGT**GTGAATTTT
12#	GCAACCACC**GGT**GTGAATTTT	**46#**	GCAACCACC**AAG**GTGAATTTT
13#	GCAACCACC**GGT**GTGAATTTT	**47#**	GCAACCACC**AAG**GTGAATTTT
14#	GCAACCACC**AAG**GTGAATTTT	**48#**	GCAACCACC**GGT**GTGAATTTT
15#	GCAACCACC**AAG**GTGAATTTT	**49#**	GCAACCACC**AAG**GTGAATTTT
16#	GCAACCACC**AAG**GTGAATTTT	**50#**	GCAACCACC**GGT**GTGAATTTT
17#	GCAACCACC**AAG**GTGAATTTT	**51#**	GCAACCACC**AAG**GTGAATTTT
18#	GCAACCACC**AAG**GTGAATTTT	**52#**	GCAACCACC**AAG**GTGAATTTT
19#	GCAACCACC**GGT**GTGAATTTT	**53#**	GCAACCACC**AAG**GTGAATTTT
20#	GCAACCACC**AAG**GTGAATTTT	**54#**	GCAACCACC**GGT**GTGAATTTT
21#	GCAACCACC**AAG**GTGAATTTT	**55#**	GCAACCACC**AAG**GTGAATTTT
22#	GCAACCACC**AAG**GTGAATTTT	**56#**	GCAACCACC**AAG**GTGAATTTT
23#	GCAACCACC**AAG**GTGAATTTT	**57#**	GCAACCACC**GGT**GTGAATTTT
24#	GCAACCACC**AAG**GTGAATTTT	**58#**	GCAACCACC**GGT**GTGAATTTT
25#	GCAACCACC**AAG**GTGAATTTT	**59#**	GCAACCACC**AAG**GTGAATTTT
26#	GCAACCACC**AAG**GTGAATTTT	**60#**	GCAACCACC**AAG**GTGAATTTT
27#	GCAACCACC**AAG**GTGAATTTT	**61#**	GCAACCACC**AAG**GTGAATTTT
28#	GCAACCACC**AAG**GTGAATTTT	**62#**	GCAACCACC**GGT**GTGAATTTT
29#	GCAACCACC**GGT**GTGAATTTT	**63#**	GCAACCACC**GGT**GTGAATTTT
30#	GCAACCACC**GGT**GTGAATTTT	**64#**	GCAACCACC**AAG**GTGAATTTT
31#	GCAACCACC**AAG**GTGAATTTT	**65#**	GCAACCACC**AAG**GTGAATTTT
32#	GCAACCACC**GGT**GTGAATTTT	**66#**	GCAACCACC**AAG**GTGAATTTT
33#	GCAACCACC**AAG**GTGAATTTT		

[a]The bold letters are the bases corresponding to the 149th amino acid of *proB*. Red represents base substitution.

113 **Table S2.** Primers used in this study

Primer	**Sequence (5'→3')** [a]
sgRNA-proB-F	ggaattcaatgacacgtggcaaccacgttttagagctagaaatagcaagttaaaataaggctagtcc
sgRNA-proB-R	cccaagcttaaaaaagcaccgactcggtgccacttttcaagttgataacggactagccttattttaact
pFST-vector-F	tctgatcaagagacaggat
pFST-vector-R	tcttgatcccctgcgccat
$proB^{G149K}$-F1	atggcgcaggggatcaagacctacagccaggaagcaa
$proB^{G149K}$-R1	attcaccttggtggttgccacggtgtcattttcattgacga
$proB^{G149K}$-F2	tggcaaccaccaaggtgaattttggtgacaacgac
$proB^{G149K}$-R2	atcctgtctcttgatcagacccaagatctccacgatc
pJYS3_0-F	agcctttcgcctatccagcagtcttcct
pJYS3_0-R	ctggataggcgaaaggctcagtcgaaag
sgRNA-zwf-1-F	ggaattccaagccaatatcttcagccgttttagagctagaaatagcaagttaaaataaggctagtcc
sgRNA-zwf-2-F	ggaattctgaccacgtccagatcaccagttttagagctagaaatagcaagttaaaataaggctagtcc
sgRNA-gnd-F	ggaattcaacgtcccagttgttctcgtgttttagagctagaaatagcaagttaaaataaggctagtcc
crRNA-zwf-F	ccatggtgatctggacgtggtcaaataaaacgaaaggctc
crRNA-zwf-R	acgtccagatcaccatggctatctacaacagtagaaattc
crRNA-gnd-F	tcggagccagccttgatctcgaaataaaacgaaaggctc
crRNA-gnd-R	agatcaaggctggctccgacgaatctacaacagtagaaattc
crRNA-$odhA_{RBS}$-F	cttgcttcttgagggtttattgagaaataaaacgaaaggctc
crRNA-$odhA_{RBS}$-R	ctcaataaaccctcaagaagcaagatctacaacagtagaaattc
pJYS3-vector-F	ctagattgacagctagctcagt
pJYS3-vector-R	ccggtgaacagttgttctact
$odhA_{RBS}$-F	gtagaacaactgttcaccgggtacatgggcctgatgtt
$odhA_{RBS}$-R	gagctagctgtcaatctaggatcgtaggtggaggtgat
$odhA_{RBS}$-LIB-F	cctcaagaagcaaggtcaagNNNNNNgtacctgccgtgagcagc
$odhA_{RBS}$-LIB-R	cttgaccttgcttcttgagggtttattgagc
Zwf^{A243T}-up-F	gctctagacaaacaccgtcaacaccat
Zwf^{A243T}-up-R	catggtgatctggacgtggt
Zwf^{A243T}-down-F	accacgtccagatcaccatgaccgaagatattggcttgggtgg
Zwf^{A243T}-down-R	cccaagcttgtggactcggtaactgcag
Gnd^{S361F}-up-F	cgggatccgcaacactgtcgctgtct
Gnd^{S361F}-up-R	gttctcgtcaaagccagccttgatctcgtc
Gnd^{S361F}-down-F	ggctggctttgacgagaacaactgggacg
Gnd^{S361F}-down-R	gctctagagggatttaccggcgctat
15-AAG-F1	ccgtggcaaccacc**a**
15-AAG-F2	cgtggcaaccacc**aa**
15-AAG-F3	gtggcaaccacc**aag**
16-AAG-F1	accgtggcaaccacc**a**
16-AAG-F2	ccgtggcaaccacc**aa**
16-AAG-F3	cgtggcaaccacc**aag**
15-AAG-R	gttgatgcggtcggcg
17-AAG-R	tagacaggttgatgcgg

ilvC^{S34G}-V-F	aatcgcggaggttctgg**cc**
ilvC^{S34G}-V-R	aacatcttccgcgcccgag
ilvC^{L48ER49F}-V-F	ttggagccctcg**aa**c**tc**
ilvC^{L48ER49F}-V-R	ccacatcctgtccgtact
Zwf^{A243T}-V-F	ccagatcaccatg**a**c**c**
Zwf^{A243T}-V-R	gccaaccaccaaaatgatc
Gnd^{S361F}-V-F	ccagttgttctcgtc**aa**
Gnd^{S361F}-V-R	ggagataatctcgcacaga
odhA$_{RBS}$-V-F	cctcaagaagcaagg**tc**
odhA$_{RBS}$-V-R	ccagaaggaatcatcggt

[a]The bold letters in red represent the mismatched bases on the MAMA PCR primer with the unmutated template.

Table S3. L-proline fermentation of transformants screened by MAMA PCR from the RBS engineering library

Transformants	L-proline (g/L)	Transformants	L-proline (g/L)
P12	8.04±0.46	86#	6.24±1.12
1#	11.26±0.28	87#	10.41±0.30
2#	6.93±0.15	88#	9.36±0.54
4#	8.64±0.60	89#	6.18±0.42
5#	5.92±0.38	90	4.15±0.62
6#	7.71±0.64	91#	10.72±0.27
7#	8.55±0.51	92#	12.93±0.80
8#	7.42±0.43	93#	5.45±0.19
10#	9.93±0.79	94#	7.18±0.43
11#	8.28±0.43	95#	2.37±0.69
13#	6.75±0.21	99#	10.46±0.78
16#	7.29±0.76	101#	1.42±0.19
17#	4.18±0.67	104#	4.20±0.45
19#	5.11±0.29	105#	10.12±0.55
20#	8.14±0.71	108#	11.70±0.48
21#	7.26±0.44	111#	4.15±0.61
22#	10.27±0.66	114#	6.36±0.32
23#	12.22±0.68	117#	6.24±0.28
25#	8.59±0.65	118#	9.43±0.35
27#	9.31±0.56	119#	4.07±0.82
30#	6.09±0.57	120#	8.37±1.13
32#	8.76±0.68	121#	7.20±0.5
36#	5.32±0.35	123#	6.89±0.75
37#	7.71±0.72	125#	8.91±0.55
40#	6.64±0.99	126#	10.86±0.25
42#	8.55±0.52	130#	5.19±0.61
47#	6.66±0.76	131#	9.28±0.52
48#	7.87±0.63	132#	3.59±0.31
49#	8.94±0.79	133#	13.60±0.52
50#	9.26±0.32	137#	5.56±0.12
52#	8.43±0.64	138#	6.72±0.41
54#	12.62±0.71	142#	1.91±0.61
55#	6.91±0.91	146#	10.04±0.71
56#	9.15±0.52	149#	6.64±0.31
58#	8.58±0.65	151#	5.25±0.81
60#	7.22±0.45	152#	7.21±0.51
62#	8.33±0.77	154#	8.90±0.61
63#	8.80±0.58	158#	11.67±0.91

64#	4.12±0.89	161#	9.82±1.16
66#	8.26±0.40	162#	4.17±0.51
67#	5.09±1.01	164#	10.21±0.71
68#	9.47±0.73	169#	6.12±0.82
69#	3.35±0.61	171#	5.54±0.99
70#	6.18±0.25	172#	7.19±0.67
71#	7.79±0.44	174#	9.16±0.72
72#	13.47±0.81	175#	10.27±0.28
73#	6.93±0.72	177#	7.80±1.21
74#	6.77±0.45	179#	11.81±0.41
75#	8.40±0.69	180#	9.62±0.33
76#	9.81±0.48	182#	5.88±0.86
78#	3.04±0.34	183#	10.09±0.63
79#	10.42±0.56	184#	6.32±0.5
80#	9.21±0.65	185#	7.51±0.43
81#	4.13±0.32	187#	9.49±0.66
82#	11.59±0.53	189#	6.60±0.88
84#	9.40±0.36	191#	10.03±0.32

References

Wang YY, Zhang F, Xu JZ, Zhang WG, Chen XL, Liu LM (2019) Improvement of l-Leucine Production in *Corynebacterium glutamicum* by Altering the Redox Flux. Int J Mol Sci 20(8):2020. doi:10.3390/ijms20082020