

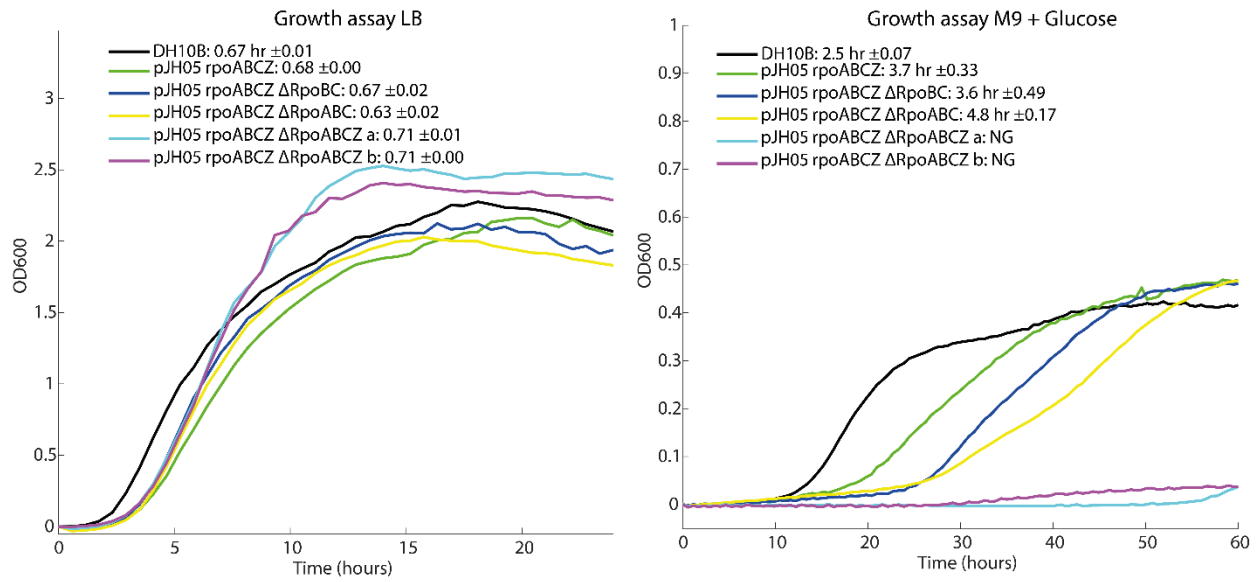
## Supplementary data

### **Design, construction and optimization of a synthetic RNA polymerase operon in *Escherichia coli*.**

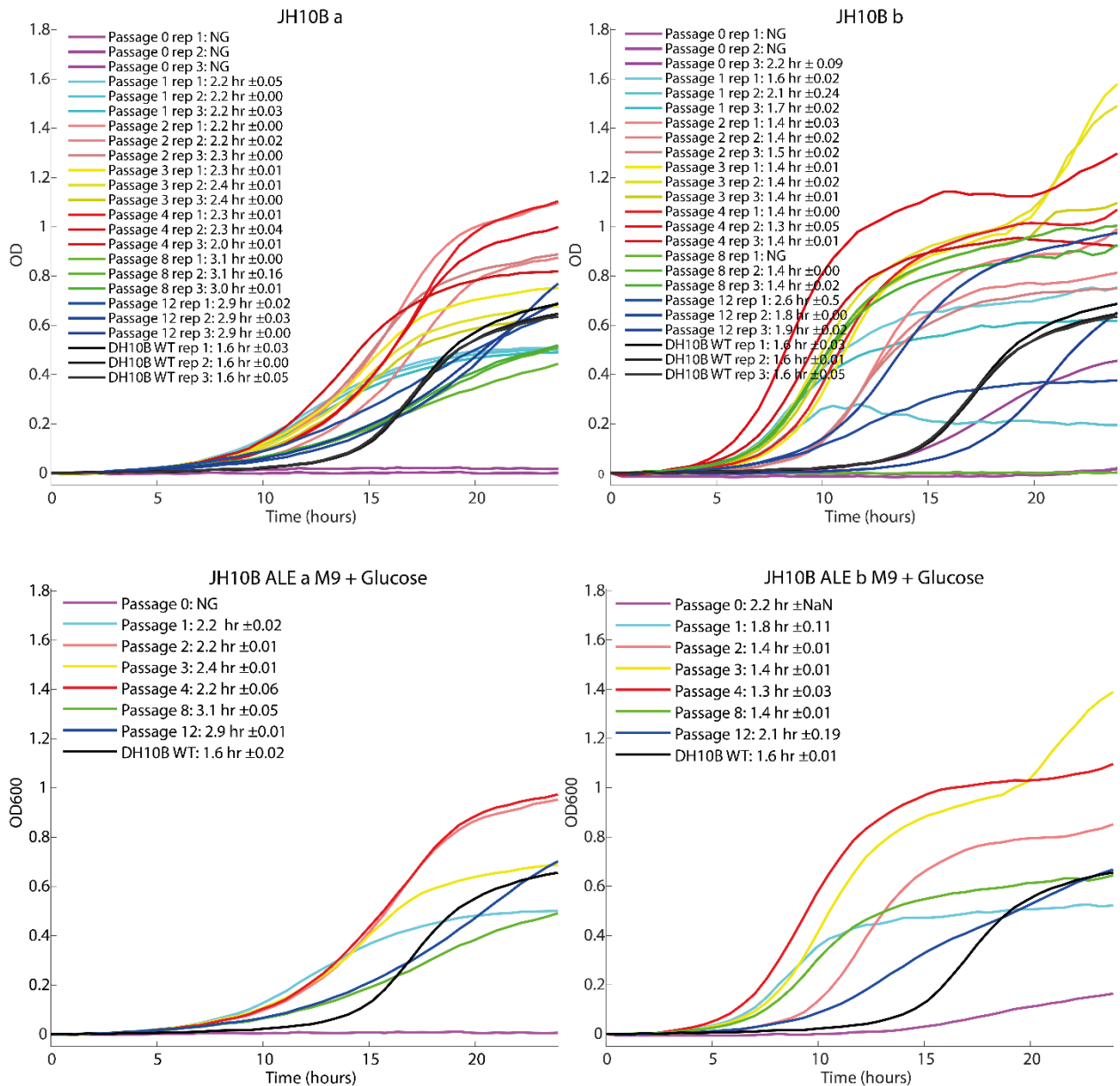
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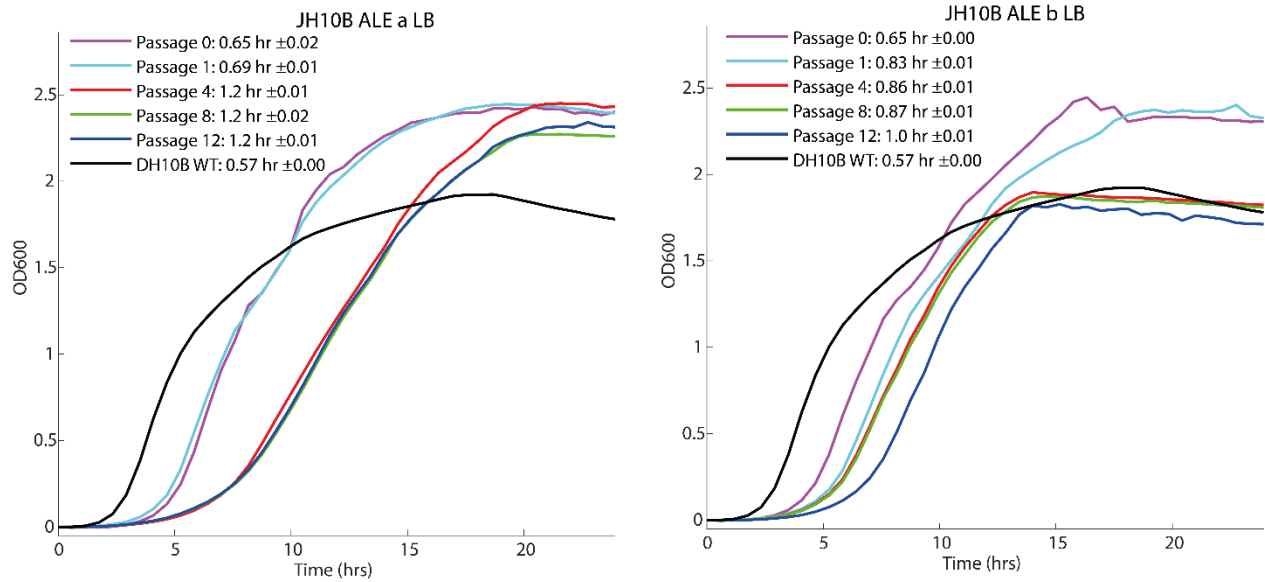
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**Suppl. Fig. 1. Growth assays of *E. coli* strains harboring the RNAP operon on LB (left) and M9 medium with glucose (right).** Representation of each line shown in figure legend, numbers are doubling time, time point of highest doubling time, and max OD reached, respectively. Lines are means of 6 technical replicates.



**Suppl. fig. 2. Growth assays of *E. coli* strains harboring the RNAP operon after ALE on M9+glucose, all replicates.** Top graphs has all three biological replicates per passage consisting out of 2 technical replicates split out. Bottom graphs have biological replicates combined. Each Representation of each line shown in figure legend. Doubling time and standard deviation are indicated.



**Suppl. Fig. 3 Growth assays of *E. coli* strains harboring the RNAP operon after ALE on LB medium.** Representation of each line shown in figure legend. Doubling time and standard deviation of 6 replicates are indicated.

**Suppl. table 1. Combinations of promoters and RBSs tested for *rpoA*.** Combination in red was not obtained, green combinations allowed complementation of the lethal  $\Delta rpoA$  mutation, doubling times measured in the consequent growth assay are included.

	P <sub>Weak</sub>	P <sub>Moderate</sub>		P <sub>Strong</sub>		P <sub>Native</sub>	
RBS <sub>20</sub>	pJL_wA20	pJL_mA20		pJL_sA20		pJL_nA20	
RBS <sub>40</sub>	pJL_wA40	pJL_mA40		pJL_sA40		pJL_nA40	
RBS <sub>60</sub>	pJL_wA60	pJL_mA60		pJL_sA60	0.54	pJL_nA60	
RBS <sub>80</sub>	pJL_wA80	pJL_mA80	0.553	pJL_sA80	0.539	pJL_nA80	
RBS <sub>98</sub>	pJL_wA98	pJL_mA98		pJL_sA98		pJL_nA98	
RBS <sub>N</sub>	pJL_wAN	pJL_mAN	0.549	pJL_sAN	0.544	pJL_nAN	0.543

**Suppl. table 2. List of strains**

Strain	Comment	Source
<i>E. coli</i> DH10B	Used for cloning purposes	Invitrogen
<i>E. coli</i> DH10B pJL_mA80 $\Delta rpoA$	DH10B $\Delta rpoA$ harboring pJL_mA80	This work
<i>E. coli</i> DH10B pJL_mAN $\Delta rpoA$	DH10B $\Delta rpoA$ harboring pJL_mAN	This work
<i>E. coli</i> DH10B pJL_sA60 $\Delta rpoA$	DH10B $\Delta rpoA$ harboring pJL_sA60	This work
<i>E. coli</i> DH10B pJL_sA80 $\Delta rpoA$	DH10B $\Delta rpoA$ harboring pJL_sA80	This work
<i>E. coli</i> DH10B pJL_sAN $\Delta rpoA$	DH10B $\Delta rpoA$ harboring pJL_sAN	This work
<i>E. coli</i> DH10B pJL_nAN $\Delta rpoA$	DH10B $\Delta rpoA$ harboring pJL_nAN	This work
<i>E. coli</i> DH10B pJH05 $\Delta rpoA$	DH10B $\Delta rpoA$ harboring pJH05	This work
<i>E. coli</i> DH10B pJH05 $\Delta rpoA$ $\Delta rpoB/rpoC$	DH10B $\Delta rpoA$ $\Delta rpoB/rpoC$ harboring pJH05	This work
<i>E. coli</i> JH10B	DH10B $\Delta rpoA$ $\Delta rpoB/rpoC$ $\Delta rpoZ$ harboring pJH05	This work
<i>E. coli</i> JH10B 1-12 a	DH10B $\Delta rpoA$ $\Delta rpoB/rpoC$ $\Delta rpoZ$ harboring pJH05. Evolved for 1-12 generations in M9+glucose	This work
<i>E. coli</i> JH10B 1-12 b	DH10B $\Delta rpoA$ $\Delta rpoB/rpoC$ $\Delta rpoZ$ harboring pJH05. Evolved for 1-12 generations in M9+glucose	This work
<i>S. cerevisiae</i> CEN.PK2-1D	MATa/ $\alpha$ ura3-52/ura3-52 trp1-289/trp1-289 leu2-3_112/leu2-3_112 his3 $\Delta$ 1/his3 $\Delta$ 1 MAL2-8C/MAL2-8C SUC2/SUC2	Euroscarf

**Suppl. table 3. List of plasmids**

Plasmid	Description and relevant characteristics	Reference
pHLUM	Harbors CEN/ARS, <i>his3</i> and <i>ura3</i> used in shuttle vectors	(1)
pBeloBAC11	Harbors BAC replication system <i>sopA</i> , <i>sopB</i> , <i>sopC</i> and <i>repE</i>	(2)
pSC020	Harbors $\lambda$ -red system and <i>cre</i>	(3)
pJL_wA20	BeloBAC backbone, $\lambda$ -red system, <i>cre</i> , <i>kan</i> and <i>rpoA</i> under weak promoter and RBS <sub>20</sub>	This work
pJL_wA40	BeloBAC backbone, $\lambda$ -red system, <i>cre</i> , <i>kan</i> and <i>rpoA</i> under weak promoter and RBS <sub>40</sub>	This work
pJL_wA60	BeloBAC backbone, $\lambda$ -red system, <i>cre</i> , <i>kan</i> and <i>rpoA</i> under weak promoter and RBS <sub>60</sub>	This work
pJL_wA98	BeloBAC backbone, $\lambda$ -red system, <i>cre</i> , <i>kan</i> and <i>rpoA</i> under weak promoter and RBS <sub>98</sub>	This work
pJL_wAN	BeloBAC backbone, $\lambda$ -red system, <i>cre</i> , <i>kan</i> and <i>rpoA</i> under weak promoter and RBS <sub>N</sub>	This work
pJL_mA20	BeloBAC backbone, $\lambda$ -red system, <i>cre</i> , <i>kan</i> and <i>rpoA</i> under medium promoter and RBS <sub>20</sub>	This work
pJL_mA40	BeloBAC backbone, $\lambda$ -red system, <i>cre</i> , <i>kan</i> and <i>rpoA</i> under medium promoter and RBS <sub>40</sub>	This work
pJL_mA60	BeloBAC backbone, $\lambda$ -red system, <i>cre</i> , <i>kan</i> and <i>rpoA</i> under medium promoter and RBS <sub>60</sub>	This work
pJL_mA80	BeloBAC backbone, $\lambda$ -red system, <i>cre</i> , <i>kan</i> and <i>rpoA</i> under medium promoter and RBS <sub>80</sub>	This work
pJL_mA98	BeloBAC backbone, $\lambda$ -red system, <i>cre</i> , <i>kan</i> and <i>rpoA</i> under medium promoter and RBS <sub>98</sub>	This work
pJL_mAN	BeloBAC backbone, $\lambda$ -red system, <i>cre</i> , <i>kan</i> and <i>rpoA</i> under medium promoter and RBS <sub>N</sub>	This work
pJL_sA20	BeloBAC backbone, $\lambda$ -red system, <i>cre</i> , <i>kan</i> and <i>rpoA</i> under strong promoter and RBS <sub>20</sub>	This work
pJL_sA40	BeloBAC backbone, $\lambda$ -red system, <i>cre</i> , <i>kan</i> and <i>rpoA</i> under strong promoter and RBS <sub>40</sub>	This work
pJL_sA60	BeloBAC backbone, $\lambda$ -red system, <i>cre</i> , <i>kan</i> and <i>rpoA</i> under strong promoter and RBS <sub>60</sub>	This work
pJL_sA80	BeloBAC backbone, $\lambda$ -red system, <i>cre</i> , <i>kan</i> and <i>rpoA</i> under strong promoter and RBS <sub>80</sub>	This work
pJL_sA98	BeloBAC backbone, $\lambda$ -red system, <i>cre</i> , <i>kan</i> and <i>rpoA</i> under strong promoter and RBS <sub>98</sub>	This work

pJL_sAN	BeloBAC backbone, $\lambda$ -red system, <i>cre</i> , <i>kan</i> and <i>rpoA</i> under strong promoter and RBS <sub>N</sub>	This work
pJL_nA20	BeloBAC backbone, $\lambda$ -red system, <i>cre</i> , <i>kan</i> and <i>rpoA</i> under native promoter and RBS <sub>20</sub>	This work
pJL_nA40	BeloBAC backbone, $\lambda$ -red system, <i>cre</i> , <i>kan</i> and <i>rpoA</i> under native promoter and RBS <sub>40</sub>	This work
pJL_nA60	BeloBAC backbone, $\lambda$ -red system, <i>cre</i> , <i>kan</i> and <i>rpoA</i> under native promoter and RBS <sub>60</sub>	This work
pJL_nA80	BeloBAC backbone, $\lambda$ -red system, <i>cre</i> , <i>kan</i> and <i>rpoA</i> under native promoter and RBS <sub>80</sub>	This work
pJL_nA98	BeloBAC backbone, $\lambda$ -red system, <i>cre</i> , <i>kan</i> and <i>rpoA</i> under native promoter and RBS <sub>98</sub>	This work
pJL_nAN	BeloBAC backbone, $\lambda$ -red system, <i>cre</i> , <i>kan</i> and <i>rpoA</i> under native promoter and RBS <sub>N</sub>	This work
pJH05	BeloBAC backbone, CEN/ARS, <i>his3</i> , <i>ura3</i> , $\lambda$ -red system, <i>cre</i> , <i>kan</i> and RNAP operon with strong promoter, RBS <sub>80</sub> <i>rpoA</i> RBS <sub>N</sub> <i>rpoB</i> RBS <sub>80</sub> <i>rpoC</i> RBS <sub>80</sub> <i>rpoZ</i>	This work



**Suppl. Table 4. List of primers**

Primer name	Primer sequence	Description
RpoA fw	ATGCAGGGTTCTGTGACAGAG	Used for amplifying genes from <i>E. coli</i> genome
RpoA rv	TTACTCGTCAGCGATGCTTG	
RpoB fw	ATGGTTTACTCCTATACCGAG	
RpoB rv	TTACTCGTCTTCCAGTTCG	
rpoC fw	GTGAAAGATTTATTAAGTTTTCTG	
rpoC rv	TTACTCGTTATCAGAACCGCC	
rpoZ fw	ATGGCACGCGTAACTGTTTCAG	
rpoZ rv	TTAACGACGACCTTCAGCAATAG	
rpoA+R20+spacer_fw	GACGTAATCGTCCAACCTTTGAGTAGTGACACAATGCAGGGTTCTGTGACAGAG	Used for amplifying rpoA with respective RBSs
rpoA+R40+spacer_fw	GACGTAATCGTCCAACCTTTGAGACATGACACAATGCAGGGTTCTGTGACAGAG	
rpoA+R60+spacer_fw	GACGTAATCGTCCAACCTTTGCAAGAGGACACAATGCAGGGTTCTGTGACAGAG	
rpoA+R80+spacer_fw	GACGTAATCGTCCAACCTTTGGGAATGACACAATGCAGGGTTCTGTGACAGAG	
rpoA+R98+spacer_fw	GACGTAATCGTCCAACCTTTGAGGAGTGACACAATGCAGGGTTCTGTGACAGAG	
rpoA+Rn+spacer_fw	GACGTAATCGTCCAACCTTTGAGAGAGGACACAATGCAGGGTTCTGTGACAGAG	
Ps+spacer_fw	AGGCCGTGCCGGCACGTTGCAATACTTGACATATCACTGTGATTCACATATAATATGCGGACGTAATCGTCCAACCTTTG	Used for amplifying RpoA with respective promoters
Pm+spacer_fw	AGGCCGTGCCGGCACGTTGCACCTATTGACAATTAAAGGCTAAAATGCTATAATTCACGACGTAATCGTCCAACCTTTG	
Pw+spacer_fw	AGGCCGTGCCGGCACGTTGCTCCCTTTGATATTGCATCCCGGTATATAATATGTCGACGTAATCGTCCAACCTTTG	
Pn+spacer_fw	AGGCCGTGCCGGCACGTTGCGATCGTCGAGCTTTACTCCAAGTAAAGCTTAGTACCAAAGAGACGTAATCGTCCAACCTTTG	
rbs20+rpoB_fw	TGGGCATGCGCCTGGAAAAC TGCCACCGGCAAGCATCGCTGACGAGTAAGATGGCAACCCTATGGTTTACTCCTATACCGAG	Used for amplifying rpoB with respective RBSs
rbs40+rpoB_fw	TGGGCATGCGCCTGGAAAAC TGCCACCGGCAAGCATCGCTGACGAGTAAAGCTGGCAACCCTATGGTTTACTCCTATACCGAG	
rbs60+rpoB_fw	TGGGCATGCGCCTGGAAAAC TGCCACCGGCAAGCATCGCTGACGAGTAAACAAGAGAACCCTATGGTTTACTCCTATACCGAG	
rbs80+rpoB_fw	TGGGCATGCGCCTGGAAAAC TGCCACCGGCAAGCATCGCTGACGAGTAATAGAGAAACCCTATGGTTTACTCCTATACCGAG	
rbs98+rpoB_fw	TGGGCATGCGCCTGGAAAAC TGCCACCGGCAAGCATCGCTGACGAGTAATAGAGGAACCCTATGGTTTACTCCTATACCGAG	
rbsN+rpoB_fw	TGGGCATGCGCCTGGAAAAC TGCCACCGGCAAGCATCGCTGACGAGTAACTGAGGAACCCTATGGTTTACTCCTATACCGAG	
rpoB+overlap_rv	AAAAAAACCCCGCCGAAGCGGGCGCCAGTAGAAGCAGCAACTGTTAATTAATTA CTCGTCTTCCAGTTCG	
rbs20+rpoC_fw	TGAAAGAGATTTCGTTTCGCTGGGTATCAACATCGAACTGGAAGACGAGTAAAGCTGCCAAATCCGTGAAAGATTTATTAAGTTTTCTG	Used for amplifying

rbs40+rpoC_fw	TGAAAGAGATTCGTTGCTGGGTATCAACATCGAACTGGAAGACGAGTAAAGCTGG CAAATCCGTGAAAGATTTATTAAGTTTCTG	rpoC with respective RBSs
rbs60+rpoC_fw	TGAAAGAGATTCGTTGCTGGGTATCAACATCGAACTGGAAGACGAGTAAAGTAGG CAAATCCGTGAAAGATTTATTAAGTTTCTG	
rbs80+rpoC_fw	TGAAAGAGATTCGTTGCTGGGTATCAACATCGAACTGGAAGACGAGTAAAGGAG CAAATCCGTGAAAGATTTATTAAGTTTCTG	
rbs99+rpoC_fw	TGAAAGAGATTCGTTGCTGGGTATCAACATCGAACTGGAAGACGAGTAACGGAGG CAAATCCGTGAAAGATTTATTAAGTTTCTG	
rbsN+rpoC_fw	TGAAAGAGATTCGTTGCTGGGTATCAACATCGAACTGGAAGACGAGTAACGGGAG CAAATCCGTGAAAGATTTATTAAGTTTCTG	
rpoC+overlap_rv	AAAAAAACCCCGCCGAAGCGGGCGCCAGTAGAAGCAGCAACTGTTAATTAATTA CTCGTTATCAGAACCGCC	
rbs20+rpoz_fw	TTACTCGTTATCAGAACCGCCAGACCTGCGTTCAGCAGTTCTGCCAGGCAGCTAC TTTAAGTATGGCACGCGTAACGTTCAG	Used for amplifying rpoZwith respective RBSs
rbs40+rpoZ_fw	TTACTCGTTATCAGAACCGCCAGACCTGCGTTCAGCAGTTCTGCCAGGCAGACAT TTTAAGTATGGCACGCGTAACGTTCAG	
rbs60+rpoZ_fw	TTACTCGTTATCAGAACCGCCAGACCTGCGTTCAGCAGTTCTGCCAGGCACGAGT TTTAAGTATGGCACGCGTAACGTTCAG	
rbs80+rpoZ_fw	TTACTCGTTATCAGAACCGCCAGACCTGCGTTCAGCAGTTCTGCCAGGCAGGCAC TTTAAGTATGGCACGCGTAACGTTCAG	
rbs98+rpoZ_fw	TTACTCGTTATCAGAACCGCCAGACCTGCGTTCAGCAGTTCTGCCAGGCAGGAGC TTTAAGTATGGCACGCGTAACGTTCAG	
rbsN+rpoZ_fw	TTACTCGTTATCAGAACCGCCAGACCTGCGTTCAGCAGTTCTGCCAGGCAGGCTT TTTAAGTATGGCACGCGTAACGTTCAG	
rpoZ+T+HR_rv	ACTCCGTTACAAAGCGAGGCTGGGTATTTCCCGCCTTTCTGTTATCCGCAAAAAA ACCCCGCCGAAGCGGGGCGCCAGTAGAAGCAGCAACTGTTAATTAATTAACGACG ACCTTCAGCAATAG	RpoZ reverse with terminator
pHLUM_OH_fw	CCTTTTACAGCCAGTAGTGCTGCGCCAGTCGAGCGACAGGGCGAAGCCCGATCGC TTGCCTGTAACCTACACGC	Used for amplifying <i>his3</i> and CEN/ARS
pHLUM_OH_rv	GTGTGTAAGCAGAATATATAAGTGCTGTTCCCTGGTGCTTCCCTCGCTACTAATCG GTGTCACTACATAAGAAC	
Bac_fw	TACCGCTGAAAGTTCTGCAAAG	pBelobac11 backbone amplification
Bac_rv	AACGTGCCGGCACGG	
pSC020_OH_fw	TTACCCAGGCCGTGCCGGCACGTTGCGGCCGCGGATAACAGAAAGGCCGG	Amplification of $\lambda$ -red and <i>cre</i>
pSC020_OH_rv	TGAGTTTTTCTAATTGCGTTGCGCTCACTG	
Ura3_OH_fw	CATATTCCATTTTGTAAATTCGTGTCGTTTCTATTATGAATTTCAATTTATCACTCA ACCCTATCTCGGTC	Amplification of <i>ura3</i>
Ura3_OH_rv	ACTCCGTTACAAAGCGAGGCTGGGTATTTCCCGCCTTTCTGTTATCCGCTCAATT CATCATTTTTTTTTATTCTTTTTTTTTG	
rpoA_KO_fw	GATCGTCGAGCTTTACTCCAAGTAAAGCTTAGTACCAAAGAGAGGACACATAGAGT CGGACTTCGCGTTCGC	Amplification of KO template for <i>rpoA</i>
rpoA_KO_rv	GATGGCGCATGACCTTATCCTTCTCAGTAAACCTTAACCTGTGATCCGGGCTAGT TATTGCTCAGCGGTGG	
rpoB_KO_fw	GTCCGCTCAATGGACAGATGGGTCGACTTGTGACGCGAGCTGAGGAACCCCTTAGAGT CGGACTTCGCGTTCGC	Amplification of KO

rpoC_KO_rv	CATAAAAAACCCGCCGAAGCGGGTTTTTACGTTATTTGCGGATTAACGAGCTAGT TATTGCTCAGCGGTGG	template for <i>rpoB</i> and <i>rpoC</i>
rpoZ_KO_fw	GATTTCAGTATCATGCCAGTCATTTCTTACCTGTGGAGCTTTTAAAGTTAGAGT CGGACTTCGCGTTCGC	Amplification of KO
rpoZ_KO_rv	ATCAGTTGATTCAGGCTTTCAAACAGATACAAGGGCGACCCGCTTTGTGAGCTAGT TATTGCTCAGCGGTGG	template for <i>rpoZ</i>

## REFERENCES

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