

Supporting Information for:
A Modular Toolkit for Generating *Pichia pastoris* Secretion Libraries

Ulrike Obst, Timothy K. Lu, Volker Sieber

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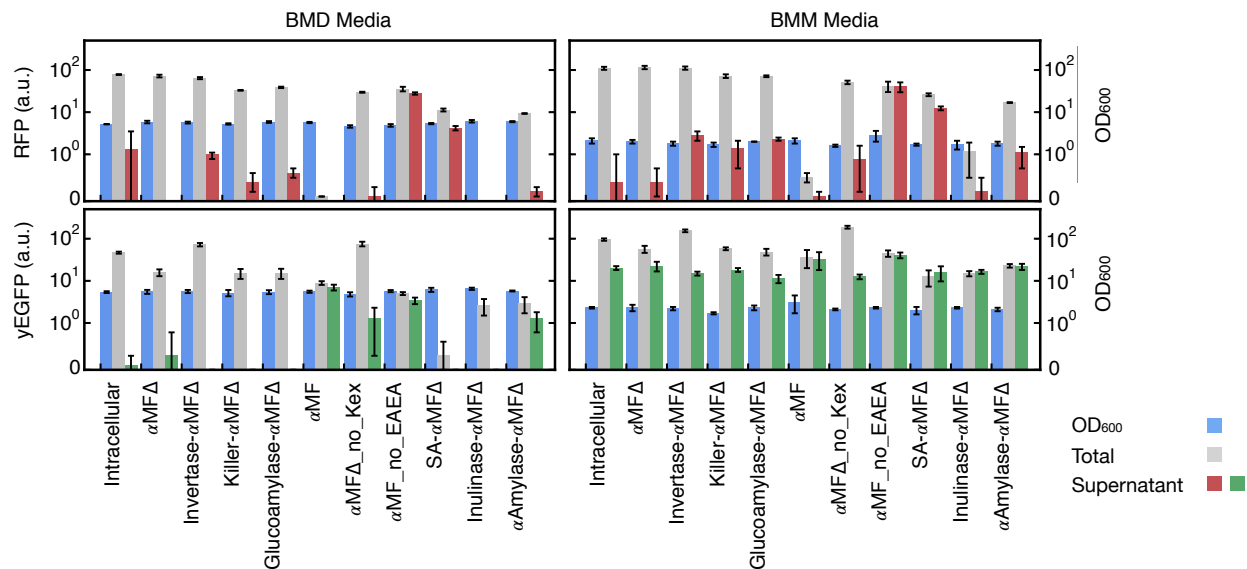


Figure S1: Comparison of pGAP-guided secretion in 1% BMM and 1% BMD media. Total OD₆₀₀ and fluorescence as well as fluorescence of the supernatant after centrifugation was determined from three biological replicates. The mean of these experiments is shown with error bars that denote ± 1 standard deviation.

Table S1: *Pichia* toolkit plasmids.

Plasmid	Part Name	Type	Description	Refs.
pPTK001	pAOX1	2	Alcohol oxidase 1	1
pPTK002	pGAP	2	Glyceraldehyde-3-phosphate dehydrogenase, Bsal site removed	2
pPTK003	pENO1	2	Enolase 1, Bsal and BsmBI site removed	3
pPTK004	pTPI1	2	Triose phosphate isomerase 1	4
pPTK005	α MF	3a	α -mating factor	5,6
pPTK006	α MF_no_EAEA	3a	α -mating factor no EAEA	5,6
pPTK007	α MFD	3a	α -mating factor, pre-sequence shortened	5,6
pPTK008	α MFD_no_Kex	3a	α -mating factor no Kex recognition site	5,6
pPTK009	α Amylase- α MFD	3a	α -Amylase followed by α MFD	7
pPTK010	Glucoamylase- α MFD	3a	Glucoamylase followed by α MFD	8
pPTK011	SA- α MFD	3a	Serum albumin followed by α MFD	9
pPTK012	Inulinase- α MFD	3a	Inulinase followed by α MFD	10
pPTK013	Invertase- α MFD	3a	Invertase followed by α MFD	4,11
pPTK014	Killer- α MFD	3a	Killer followed by α MFD	12
pPTK015	yEGFP_Intra	3	Green fluorescent protein	
pPTK016	RFP_Intra	3	Red fluorescent protein, Bsal removed	
pPTK017	yEGFP_Sec	3b	Green fluorescent protein	
pPTK018	RFP_Sec	3b	Red fluorescent protein, Bsal removed	
pPTK019	tAOX1	4	Alcohol oxidase 1	1
pPTK020	<i>attB</i>	7	BxbI recognition site, Bsal site removed	13
pPTK021	PARS	7	<i>Pichia</i> autonomously replicating sequence	14

Table S2: *Pichia* toolkit part sequences.

Part Name	Type	Sequence
pAOX1	2	GATCTAACATCCAAAGACGAAAGGTTGAATGAAACCTTTTGCCATCCGACATCCACAGGTCCA TTCTCACACATAAGTGCCAAACGCAACAGGAGGGGATACACTAGCAGCAGACCGTTGCCAAACG CAGGACCTCCACTCCTCTTCTCCTCAACACCCACTTTTGCCATCGAAAAACCAGCCCAGTTATTG GGCTTGATTGGAGCTCGCTCATTCCAATTCCTTCTATTAGGCTACTAACACCATGACTTTATTAG CCTGTCTATCCTGGCCCCCTGGCGAGGTTTCATGTTTGTATTTCGAATGCAACAAGCTCCGC ATTACACCCGAACATCACTCCAGATGAGGGCTTTCTGAGTGTGGGGTCAAATAGTTTCATGTTT CCCAATGGCCCAAACTGACAGTTTAAACGCTGTCTTGGAACCTAATATGACAAAAGCGTGAT CTCATCCAAGATGAACTAAGTTTGGTTCGTTGAAATGCTAACGGCCAGTTGGTCAAAAAGAAAC TTCCAAAAGTCGGCATACCGTTTGTCTTGTGGTATTGATTGACGAATGCTCAAAAATAATCTC ATTAATGCTTAGCGCAGTCTCTATCGCTTCTGAACCCCGGTGCACCTGTGCCGAAACGCAAA TGGGGAAACACCCGCTTTTGGATGATTATGCATTGTCTCCACATTGTATGCTTCCAAGATTCTG GTGGGAATACTGCTGATAGCCTAACGTTTCATGATCAAAATTTAACTGTTCTAACCCCTACTTGAC AGCAATATATAAACAGAAGGAAGCTGCCCTGTCTTAAACCTTTTTTTTTATCATCATTATTAGCT TACTTTTCATAATTGCGACTGGTCCAATTGACAAGCTTTTGATTTTAACGACTTTTAACGACAAC TTGAGAAGATCAAAAAACAATAATTATTCGAAACG
pGAP	2	TTTTGTAGAAATGTCTTGGTGTCTCGTCCAATCAGGTAGCCATCTCTGAAATATCTGGCTCCG TTGCAACTCCGAACGACCTGCTGGCAACGTAATAATTCTCCGGGGTAAACTTAAATGTGGAGT AATGGAACCAGAAACGTGTCTTCCCTTCTCTCTTCCACGCCCGTTACCGTCCCTAGGAAAT TTTACTCTGCTGGAGAGCTTCTTACGGCCCCCTTGACGAATGCTTCCAGCATTACGTTG CGGGTAAACGGAGGTCGTGTACCCGACCTAGCAGCCCAGGGATGGAAAAGTCCCGGCCGTC GCTGGCAATAATAGCGGGCGGACGCATGTCATGAGATTATTGAAACCACCAGAATCGAATAT AAAAGGCGAACACCTTTCCCAATTTTGGTTTCTCTGACCCAAAGACTTTAAATTTAATTTATT GTCCCTATTTCAATCAATTGAACAATAT
pENO1	2	AGAAAGCATACTATACTATTGACATTCTTTCAATCCTGGAATTAACAGTCACTTTTAAAAAAG ACATCTACCGTGAAGGTGCCGTAGAGTATCGCGTTACCATATCGCCAAAAACTGATATACGCCG CGGAAACCAGGCAACAATTGAAAAGAAAAATTTGAGGAACTCTCTGCATCGAAGCCGTCTA GAGTTACCACTAGTCAGATGCCGCGGGCACTTGAGCACCTCATGCACAGCAATAACACAACAC AATGGTTAGTAGCAACCTGAATTCGGTCATTGATGCATGCATGTGCCGTGAAGCGGGACAACC AGAAAAGTCGTCTATAAATGCCGGCACGTGCGATCATCGTGGCGGGGTTTTAAGAGTGCATAT CACAAATTGTCGATTACCGCGGAACCGCCAGATATTCACTTACGCAAAAGCGTTTGAAA TAATGACGAAAAAGAAGGAAGAAAAAAGAAAAATACCGCTTCTAGGCGGGTTATCTACT GATCCGAGCTTCCACTAGGATAGCACCCAAACACCTGCATATTTGGACGACCTTTACTTACACC ACCAAAAACCACTTTCGCTCTCCCGCCCTGATAACGTCCACTAATTGAGCGATTACCTGAGCG GTCTCTTTTGTGTTGCAGCATGAGACTTGCATACTGCAAATCGTAAGTAGCAACCTCTCAAGGTC AAAAGTGTATGGAAACCTGTCACTCACTTAATTCTAGCTAGCCTACCCTGCAAGTCAAGAGCT CTCCGTGATTCTAGCCACCTCAAGGTATGCCTCTCCCGGAAACTGTGGCCTTTCTGGCACAC ATGATCTCCACGATTTCAACATATAAATAGCTTTTGATAATGGCAATATTAATCAAAATTTATTTA CTTCTTTCTGTAACATCTCTCTGTAATCCCTTATTCTTCTAGCTATTTTTCATAAAAAACCAAG CAACTGCTTATCAACACACAAACACTAAATCAAA
pTPI1	2	GTGTTTAAAGATTACGGATATTTAACTTACTTAGAATAATGCCATTTTTTTGAGTTATAATAATC CTACGTTAGTGTGAGCGGGATTAAACTGTGAGGACCTTAATACATTCAGACACTTCTGCGGTA TCACCCTACTTATCCCTTCGAGATTATATCTAGGAACCCATCAGGTTGGTGGAAGATTACCCGT TCTAAGACTTTTCAGCTTCTCTATTGATGTTACACCTGGACACCCCTTTCTGGCATCCAGTTTT TAATCTTCAGTGGCATGTGAGATTCTCGAAATTAATAAGCAATCACACAATTCTCTCGGATA CCACCTCGGTTGAAACTGACAGGTGGTTTGTACGCATGCTAATGCAAAGGAGCCTATATACCT

		TTGGCTCGGCTGCTGTAACAGGGAATATAAAGGGCAGCATAATTTAGGAGTTTAGTGAACCTG CAACATTTACTATTTTCCCTTCTACGTAAATATTTTCTTTTAATTCTAAATCAATCTTTTCAAT TTTTTGTTGTATTCTTTCTTGCTTAAATCTATAACTACAAAAACACATACATAAACTAAAA
α MF	3a	ATGAGATTTCTTCAATTTTACTGCTGTTTTATTCGCAGCATCCTCCGATTAGCTGCTCCAGTC AACACTACAACAGAAGATGAAACGGGCACAAATTCGGCTGAAGCTGTCATCGGTTACTCAGAT TTAGAAGGGGATTTTCGATGTTGCTGTTTTGCCATTTTCCAACAGCACAAATAACGGGTTATTGTT TATAAATACTACTATTGCCAGCATTGCTGCTAAAGAAGAAGGGGTATCTCTCGAGAAAAGAGA GGCTGAAGCT
α MF_no_ EAEA	3a	ATGAGATTTCTTCAATTTTACTGCTGTTTTATTCGCAGCATCCTCCGATTAGCTGCTCCAGTC AACACTACAACAGAAGATGAAACGGGCACAAATTCGGCTGAAGCTGTCATCGGTTACTCAGAT TTAGAAGGGGATTTTCGATGTTGCTGTTTTGCCATTTTCCAACAGCACAAATAACGGGTTATTGTT TATAAATACTACTATTGCCAGCATTGCTGCTAAAGAAGAAGGGGTATCTCTCGAGAAAAGAG
α MFD	3a	ATGAGATTTCTAGTATTTTACTGCTGTGCTATTTGCCGCTAGTTCGGCTCTAGCTGCTCCAGTT AATACTACTACTGAAGATGAATTGGAGGGTGACTTCGATGTTGCTGTTCTGCCTTTTCCGCTTC TATCGCAGCCAAGGAAGAAGGTGTATCTCTAGAGAAGCGT
α MFD_no_ Kex	3a	ATGAGATTTCCATCAATTTTACTGCTGTTCTGTTCCGCTTCTAGTGCACTTGCCATGAGATTT CCTAGTATTTTCACTGCTGTGCTATTTGCCGCTAGTTCGGCTCTAGCTGCTCCAGTTAATACTACT ACTGAAGATGAATTGGAGGGTGACTTCGATGTTGCTGTTCTGCCTTTTCCGCTTCTATCACAGC CAAGGAAGAAGGTGTATCTCTAGAGAAGCGT
α Amylase- α MFD	3a	ATGGTGGCATGGTGGTCTTATTCTTATATGGTCTTCAAGTTGCTGCTCCTGCCCTTGCTATGAG ATTTCTAGTATTTTCACTGCTGTGCTATTTGCCGCTAGTTCGGCTCTAGCTGCTCCAGTTAATAC TACTACTGAAGATGAATTGGAGGGTGACTTCGATGTTGCTGTTCTGCCTTTTCCGCTTCTATCG CAGCCAAGGAAGAAGGTGTATCTCTAGAGAAGCGT
Glucoamyla se- α MFD	3a	ATGTCTTTCAGATCCCTATTGGCATTGTCAGGGTTGGTCTGTTCTGGATTGGCTATGAGATTTCC TAGTATTTTCACTGCTGTGCTATTTGCCGCTAGTTCGGCTCTAGCTGCTCCAGTTAATACTACTAC TGAAGATGAATTGGAGGGTGACTTCGATGTTGCTGTTCTGCCTTTTCCGCTTCTATCGCAGCCA AGGAAGAAGGTGTATCTCTAGAGAAGCGT
SA- α MFD	3a	ATGAAGTGGGTAACCTTTCATCTCATTGTTATTCTTGTCTCCTCTGCTTACTCTATGAGATTTCT AGTATTTTCACTGCTGTGCTATTTGCCTCTAGTTCGGCTCTAGCTGCTCCAGTTAATACTACTACT GAAGATGAATTGGAGGGTGACTTCGATGTTGCTGTTCTGCCTTTTCCGCTTCTATCGCAGCCA AGGAAGAAGGTGTATCTCTAGAGAAGCGT
Inulinase- α MFD	3a	ATGAACTGGCTTACTCCCTGTTGCTACCTCTGGCTGGAGTTTCCGCTATGAGATTTCTAGTAT TTTCACTGCTGTGCTATTTGCCGCTAGTTCGGCTCTAGCTGCTCCAGTTAATACTACTACTGAAG ATGAATTGGAGGGTGACTTCGATGTTGCTGTTCTGCCTTTTCCGCTTCTATCGCAGCCAAGGA AGAAGGTGTATCTCTAGAGAAGCGT
Invertase- α MFD	3a	ATGTTATTGCAAGCTTTTTTATTTCTGCTGGCAGGTTTTGCAGCAAAGATTTCTGCCATGAGATT TCCTAGTATTTTCACTGCTGTGCTATTTGCCGCTAGTTCGGCTCTAGCTGCTCCAGTTAATACTAC TACTGAAGATGAATTGGAGGGTGACTTCGATGTTGCTGTTCTGCCTTTTCCGCTTCTATCGCAG CCAAGGAAGAAGGTGTATCTCTAGAGAAGCGT
Killer- α MFD	3a	ATGACCAAACCAACGCAAGTCTAGTTCGTTCACTCTATTTTATTCTTCATCACACTGTTGCAC TTGGTTGTTGCAATGAGATTTCTAGTATTTTCACTGCTGTGCTATTTGCCGCTAGTTCGGCTCTA GCTGCTCCAGTTAATACTACTACTGAAGATGAATTGGAGGGTGACTTCGATGTTGCTGTTCTGC CTTTTCCGCTTCTATCGCAGCCAAGGAAGAAGGTGTATCTCTAGAGAAGCGT
yEGFP	3/3b	GTGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGCCATCCTGGTCGAGCTGGACGGCGA CGTAAACGGCCACAAGTTCAGCGTGTCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGC TGACCCTGAAGTTCATCTGCACCACGGCAAGCTGCCCCTGCCCTGGCCACCCCTCGTGACCAC CCTGACCTACGGCGTGAGTGCTTCAGCCGCTACCCGACCACATGAAGCAGCACGACTTCTTC

		AAGTCCGCCATGCCCCAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAAC TACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAA GGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACCTACAACA GCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCC GCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCG GCGACGGCCCCGTGCTGCTGCCCCGACAACCACTACCTGAGCACCCAGAGCGCCCTGAGCAAAG ACCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTC TCGGCATGGACGAGCTGTACAAG
RFP	3/3b	GCAACTTCCGGTATGGTGTCAAAGGGAGAGGAAAATAATATGGCTATTATTAAGGAGTTTATG CGTTTTAAGGTACATATGGAAGGTTCTGTCAACGGTCACGAATTCGAAATTGAAGGTGAGGGG GAGGGGAGGCCATACGAGGGAACTCAGACTGCTAAGTTAAAGGTCACTAAAGGTGGTCCTTT ACCTTTGCGCTGGGATATCCTGTCTCCACAGTTTATGTACGGTTCAAAGGCTTATGTGAAACATC CTGCCGATATCCAGATTATCTTAACTTTCTTCCCTGAGGGTTTTAAGTGGGAGAGGGTAAT GAACTTTGAAGACGGTGGTGTGGTCACTGTTACTCAGGACTCAAGTCTGCAGGACGGTGAGTT CATCTACAAGGTGAAGCTGAGAGGTACCAATTTTCCATCAGATGGTCCCGTGATGCAAAAAA GACAATGGGTTGGGAAGCTTCTAGTGAACGTATGTATCCCGAAGATGGAGCTTTGAAAGGTGA AATTAAGCAAAGACTAAACTTAAGGATGGTGGACATTACGATGCTGAAGTTAAGACGACCTA CAAGGCCAAAAAGCCAGTCCAGTTGCCTGGAGCATAACAATGTTAACATCAAATTGGATATAACT TCCCATATGAAGACTATACCATCGTCGAGCAATACGAACGAGCCGAAGGGAGACACAGTACT GGTGGTATGGATGAACTTTATAAAGGATCCGGAACCGCA
tAOX1	4	TCAAGAGGATGTCAGAATGCCATTTGCCTGAGAGATGCAGGCTTCATTTTTGATACTTTTTTATT TGTAACCTATATAGTATAGGATTTTTTTTGTCATTTTGTTTCTTCTCGTACGAGCTTGCTCCTGAT CAGCCTATCTCGCAGCTGATGAATATCTTGTGGTAGGGGTTTGGGAAAATCATTGAGTTTGAT GTTTTCTTGGTATTTCCCACTCCTCTTCAGAGTACAGAAGATTAAGTGAGA
<i>attB</i>	7	TGGCCGTGGCCGTGCTCGTCTCGTCGGCCGGCTTGTCGACGACGGCGGTACCGTCGTCAGG ATCATCCGGGCCACAAGCTTGCTGACAGAAGCCTCAAGAAAAAAAAAATTCTTCTCGACTATG CTGGAGGCAGAGATGATCGAGCCGGTAGTTAACTATATATAGCTAAATTGGTTCCATCAC
PARS	7	CCTTCGTTTGTGCGGATCCAATTAATATTTACTTATTTTGGTCAACCCCAATAGGTTGATTTTCA TCTTGGTTCATTCAAAAATAAGTAGTCTTTTGGAGATCTTCAATATTATAATAAATACTATAAC AGCCGACTTGTTTCATTTTCGCGAATGTTCCCCCAGCTTATCG

Table S3: *Saccharomyces* toolkit plasmids.¹⁵

Plasmid	Part Name	Type	<i>E. coli</i> Antibiotic Marker
pYTK001	Entry Vector	Entry vector	Chloramphenicol
pYTK002	ConLS	1	Chloramphenicol
pYTK011	pPGK1	2	Chloramphenicol
pYTK013	pTEF1	2	Chloramphenicol
pYTK054	tPGK1	4	Chloramphenicol
pYTK072	ConRE	5	Chloramphenicol
pYTK080	ZeoR	6	Chloramphenicol
pYTK084	KanR-ColE1	8	Kanamycin

Table S4: Expression plasmids.

Intracellular

ID	Type 1	Type 2	Type 3	Type 4	Type 5	Type 6	Type 7	Type 8
1	ConLS	pAOX1	RFP	tAOX1	ConRE	ZeoR	<i>attB</i>	KanR-ColE1
2		pGAP		tAOX1				
3		pTEF1		tPGK1				
4		pENO1		tAOX1				
5		pPGK1		tPGK1				
6		pTPI1		tAOX1				
7	ConLS	pAOX1	GFP	tAOX1	ConRE	ZeoR	<i>attB</i>	KanR-ColE1
8		pGAP		tAOX1				
9		pTEF1		tPGK1				
10		pENO1		tAOX1				
11		pPGK1		tPGK1				
12		pTPI1		tAOX1				
13	ConLS	pAOX1	RFP	tAOX1	ConRE	ZeoR	PARS	KanR-ColE1
14		pGAP		tAOX1				
15		pTEF1		tPGK1				
16		pENO1		tAOX1				
17		pPGK1		tPGK1				
18		pTPI1		tAOX1				
19	ConLS	pAOX1	GFP	tAOX1	ConRE	ZeoR	PARS	KanR-ColE1
20		pGAP		tAOX1				
21		pTEF1		tPGK1				
22		pENO1		tAOX1				
23		pPGK1		tPGK1				
24		pTPI1		tAOX1				

pAOX1

ID	Type 1	Type 2	Type 3a	Type 3b	Type 4	Type 5	Type 6	Type 7	Type 8
1	ConLS	pAOX1	RFP		tAOX1	ConRE	ZeoR	attB	KanR-ColE1
25			αMFD	RFP					
26			Invertase-αMFD						
27			Killer-αMFD						
28			Glucoamylase-αMFD						
29			αMF						
30			αMFD_no_Kex						
31			αMF_no_EAEA						
32			SA-αMFD						
33			Inulinase-αMFD						
34			αAmylase-αMFD						
7			ConLS	pAOX1					
35	αMFD	GFP							
36	Invertase-αMFD								
37	Killer-αMFD								
38	Glucoamylase-αMFD								
39	αMF								
40	αMFD_no_Kex								
41	αMF_no_EAEA								
42	SA-αMFD								
43	Inulinase-αMFD								
44	αAmylase-αMFD								

pGAP

ID	Type 1	Type 2	Type 3a	Type 3b	Type 4	Type 5	Type 6	Type 7	Type 8
2	ConLS	pGAP	RFP		tAOX1	ConRE	ZeoR	attB	KanR-ColE1
45			αMFD	RFP					
46			Invertase-αMFD						
47			Killer-αMFD						
48			Glucoamylase-αMFD						
49			αMF						
50			αMFD_no_Kex						
51			αMF_no_EAEA						
52			SA-αMFD						
53			Inulinase-αMFD						
54			αAmylase-αMFD						
8	ConLS	pGAP	GFP		tAOX1	ConRE	ZeoR	attB	KanR-ColE1
55			αMFD	GFP					
56			Invertase-αMFD						
57			Killer-αMFD						
58			Glucoamylase-αMFD						
59			αMF						
60			αMFD_no_Kex						
61			αMF_no_EAEA						
62			SA-αMFD						
63			Inulinase-αMFD						
64			αAmylase-αMFD						

pTEF1

ID	Type 1	Type 2	Type 3a	Type 3b	Type 4	Type 5	Type 6	Type 7	Type 8
3	ConLS	pTEF1	RFP		tPGK1	ConRE	ZeoR	attB	KanR-ColE1
65			αMFD	RFP					
66			Invertase-αMFD						
67			Killer-αMFD						
68			Glucoamylase-αMFD						
69			αMF						
70			αMFD_no_Kex						
71			αMF_no_EAEA						
72			SA-αMFD						
73			Inulinase-αMFD						
74			αAmylase-αMFD						
9	ConLS	pTEF1	GFP		tPGK1	ConRE	ZeoR	attB	KanR-ColE1
75			αMFD	GFP					
76			Invertase-αMFD						
77			Killer-αMFD						
78			Glucoamylase-αMFD						
79			αMF						
80			αMFD_no_Kex						
81			αMF_no_EAEA						
82			SA-αMFD						
83			Inulinase-αMFD						
84			αAmylase-αMFD						

pENO1

ID	Type 1	Type 2	Type 3a	Type 3b	Type 4	Type 5	Type 6	Type 7	Type 8
4	ConLS	pENO1	RFP		tAOX1	ConRE	ZeoR	attB	KanR-ColE1
85			α MFD	RFP					
86			Invertase- α MFD						
87			Killer- α MFD						
88			Glucoamylase- α MFD						
89			α MF						
90			α MFD_no_Kex						
91			α MF_no_EAEA						
92			SA- α MFD						
93			Inulinase- α MFD						
94			α Amylase- α MFD						
10	ConLS	pENO1	GFP		tAOX1	ConRE	ZeoR	attB	KanR-ColE1
95			α MFD	GFP					
96			Invertase- α MFD						
97			Killer- α MFD						
98			Glucoamylase- α MFD						
99			α MF						
100			α MFD_no_Kex						
101			α MF_no_EAEA						
102			SA- α MFD						
103			Inulinase- α MFD						
104			α Amylase- α MFD						

pPGK1

ID	Type 1	Type 2	Type 3a	Type 3b	Type 4	Type 5	Type 6	Type 7	Type 8
5	ConLS	pPGK1	RFP		tPGK1	ConRE	ZeoR	attB	KanR-ColE1
105			α MFD	RFP					
106			Invertase- α MFD						
107			Killer- α MFD						
108			Glucoamylase- α MFD						
109			α MF						
110			α MFD_no_Kex						
111			α MF_no_EAEA						
112			SA- α MFD						
113			Inulinase- α MFD						
114			α Amylase- α MFD						
11	ConLS	pPGK1	GFP		tPGK1	ConRE	ZeoR	attB	KanR-ColE1
115			α MFD	GFP					
116			Invertase- α MFD						
117			Killer- α MFD						
118			Glucoamylase- α MFD						
119			α MF						
120			α MFD_no_Kex						
121			α MF_no_EAEA						
122			SA- α MFD						
123			Inulinase- α MFD						
124			α Amylase- α MFD						

Table S5: Oligonucleotides for part plasmid generation.

Name	Sequence
FW-2-pp-pAOX1	GCATCGTCTCATCGGTCTCAAACGGATCTAACATCCAAAGACGAAAGGTTGAATGA
REV-2-pp-pAOX1	ATGCCGTCTCAGGTCTCACATACGTTTCGAATAATTAGTTGTTTTTGGATCTTCTCAAGT
FW-3-pp-GFP	GCATCGTCTCATCGGTCTCATATGGTGAGCAAGGGCGAG
FW-3b-pp-GFP	GCATCGTCTCATCGGTCTCATTCTGTGAGCAAGGGCGAGGAG
REV-3/3b-pp-GFP	ATGCCGTCTCAGGTCTCAGGATCCCTTGTACAGCTCGTCCATGC
FW-3-pp-RFP	GCATCGTCTCATCGGTCTCATATGG
FW-3b-pp-RFP	GCATCGTCTCATCGGTCTCATTCTGCAACTTCCGGTATGGTGTCAAAG
REV-3/3b-pp-RFP	ATGCCGTCTCAGGTCTCAGGATCCTGCGGTTCCGG
FW-4-pp-tAOX1	GCATCGTCTCATCGGTCTCAATCCTAATCAAGAGGATGTCAGAATGCC
REV-4-pp-tAOX1	ATGCCGTCTCAGGTCTCACAGCTCTCACTTAATCTTCTGTACTCTGAAGAGGAGTGGG
FW-3a-pp- α Kex	GCATCGTCTCATCGGTCTCATATGAGATTTCTAGTATTTTCACTGCTGTG
FW-3a-pp-Invertase	GCATCGTCTCATCGGTCTCATATGTTATTGCAAGCTTTTTTATTTCTGCTGG
FW-3a-pp-Killer	GCATCGTCTCATCGGTCTCATATGACCAAACCAACGCAAGTCTTAG
FW-3a-pp-Glucoamylase	GCATCGTCTCATCGGTCTCATATGTCTTTCAGATCCCTATTGGCATTG
FW-3a-pp- α MF_no_Kex	GCATCGTCTCATCGGTCTCATATGAGATTTCCCATCAATTTTACTGCTGTTC
FW-3a-pp-SA	GCATCGTCTCATCGGTCTCATATGAAGTGGGTAACTTTCATCTCATTGTTATTC
FW-3a-pp-Inulinase	GCATCGTCTCATCGGTCTCATATGAAACTGGCTTACTCCCTGTTGC
FW-3a-pp- α Amylase	GCATCGTCTCATCGGTCTCATATGGTGGCATGGTGGTCC
REV-3a-pp-Kex α MF	ATGCCGTCTCAGGTCTCAAGAACCACGCTTCTCTAGAGATACACCTTCTTCC

Table S6: Primers for testing genome integration and plasmid verification.

Name	Sequence
FW-BxbILocus ^a	TGGTTTCTCCTGACCCAAAGACTTTAAATTT
REV-BxbILocus ^a	GAACCAATTTAGCTATATATAGTTAACTACCGGCTCG
FW-ConE ^b	GAACCAGCGCCGGCGAAC
FW-ZeoTerm ^b	GCGAAGTTAAGTGCGCAGAAAG
FW-ColE1 ^b	GCGGAGCCTATGGAAAAACGC
REV-ConS ^b	CAGATGGTCCTGGAGATCGTTG
REV-ConE ^b	CATCGGTATGATCTGTACATGATTG
REV-Kana ^b	CTCACCGGATTGAGTCGTCCTC
REV-Mid_RFP ^c	CACCTTCAATTTGGAATTCGTGACCG
REV-Mid_GFP ^c	CAGCTTGCCGTAGGTGGCATC

- a. Used for PCR verification of transformation based on genomic integration at the *attP* locus (FW-BxbILocus+REVBxbILocus).
- b. Used for verification of the plasmid and verification of PARS-based transformation (FW-ColE1+REV-ConE, FW-ZeoTerm+REV-ConS, FW-ConE+REV-Kana).
- c. Used for sequencing (REV-Mid_RFP or REV-Mid_GFP)

Supporting References

- (1) Tschopp, J., Sverlow, G., Kosson, R., Craig, W., and Grinna, L. (1987) High-level secretion of glycosylated invertase in the methylotrophic yeast, *Pichia pastoris*. *Nat. Biotechnol.* **5**, 1305–1308.
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