

SUPPLEMENTARY MATERIAL

Multiplexed *in vivo* His-tagging of enzyme pathways for *in vitro* single-pot multi-enzyme catalysis

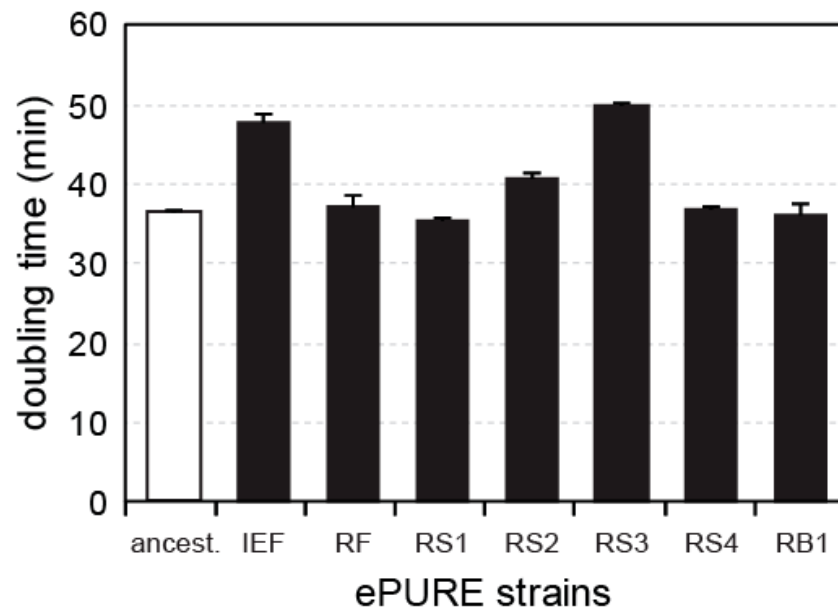
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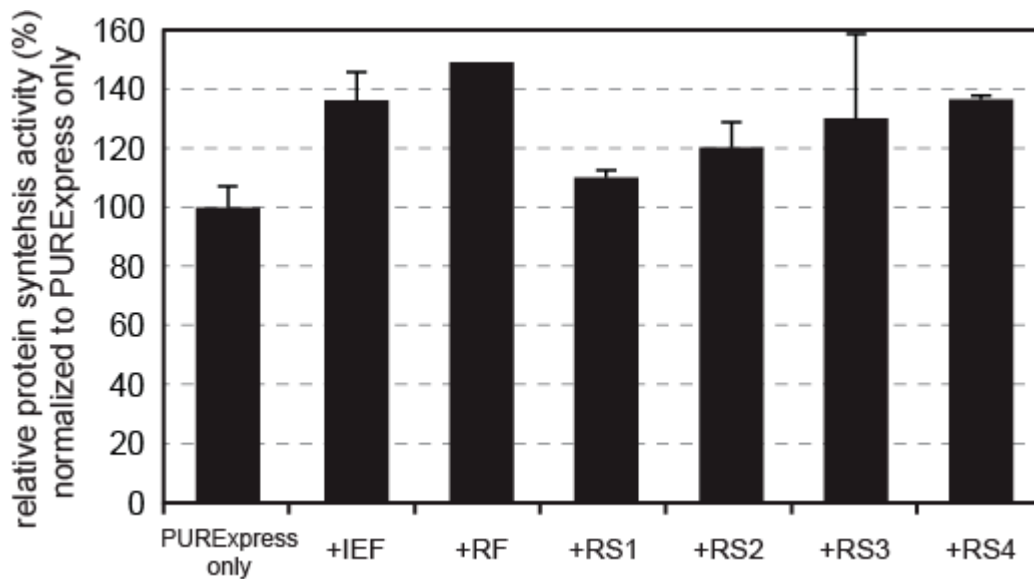
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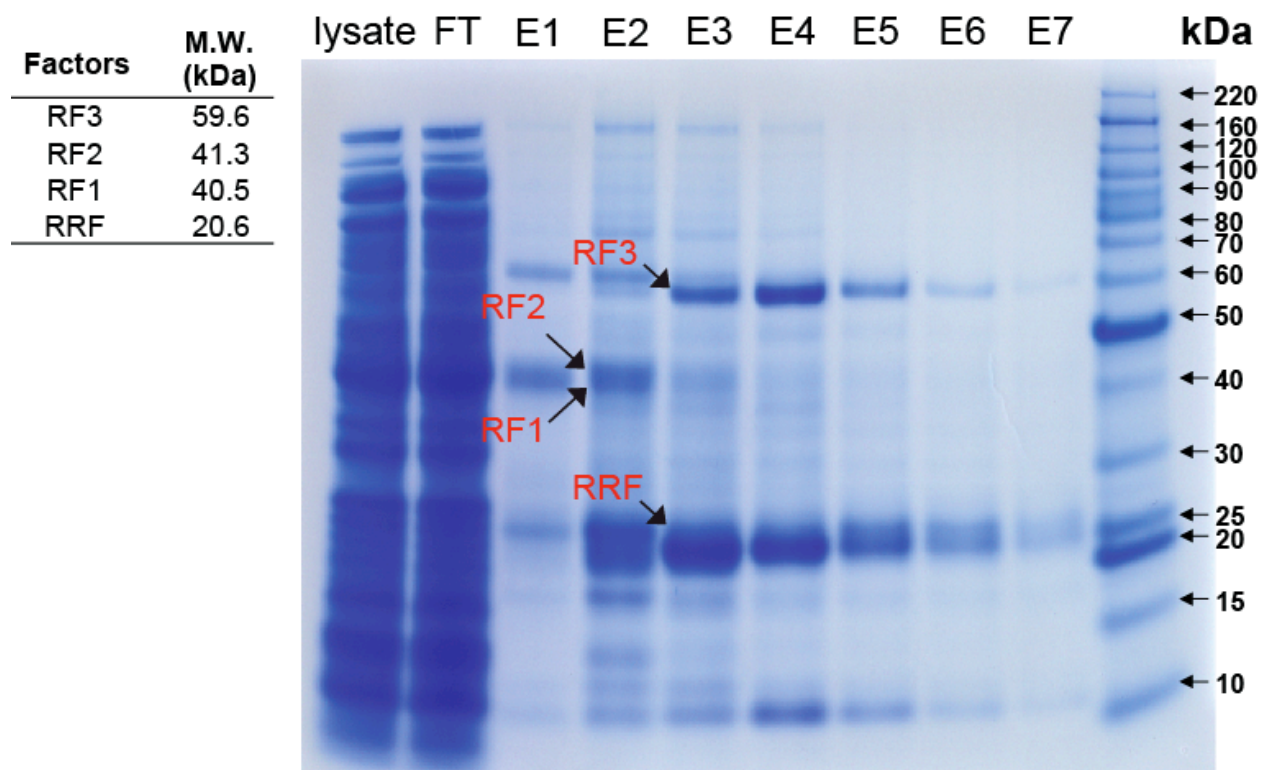
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Supplementary Figure 1. Growth rates of strains (black bar) in comparison to the ancestral strain (white bar) as determined by time-course spectrophotometry in a 96-well plate. Error bars are \pm standard deviations, with $n=3$. RB2 and RB3 growth rates are not shown since they were not used for subsequent ePURE systems.



Supplementary Figure 2. Supplement PURE in vitro translation reaction with of each ePURE pool of factors to examine the presence of any hypothetical inhibitory contaminants. Translation reactions producing luciferase are performed in 10 μ L PURExpress reaction with the following amount of ePURE factors added: 0.372 mg/mL of IEF, 0.311 mg/mL of RF, 0.176 mg/mL of RS1, 0.663 mg/mL of RS2, 0.172 mg/mL of RS3, and 0.116 mg/mL of RS4. Activities are measured by luminescence and PURExpress reaction without supplement is set as standard for comparison and normalized to 100%. Error bars are \pm standard deviations, with n=3



Supplementary Figure 3.

Protein gel of His-tagged RF factors purified from Ni-NTA resin. The resin is washed with lower concentration of 35mM Imidazole in order to prevent RF factors detaching during wash step. Factors eluted consecutively with a linear gradient from 35mM to 400mM Imizadole.

Sequencing Verification Forward Primers

gene	primer sequence
tsf.f	glaaaacgacggcaGGCCGCATGAAGAAATTCAC
infA.f	glaaaacgacggcaTTTTTCGGCTCAGGTAACG
infC.f	glaaaacgacggcaTTGGCTTTAAAATCCGCGAG
lepA.f	glaaaacgacggcaACGCACATCATTGCGC
infB.f	glaaaacgacggcaAAGGGGTAGATCGTGATTGG
tufA.f	glaaaacgacggcaCCGCAGTTCTACTCCGTAC
fusA.f	glaaaacgacggcaCGTGATGCTCAAAGGTCA
tufB.f	glaaaacgacggcaCCGCAGTTCTACTCCGTAC
frr.f	glaaaacgacggcaGAAGCAGAACAGCGCG
prfA.f	glaaaacgacggcaCTTGAGCAGGGCGGC
prfB.f	glaaaacgacggcaGGCCGGATAACGGCG
prfC_C.f	glaaaacgacggcaGAGTCAGTCAACGTTGCCA
prfC_N.f	glaaaacgacggcaTTGCGACCTGCTGGC
ileS.f	glaaaacgacggcaATGTTGCAATGGACCTTACG
proS.f	glaaaacgacggcaGTGCTGCTGGATGACCG
cysS.f	glaaaacgacggcaCAAGAACCGGAAGCGTTCC
leuS.f	glaaaacgacggcaATGGTGAAGACTCCACG
glnS.f	glaaaacgacggcaTGCTTTATGCCTGATGCGA
serS.f	glaaaacgacggcaTTCAGGCACGTCGTATG
asnS.f	glaaaacgacggcaTCGACCTTCCGATATTAATAAAG
tyrS.f	glaaaacgacggcaCTGATGCAGGCACTGGTC
pheT.f	glaaaacgacggcaGTTGGCGTAAATCAGGTAGTTG
pheS.f	glaaaacgacggcaTTGAAAAATAAAAGCCTGTCTCATCA
thrS.f	glaaaacgacggcaAGGTACAATCTCCCTTTTTTAGTGT
aspS.f	glaaaacgacggcaACTCCGCCGACG
argS.f	glaaaacgacggcaTTCAGTAAAAGCGTTAATTTACCCTG
metG.f	glaaaacgacggcaGTCTTCTCCGGTATTCGTTCTG
gltX.f	glaaaacgacggcaGTTTCATCAGCTATTGAGGC
hisS.f	glaaaacgacggcaAAATTGATGACCAACCCAGCGC
alaS.f	glaaaacgacggcaCCAGCGTTTTCTGCTCTATCG
lysS.f	glaaaacgacggcaTTCTGGATCAGGTTGCC
fmt.f	glaaaacgacggcaAAAGCATCGGTATTGATACGG
trpS.f	glaaaacgacggcaATGTATGGTCATCTGAAAGGTGAAG
glyS.f	glaaaacgacggcaAAGCTGGAGCCGTAATTTAC
glyQ.f	glaaaacgacggcaAATCCGCTGCCGCTG
valS.f	glaaaacgacggcaGAAGATGAGCTGGCGCG
rpsB.f	glaaaacgacggcaGCAAAACAACCTGGGTATTCC
rplC.f	glaaaacgacggcaGGTTCTATCGGTGAGAACCAG
rplL.f	glaaaacgacggcaCTCAGATCGACCGCCTG

Sequencing Verification Reverse Primers

gene	primer sequence
tsf.r	GTTCAACCAGTTCTTTGATTTCCTG
infA.r	AATAAAAAGGCCGGTTAAACCG
infC.r	TTTCCTTAACCTGGATAACCTTTTGG
lepA.r	ACCTGACGGGATCTGGAA
infB.r	GATCGCGTTTCAAAAAGGTG
tufA.r	CCCTTGACCGTAAAAACTGC
fusA.r	GTGTGATTTCAACGTGAGAAGT
tufB.r	CACCTCATCGCTCCAGCG
frr.r	AGAAGTTTCGCACCTCGCTT
prfA.r	ATTGCTGTTCCAGTTGCTCG
prfB.r	GGTTTCTTCGTCGTCAGCT
prfC_C.r	AAAAGCGATGAGTGATGAATAACG
prfC_N.r	AGAAGTCTTCGTCGCCCG
ileS.r	CCCAGCCAGGCACATAC
proS.r	CCCCAAGGCTTGAGGAGA
cysS.r	GTTTTTAACCGTCTCCTTGTATC
leuS.r	GGAATACCGATGCGGTATCTTT
glnS.r	TACCAGACCAGTGA AACCTAAC
serS.r	CGACTCACCTCAGCAGC
asnS.r	TGCTGGCCTGAATTTCAAATG
tyrS.r	CAGGTGTGAATTTATCAATGGCG
pheT.r	GCGTTTTTCGACCCGGC
pheS.r	GAGAGACATCAATCGTTTCCGC
thrS.r	GGCCGATTGCCATTTTGG
aspS.r	TTCCGCGATGGCAGC
argS.r	CATGTTTCAGCCAGGAATGC
metG.r	CCGCTGCCGACG
gltX.r	CAAGTTTGCGAGTTTATTCAGAACA
hisS.r	CCAGCCACCATAAGTATTTTTATT
alaS.r	TGCCAGCATTTTCAAGAA
lysS.r	GCTACCCGCCACC
fmt.r	CAGGTAATGCACGGTCCG
trpS.r	AATTTTCAGTAAATTGAATATTTATCTTCAGGAATT
glyS.r	TGTTTAACTGTCCAATTTTTGGG
glyQ.r	TTCTGCCGCTTTGCTCG
valS.r	CGTACCACGGTATGCTTTATCG
rpsB.r	TTACCAGGGATGCGGTAATTC
rplC.r	TGTCCTGCGACGACG
rplL.r	GAGCAGCCGGTGACG