Supporting Information

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SI Text

Construction of T7-Orbs Plasmids and Libraries. To construct the plasmids **2** and **3** (Fig. S1) we amplified the backbone of pET17b with the primers pET17bf HindIII (gttgAAGCTTagttggctgctgccaccgctgag) and pET17brNotI (gaaaGCGGCCGCcaaaattatttctagagggaaaccgttg), digested it with NotI and HindIII restriction endonucleases, and purified the digested PCR product. To create plasmid **3** we amplified *GFPmut3.1* from pGFPmut3.1 (catalog no. 6039–1; Clontech) with the primers cirG9GFP31f (gaaCTCGAGtatgcggccgctttcaTATCCCTccgcaaATGAGGAAGGGAGAAGAACTTTTCACTGGAGTTGTC) and cirGFP31r (gaacAAGCTTAtcactaGGTCTCttaTTTGTATAGTTCATCCATGCCATGTGTAATCCCAG) to generate the *GFP* insert fragment, and ligated this insert fragment to the purified pET vector fragment. To create plasmid **2** we PCR amplified *GFPmut3.1* from pGFPmut3.1 with the primers cirG9R22f (GAACTCGAGTATGCGGCCGCAATTGTGAGCGGctcgagacaatTTTCATATCCCTCCGCAAATGAGGAAGGGAGAAGAACTTTTCACTGGAGTTGTC) and cirGFP31r and ligated this insert to the purified pET vector fragment. To create plasmid **4** we performed enzymatic inverse PCR, using plasmid **3** as the template and the primers pT71504cutf (ggaaaAGATCTcgatcccgcgaaattaatacgacTCACTATAGGGAGATTTCATATCCCTCCGCAAATGc) and pT71504cutr (ggaaaAGATCTcgggcagcgttgggtcctggc).

The sequences shown schematically in Fig. S1 are spacer 1 (aattgtgagcggCTCGAGacaat), spacer 2 (ccacaacggtttccctctagaaataattttgGCGGCCGC), and O-rbs (TTTCATATCCCTCCGCAA).

To create the library pT7 N15lib we performed enzymatic inverse PCR (1, 2) with primers T7n15O-rbsf (gaaccgagatctcgatcccgcgaaattaatacgactcactatagggagannnnnnnnnnnnnnnnTTTCATATCCCTCCGCAAATGcGtAAaGGAG) and T7R (atcgagatctcgggcagcgttgggtcctggc) on plasmid C. The resulting enzymatic inverse PCR products (20 μg) were digested with BglII (10 h, 37 °C), digested with DpnI (2 h, 37 °C), and ligated (T4 DNA ligase, 16h, 16 °C). The library DNA was ethanol-precipitated and transformed into Mega X DH10B (Invitrogen).

To construct a plasmid for constitutive expression of *GFP* from an O-rbs the sequence ATA in lac operator of pGFPmut3.1 was replaced by CTCGAG. The entire flanking sequence between lac operator and *GFP* was replaced by the conserved 18-nt (TTTCATATCCCTCCGCAA), producing the vector pR22. The *GFP* gene, O-rbs, flanking sequence and terminator were amplified from R22 by using the primers xr1GFPnotIf (ATATGCGGCCGCAACCGTATTACCGCCTTTGA) and xr1GFPbglr (TGACAGATCTACATTTCCCCGAAAAGTGC). The PCR product was digested with BglII and NotI. A fragment containing the tetracycline resistance gene and the p15A origin was amplified from pO-CAT with the PCR primers pcatbglf (TATAGCGGCCGCCAAAGCCGTTTTTCCATAGG) and pcatNotIr (CAGTAGATCTTCCGCGTTTCCAGACTTTAC), and digested with BglII and NotI. The pR22 fragment and the pO-CAT fragments were ligated (T4 DNA ligase, 16 h, 16 °C) to yield pXR1.

Selection of an Optimized T7O-rbs System. An optimized T7 promoter/O- ribosome binding site system was selected by 3 rounds of FACS screening (positive, negative, and positive). In the positive rounds of screening Bl21 (DE3) (Novagen) containing pSC101*O-ribosome was transformed with pT7n15lib and grown overnight (37 °C, 12 h, 250 rpm) in 100 mL of LB-AK (LB media containing 25 μg·mL $^{-1}$ ampicillin and 12.5 μg·mL^{-1} kanamycin). Five milliters of overnight culture was diluted 1:20 in fresh LB-AK and incubated (1.5–2 h, 250 rpm, 37 °C). At OD_{600} ≈0.5–0.8, IPTG (1 mM) was added, and the cells were incubated (1.5–2 h, 250 rpm, 37 °C. The cultures were filtered through 70 μm BD Falcon Cell Strainers (BD Biosciences) to remove cell debris and diluted 1:100 in PBS. The samples were subjected to FACS by MoFlo (MoFlo Cytomation), with a flow rate of 10,000 events per s using a 100-μm nozzle. A total of 3.7×10^7 cells were sorted and 3×10^6 cells were collected (region R1 in Fig. S2). The collected GFP-positive cells were amplified in LB-2AKG (LB media containing 25 μg·mL^{-1} ampicillin, 25 μg·mL^{-1} kanamycin, and 2% glucose) (37 °C, 250 rpm, 16 h). Total plasmid DNA was isolated from cells and pT7n15lib DNA was separated from pSC101*O-ribosome DNA by 1% agarose gel electrophoresis. The pT7n15lib DNA was extracted from the gel for use in the next round of screening or for characterization of individual clones.

For negative FACS sorting pT7n15lib DNA surviving the positive sort was transformed into BL21 (DE3) containing pSC101*BD (this vector produces rrnB from the native ribosomal P1P2 promoter). Cultures were prepared for FACS sorting as described for the positive FACS sort. In the negative FACS sort 10^8 cells were sorted and 88.5% of the cells were collected, as they had a level of fluorescence comparable to negative controls. The collected negative clones were amplified and their pT7n15lib DNA was resolved and extracted, as described above for positive sort clones. In the final positive sort 10^8 cells were sorted and 6×10^3 cells were collected with strong fluorescence.

Characterizing pT7 O-rbs GFP Expression Constructs. Individual pT7n15lib clones were transformed into BL21 (DE3) containing either the wild-type (pTrc RSF wt ribosome or pSC101*BD) or the O-ribosome (pTrc RSF-O-rDNA or pSC101*O-ribosome). Transformed cells were grown overnight (37 °C, 12 h, 250 rpm) in 10 mL of LB-AK. Overnight culture (0.5 mL) was diluted 1:20 in to 10 mL of fresh LB-AK and incubated (1.5–2 h, 250 rpm, 37 °C). At OD_{600} ≈0.5–0.8, IPTG (1 mM) was added, and the cells were incubated (12 h, 250 rpm, 37 °C). Fluorescence was quantified by using a fluorescent plate reader (Tecan safire II plate reader). The excitation wavelength was 488 nm and the emission was measured at 515 nm with a 10-nm bandpass. The GFP values were normalized by OD_{600} values. Clones from the selection showing good O-ribosome-dependent fluorescence were sequenced.

To further characterize clone pT71504 resulting from the selection we replaced *GFP* in pT71504 by a *GSTsfGFP* fusion to create the pT7 Orbs-*GST-GFP* [sfGFP is superfolding green fluorescent protein (3)]. A *GSTsfGFP* containing fragment was amplified by using the primers 1504G9GfGFPf (tgcccgAGATCTcgatcccgcgAAATTAATACGACTCACTATAGGGAGActatatctgttatttTTTCATATCCCTCCGCAAATGtcC) and sfGFPHindr (caactAAGCttattaatggtgatgatgatggtggctgcctttatacagttcatccatacc) and ligated between the BglII and HindIII sites in pT71504 to generate pT7 Orbs-*GST-GFP*.

To demonstrate that pT7 Orbs-*GST-GFP* displays Boolean AND logic we transformed BL21 (T1R) (Sigma/Aldrich) and BL21 (DE3) with pT7 Orbs-*GST-GFP* and either pSC101*O-ribosome or pSC101*BD. The cells were cultured in LB-2AKG media (37 °C, 250 rpm, 16 h). Overnight cultures were inoculated (1:100) into 100 mL of LB-AK and incubated (37 °C, 3 h, 250 rpm). At OD_{600} ≈0.7–0.9, IPTG (1 mM) was added and the cells,

which were incubated for a further 3–5 h (37 °C, 3–5 h, 250 rpm). Fifty milliliters of cells was harvested by centrifugation (4,000 × *g*, 10 min), and the pellets were washed once with 1 mL of ice-cold PBS. BugBuster Protein Extraction Reagent (1 mL) (Novagen) containing complete protease inhibitor mixture (Roche) and 1 mM PMSF (Sigma/Aldrich) were added to cell pellets and incubated at 25 °C for 30 min. The supernatant was collected after centrifugation (16,000 × *g*, 10 min, 4 °C). Glutathione Sepharose 4B beads (40 μL) (GE Healthcare Bioscience AB) were added to the supernatant and incubated at 4 °C (1 h) with rotating. The beads were washed (4 × 1 mL) with ice-cold PBS. Proteins were eluted from the beads by the addition of 60 μL of NuPAGE SDS sample buffer (1×) (Invitrogen). The mixture was boiled for 5 min at 95 °C and the beads were pelleted by centrifugation. Samples of the supernatant (15 μL) were subjected to SDS/PAGE on 4–12% gel (400 mA, 2 h). Proteins were visualized by InstantBlue staining (www.expedeon.com).

Total RNA Extraction and Northern and Dot Blotting. To demonstrate the T7 RNAP dependence of pT7 O-rbs GST-GFP was transformed into Bl21 (DE3) or BL21-T1R competent *E. coli* containing pSC101* O-ribosome. One milliliter of overnight culture (37 °C, 250 rpm) in LB-AK was used to innoculate 20 mL of LB-KA. IPTG (1 mM) was added into the culture media at the log-phase and cells were harvested after 3 h. Four milliliters of each sample was pelleted and resuspended in RNAlatter stabilization solution and stored at 4 °C. Cells were pelleted by centrifugation and resuspended in 4 ml of TRIZOL reagent. The samples were incubated (5 min at room temperature) to permit dissociation of nucleoprotein complexes and 800 μL of chloroform was added. The samples were incubated (3 min, room temperature) before centrifugation (12,000 × *g*, 15 min, 4 °C). RNAs in the colorless upper aqueous layer were transferred into fresh tube. RNA was precipitated by the addition of 2 mL of isopropanol and centrifugation (12,000 × *g*, 15 min, 4 °C). The resulting pellet was washed with 75% ethanol and dissolved in DEPC-treated water.

For Northern blots, total RNA was mixed (1:2 vol/vol) with RNA sample loading buffer (62.5% deionized formamide, 1.14 M formaldehyde, 1.25× Mops-EDTA-sodium acetate buffer, 200 μg/mL bromophenol blue, 200 μg/mL xylene cyanole FF, and 50 μg/mL ethidium bromide). The samples (16 μg of RNA in 10 μL) were resolved on 1% denaturing agarose in running buffer (5 mM NaOAC, 20 mM Mops, 1 mM EDTA, pH 7.0) at 100 V for 10 min, then at 50 V for 90 min. The RNAs were transferred to BiodyneB precut modified nylon 0.45-μm membranes in 20× SSC buffer (17.53% NaCl, 8.82% sodium citrate, pH 7.0) at 4 °C overnight and were UV-cross-inked on the membrane by using a Stratagene Stratalinker 2400 UV Crosslinker.

The hybridization and detection of RNAs on the membrane was performed according to the instruction of the North2South chemiluminescent hybridization and detection kit (catalog no. 17097. Pierce). Briefly, the fixed blot was washed with 0.1% SDS at 55 °C for 30 min. Hybridization buffer was added to cover the blot, and the blot was prehybridized to the membrane with rotating for 45 min at 55 °C. The biotinylated DNA probe was denatured (100 °C, 10 min) and placed on ice for 5 min. After prehybridization, the denatured biotinylated DNA probe was added to the hybridization buffer to a final concentration of 3 nM and incubated at 55 °C overnight with rotating. The membrane was washed with hybridization stringency wash buffer (2× SSC/0.1%SDS diluted in distilled water) 3 times for 20 min per wash with agitation at 55 °C. The stringency wash buffer was removed from the membrane and replaced with blocking buffer. After blocking the membrane with rotating for 15 min at room temperature, streptavidin-HRP was added into the blocking buffer at a final dilution at 1:1,500–3,000 and incubated for 30 min at room temperature with agitation. The membrane was washed 4 times for 15 min each with 1× wash buffer. Substrate equilibration buffer was added to cover the membrane and incubated for 10 min at room temperature with agitation. Luminol/enhancer solution and stable peroxide solution were mixed at 1:1 (vol/vol), and added to the equilibrated membrane on a tray for 5 min at room temperature, before the blot was exposed to X-ray film. For dot blots, 8 μg of total RNAs was autocross-linked on the BiodyneB-modified nylon membranes, followed by the same procedure as Northern blot.

The 5′-end biotin- labeled probes used were: for GFP mRNA, spx34EcGFPr: TGTCTGGTAAAAGGACAGGGCCATCG; for 16s rRNA, spx34Ec16sr: ACGCATTTCACCGCTACACcTGGAATTCTACC); for *sf*GFP, spX34sfGFPr GCTGCCTTTATACAGTTCATCCATACCGTG), which are complementary to GFP sequence (residues 567–592) 16s rDNA sequence (residues 670–701), and sfGFP sequence (residues 694–723), respectively. The 16S rDNA probe was used to check that the same amount RNA was loaded in each lane.

Construction of Plasmids that Produce rRNA from a T7 Promoter. To construct pT7 RSF O-ribosome and pT7 RSF-BD we performed enzymatic inverse PCR (2) using primers pT7pmeIRSFf and pT7pmeIRSFr with pTrc RSF O-ribosome or pTrc RSF-BD as a template. The PCR product deletes the Trc promoter and replaces it with a T7 promoter. The PCR products were digested with *Pme*I and ligated by T4 DNA ligase.

To create the truncated rrnB operon on a T7 promoter (a T7 promoter version of plasmid 6) enzymatic inverse PCR primers TG65bsaf and primers TG65bsar were designed. These primers were used to amplify the sequence 3′ to 11 nt following the 23S and 5′ to 65 nt from the 3′ end of 16S rDNA in pT7 RSF O-ribosome. Digestion of the PCR product with BsaI and DpnI and ligation with T4 DNA ligase created pT7 RSF O-16S.

Construction of Minimal O-rRNA for O-Ribosomes. The O-*rrnB* (*rrnB* containing the O-ribosome mutations in 16S rRNA) deletion constructs were produced by enzymatic inverse PCR (the primers are listed in Table S1) based on pTrc O-ribosome. To make constructs 1-9, the enzymatic inverse PCR products were digested with BamHI and ligated with T4 DNA ligase. To create construct 10 the pTrc vector backbone was amplified by primers Tg9BamHIF and Tg9xhoIR (see Table S1 for primer sequences). The O-16s rDNA sequence was amplified by primers Tg9XhoIF and Tg9BamHIR (Table S1). The PCR fragments were digested with BamHI and XhoI and ligated by T4 DNA ligase. All constructs were confirmed by sequencing.

Characterization of Minimal O-rDNA for Functional O-Ribosome Production. To compare the activity of O-ribosomes produced by each O-rrnB truncation, each construct (Fig. 4, constructs 1-10) was cotransformed with pXR1 into Genehog *E. coli* (Invitrogen). Transformed cells were grown overnight (37 °C, 12 h, 250 rpm) in 10 mL of LB-AK. Overnight culture (0.5 mL) was diluted 1:20 into fresh LB-AK and incubated (1.5–2 h, 250 rpm, 37 °C). At OD_{600} ≈0.5–0.8, IPTG (1 mM) was added, and the cells were incubated (12 h, 250 rpm, 37 °C). Fluorescence was quantified by using a fluorescent plate reader (Tecan safire II plate reader). The excitation wavelength was 488 nm and the emission was measured at 515 nm with a 10-nm bandpass. The GFP values were normalized by OD_{600} values.

Characterization of Orthogonal Gene Expression Kinetics. To characterize the orthogonal gene expression kinetics, we used BL21 (DE3) containing pT7 Orbs GST-GFP with pRSF O-ribosome, pSC101* O-ribosome, pT7 RSF O-ribosome, or pT7 RSF O-16S. Overnight culture (0.5 mL) grown in LB-AKG was used to

innoculate 10 mL of LB-AK. These cultures were incubated (37 °C, 250 rpm, 2 h to OD 600 = ≈ 0.5) and then induced with IPTG (1 mM) and incubated a further 56 h. Fluorescence was quantified by using a fluorescent plate reader (Tecan safire II plate reader). The excitation wavelength was 488 nm and the emission was measured at 515 nm with a 10-nm bandpass. To determine the effect of T7 RNAP on gene expression the experiments were carried out using BL21-TIR instead of BL21 (DE3). To determine the effect of the O-ribosome on gene expression the experiments were carried out using the wild type ribosome equivalent of the O-rRNA vectors described above. Each experiment was repeated for at least 3 independent cultures, and error bars represent the standard deviation.

1. Rackham O, Chin JW (2005) A network of orthogonal ribosome x mRNA pairs. *Nat Chem Biol* 1:159–166.
2. Stemmer WP, Morris SK (1992) Enzymatic inverse PCR: A restriction site independent, single-fragment method for high-efficiency, site-directed mutagenesis. *Biotechniques* 13:214–220.
3. Pedelacq JD, Cabantous S, Tran T, Terwilliger TC, Waldo GS (2006) Engineering and characterization of a superfolder green fluorescent protein. *Nat Biotechnol* 24:79–88.

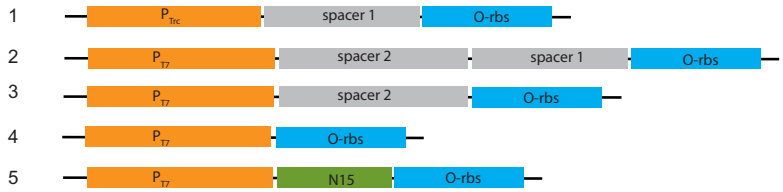

Fig. S1. Anatomy of orthogonal transcription translation control elements. Lanes 1–4 show the organization of constructs tested for orthogonal gene expression. The sequence modules used are Ptrc [Trc promoter, a hybrid of the trp and lac promoter, from pTrc HisA (Invitrogen], PT7 (the class III T7 promoter), spacer 1 (23 nt in construct 1, 5′ to the O-rbs containing sequence, spacer 2 (39 nt 5′ to the rbs is the standard pET-17A vector). O-rbs is a sequence bearing an orthogonal ribosome binding site. Lane 5 shows the anatomy of the library used to generate a minimal combination of orthogonal transcription and translation control elements.

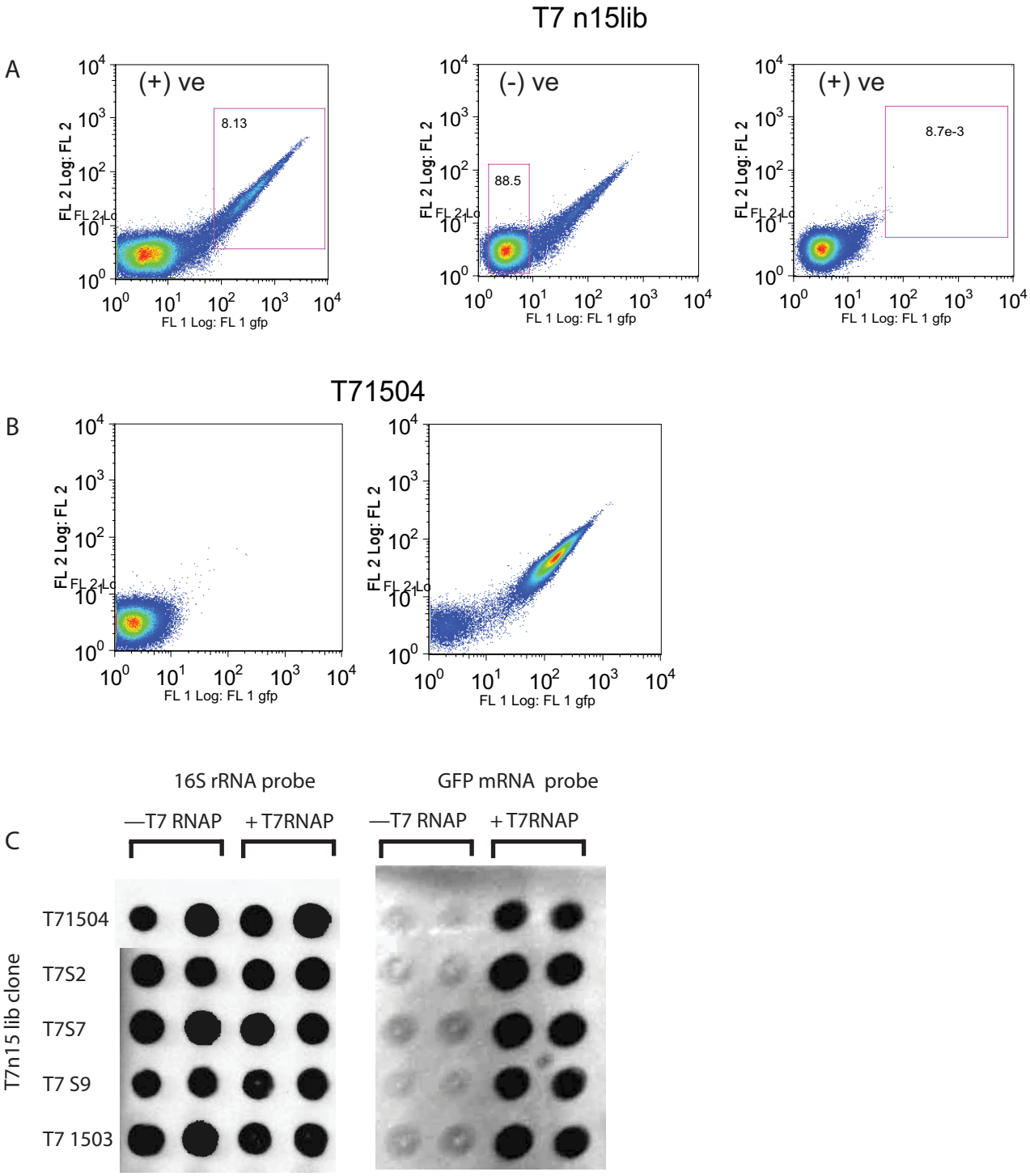

Fig. S2. (*A*) FACS sorts from the 3 rounds of screening for a module that specifically and efficiently directs transcription by T7 RNAP and translation by the O-ribosome of a target gene. (*B*) FACS plots of a selected sequence in BL21 (DE3) cells containing wild-type ribosomes (*Left*) and in BL21 (DE3) containing the O-ribosome (*Right*). (*C*) Dot blots demonstrate that each of the selected T7N15lib clones shows T7 RNAP-dependent production of GFP mRNA. The 16S rRNA probe provides a loading control.

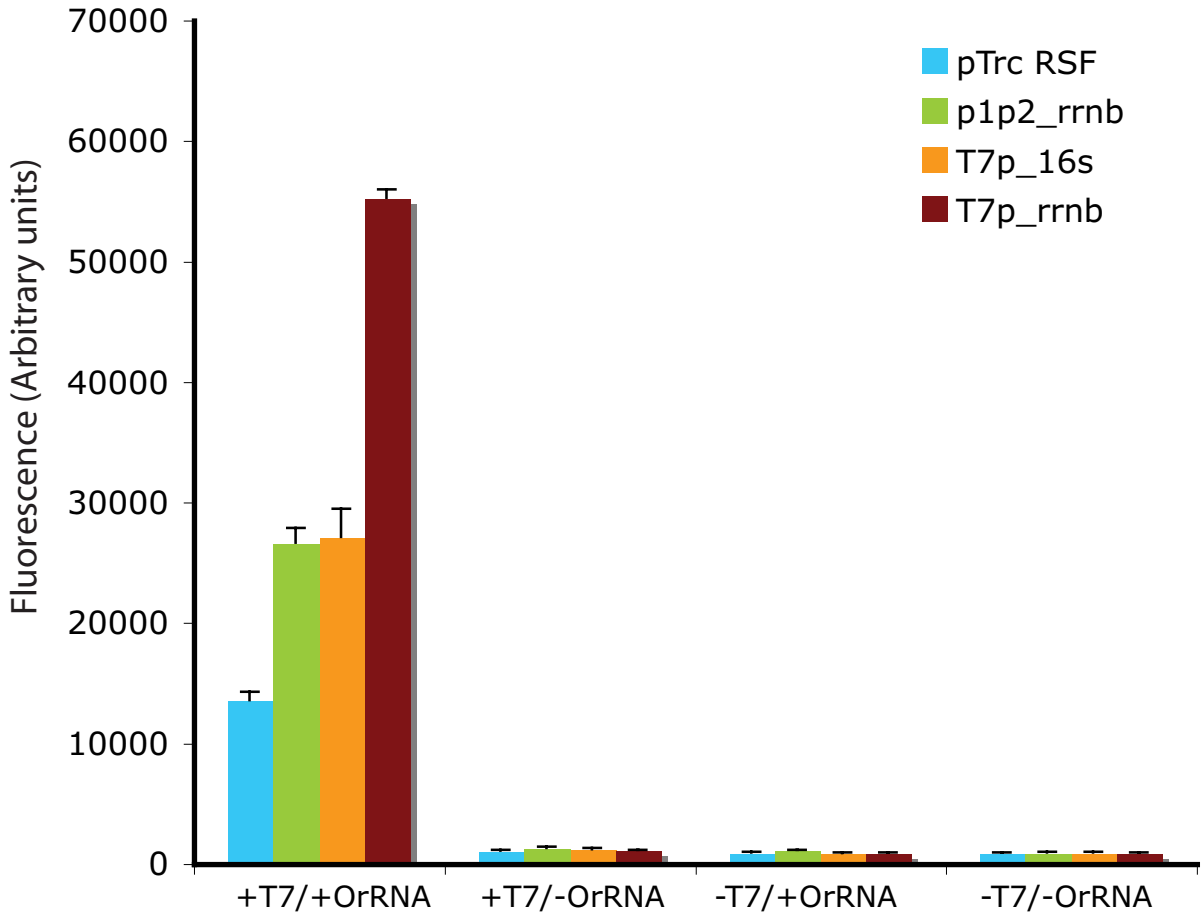

Fig. S3. Comparison of GST-GFP expression from pT7 O-rbs GST-GFP using the ribosome constructs described. Experiments that do not contain O-ribosomes were performed with cells that contain the equivalent plasmid-encoded wild-type ribosomal RNA.

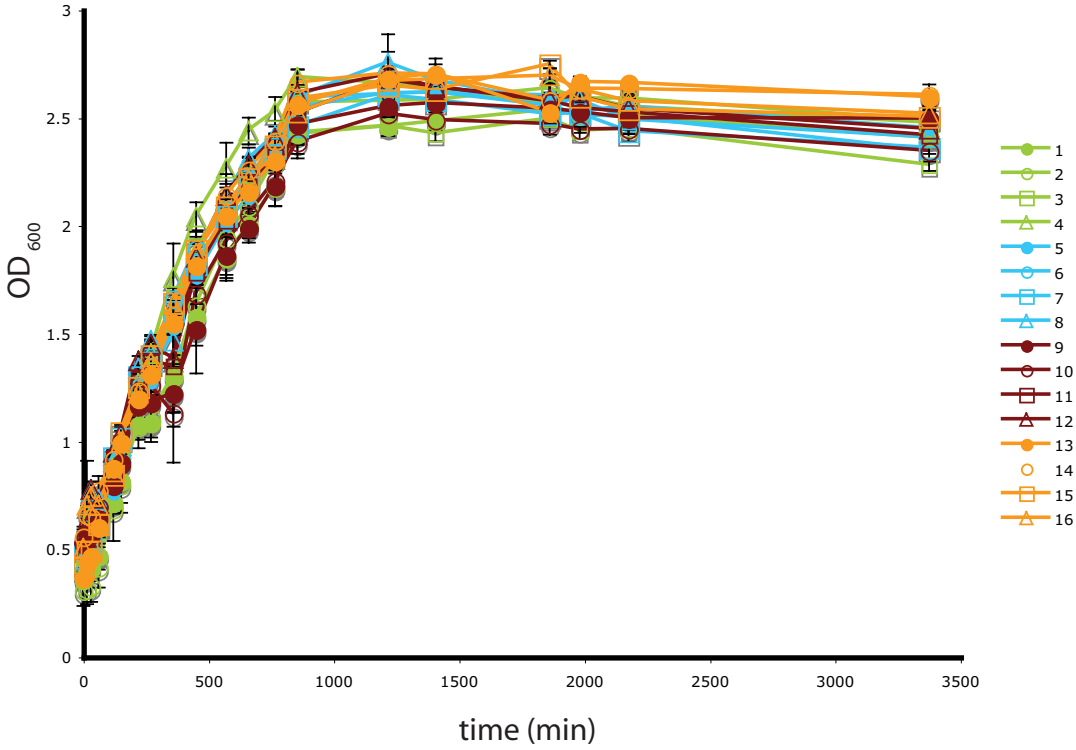

Fig. S4. The different combinations of ribosomes and T7 RNAP do not substantially affect growth rate. The data show the mean of at least 3 independent trials and the error bars represent the SD. Green solid circles: BL21 (DE3) (produce T7 RNAP from an IPTG inducible promoter), pSC101* O-ribosome, pT7 O-rbs GST-GFP. Green open circles: BL21 (DE3), pSC101* BD (produces wild-type rrnB), pT7 O-rbs GST-GFP. Green open squares: BL21 (T1R) (isogenic with BL21 (DE3), except that they do not produce T7 RNAP) pSC101* O-ribosome, pT7 O-rbs GST-GFP. Green open triangles: BL21 (T1R), pSC101* BD, pT7 O-rbs GST-GFP. Blue solid circles: BL21 (DE3) pTrc RSF O-ribosome (produces O-rRNA from an IPTG inducible Trc promoter), pT7 O-rbs GST-GFP. Blue open circles: BL21 (DE3), pTrc RSF BD, pT7 O-rbs GST-GFP. Blue open squares: BL21 (T1R), pTrc RSF O-ribosome, pT7 O-rbs GST-GFP. Blue open triangles: BL21 (T1R), pTrc RSF BD, pT7 O-rbs GST-GFP. Maroon solid circles: BL21 (DE3), pT7 RSF O-ribosome, pT7 O-rbs GST-GFP. Maroon open circles: BL21 (DE3), pT7 RSF BD, pT7 O-rbs GST-GFP. Maroon open squares: BL21 (T1R), pT7 RSF O-ribosome, pT7 O-rbs GST-GFP. Maroon open triangles: BL21 (T1R), pT7 RSF BD, pT7 O-rbs GST-GFP. Orange solid circles: BL21 (DE3), pT7 RSF O-16S, pT7 O-rbs GST-GFP. Orange open circles: BL21 (DE3), pT7 RSF wt16S, pT7 O-rbs GST-GFP. Orange open squares: BL21 (T1R), pT7 RSF O-16S, pT7 O-rbs GST-GFP. Orange open triangles: BL21 (T1R), pT7 RSF wt16S, pT7 O-rbs GST-GFP.

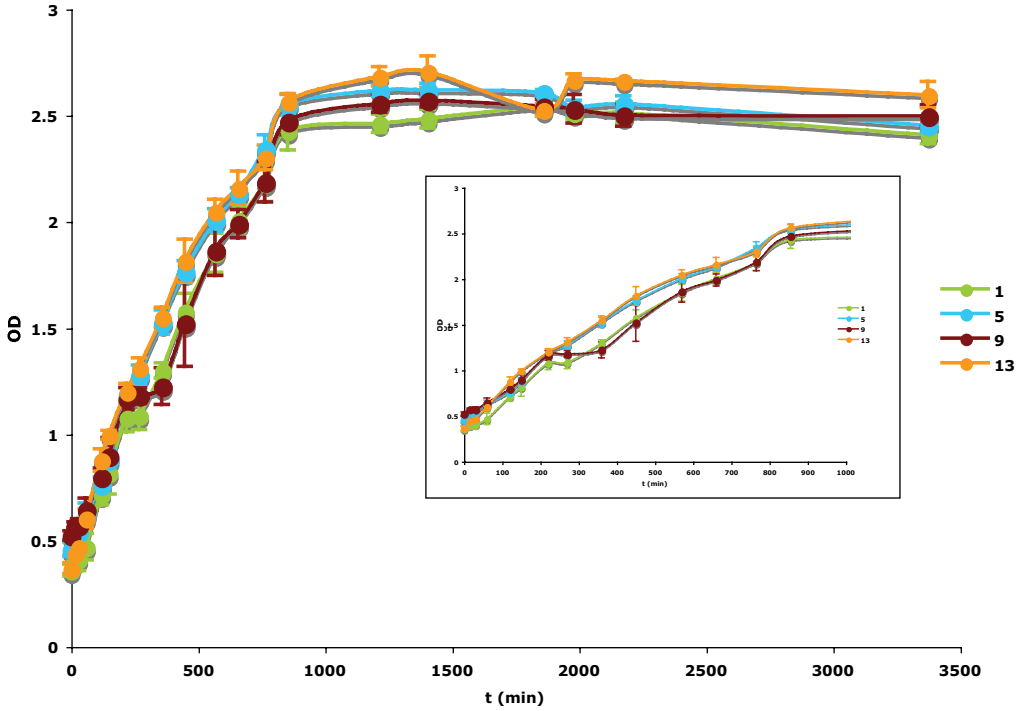

Fig. S5. The active combinations of ribosomes and T7 RNAP do not substantially affect growth rate. The data show the mean of at least 3 independent trials and the error bars represent the SD. The color coding is the same as that in Fig. S4.

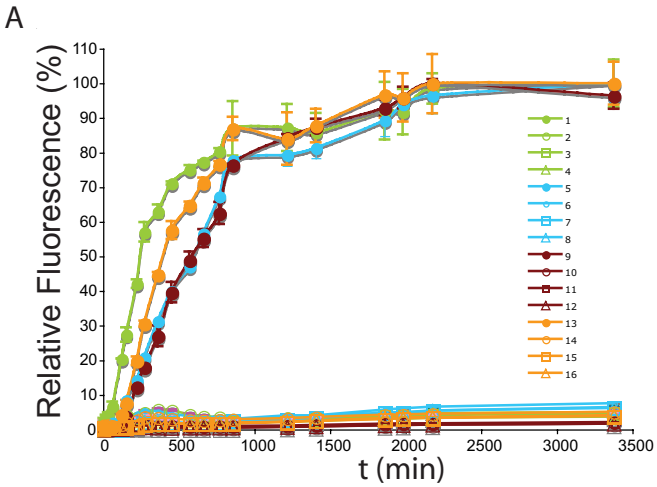

Fig. S6. Percentage of maximal sfGFP expression produced from pT7 O-rbs GST-GFP as a function of time over 60 h after the addition of 1 mM IPTG. The data show the mean of at least 3 independent trials and the error bars represent the SD. Green solid circles : BL21 (DE3) (produce T7 RNAP from an IPTG inducible promoter), pSC101* O-ribosome, pT7 O-rbs GST-GFP. Green open circles: BL21 (DE3), pSC101* BD (produces wild-type rrnB), pT7 O-rbs GST-GFP. Green open squares: BL21 (T1R) (isogenic with BL21 (DE3), except that they do not produce T7 RNAP) pSC101* O-ribosome, pT7 O-rbs GST-GFP. Green open triangles: BL21 (T1R), pSC101* BD, pT7 O-rbs GST-GFP. Blue solid circles: BL21 (DE3) pTrc RSF O-ribosome (produces O-rRNA from an IPTG inducible Trc promoter), pT7 O-rbs GST-GFP. Blue open circles: BL21 (DE3), pTrc RSF BD, pT7 O-rbs GST-GFP. Blue open squares: BL21 (T1R), pTrc RSF O-ribosome, pT7 O-rbs GST-GFP. Blue open triangles: BL21 (T1R), pTrc RSF BD, pT7 O-rbs GST-GFP. Maroon solid circles: BL21(DE3), pT7 RSF O-ribosome, pT7 O-rbs GST-GFP. Maroon open circles: BL21 (DE3), pT7 RSF BD, pT7 O-rbs GST-GFP. Maroon open squares: BL21 (T1R), pT7 RSF O-ribosome, pT7 O-rbs GST-GFP. Maroon open triangles: BL21 (T1R), pT7 RSF BD, pT7 O-rbs GST-GFP. Orange solid circles: BL21 (DE3), pT7 RSF O-16S, pT7 O-rbs GST-GFP. Orange open circles: BL21 (DE3), pT7 RSF wt16S, pT7 O-rbs GST-GFP. Orange open squares: BL21 (T1R), pT7 RSF O-16S, pT7 O-rbs GST-GFP. Orange open triangles: BL21 (T1R), pT7 RSF wt16S, pT7 O-rbs GST-GFP.

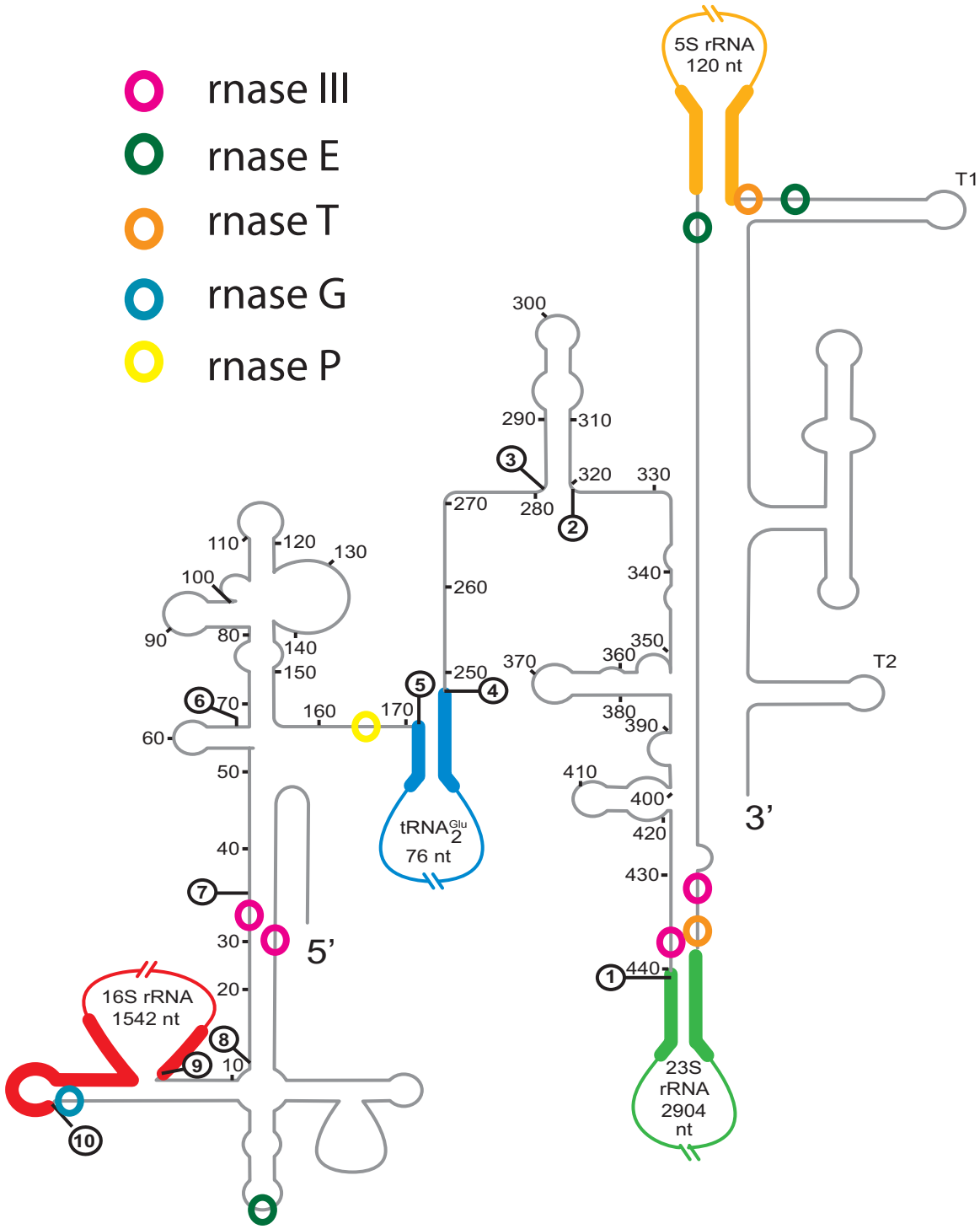

Fig. S7. The proposed fold of the rrnB primary transcript. T1 and T2 are terminator sequences. Numbered circles indicate the sites of truncation.

Table S1. Primer sequences used

Name	Primer sequence	Comments (1–10 refer to constructs in Fig. 4)
Tg9BamHIR440	ggaaaGGATCCtcacaacccgaagatgtttctttcgattcatcatcgtgttgcgaaaatttgaga-gactcacgaacaac	1
Tg9BamHIR320	ggaaaGGATCCaatatctcaaacatcacccgaag	2
Tg9BamHIR247	ggaaaGGATCCtggcgtcccctaggggattcgaacccctgttaccgc	3
Tg9BamHIR280	ggtctGGATCCAAGGTCGGCGACTTtcactcacaaaccagcaag	4
Tg9BamHIR171	ggaaaGGATCCACGAAAATTGCTTAtcacgcgttgc	5
Tg9BamHI65	ggaaaGGATCCACGCCTTgcttttcactttctatcagacaatCTGTGTGAGcactacaaagtacgcttc	6
Tg9BamHI35	tatacGGATCCtCTGTGTGAGcactacaaagtacgcttc	7
Tg9BamHI14	ggaaaGGATCCacgcttctttaaggtaatcccatgatccaaccgc	8
Tg9BamHI0	ggaaaGGATCCtaatcccatgatccaaccgcaggttcccc	9
Tg9BamHIF	ACCTTGGATCCagacctGGTACCatcgattcccgggaattcgaagcttgggcccgaacaaaaactcatctc	1–10
Tg9xhoIR	ggaaaCTCGAGctgtttcctgtgtgaaattgttatccgc	10
Tg9XhoIF	ggaaaCTCGAGaaattgaagagtttgatcatggc	10
Tg9BamHIR	AGGTCTGGATCCtaatcccatgatccaaccgcaggttcccctacggtTACcttgttacg	10
pT7pmeIRSFf	ggaaagtttAAACAGCGCCGCTGAGAAAAAGCGAAGCGGCACTGCTCTTTAACAATTTATCAG-ACAATCTGTGTGGGCActcgagGC	pT7 RSF O-ribosome
pT7pmeIRSFr	ggaaaGTTTaaacTGTGAggAATTGTTATCCGCTCACAATTCCcCTATAGTGAGTCGTATTAattt-GCTCATTTCAG	pT7 RSF O-ribosome
TG65bsaf	ggaaaGGTCTCActgtTTTGGCGGATGAGAGAAG	pT7 RSF O-ribosome
TG65bsar	ggaaaGGTCTCAacagACGCCTTGCTTTTCACTTTCTATC	pT7 RSF O-ribosome