Supplementary Information

Protocols

Restriction Site Distribution: All design and annotation of DNA constructs was done using Vector NTI (InforMax). All restriction analyses were performed with Vector NTI (InforMax), NEBcutter (Vincze et al, 2003), and REBASE (Roberts et al, 2005). A perl script was written to search for sites within coding regions where restriction sites could be introduced by silent mutation [http://web.mit.edu/endy/www/software/cuts/].

Oligonucleotide Synthesis: All oligonucleotides were synthesized by MWG, Invitrogen or using an ABI Model 394 DNA synthesizer (courtesy of Tom Knight).

Part Amplification: All parts were amplified by PCR using the following reaction mixture: 5uL 10X Thermo Pol Buffer (NEB), 20pM primer1, 20pM primer2, 3-30ng T7 genomic DNA, 1unit Vent polymerase (NEB), 10uM each dNTP and water to 50uL. The mixture was thermocycled (MJ Research PTC-200) as follows: 95°C for 2 minutes, 25-35 cycles of 95°C for 30 seconds, 50°C-60°C for 30 seconds, 72°C for 1-5 minutes, 72°C for 10 minutes.

Part Cloning: All parts and vectors (0.1 - 50pmoles) were restriction enzyme digested according to the manufacturers' directions (NEB, Fermentas). Parts and vectors were then purified by gel electrophoresis (0.5 - 2% TAE agarose gel, 3 - 8 V/cm) and extracted with Qiaquick gel extraction kit (Qiagen). Ligation reactions using T4 DNA ligase (NEB) were carried out in a 3:1 part:vector molar ratio according to the manufacturer's directions. Ligation products were dialyzed on nitrocellulose membranes (Millipore) against 1000X volume of water for 30 minutes. Ligation products were transformed by electroporation using 1800V across a 1mm gap (Bio-Rad Gene Pulser Xcell) and plated on the appropriate medium. Screening for clones was performed by colony PCR with the following protocol: Colonies were picked and diluted in 100uL of water. 1uL of that cell suspension was added to 1uL 10X Thermo Pol Buffer (NEB), 4pM primer1, 4pM primer2, 0.5U Taq Polymerase (NEB), 2uM each dNTP and water to 10uL. This mixture was thermocycled as follows: 95°C for 6 minutes, 25-35 cycles of 95°C for 30 seconds, 54°C for 30 seconds, 72°C for 1-5 minutes, 72°C for 10 minutes.

Site Directed Mutagenesis of Parts: Site specific changes were performed on the cloned parts using QuickChange Site-Directed Mutagenesis Kit (Stratagene) according to the manufacturer's directions. Primers were 5' phosphorylated using Polynucleotide Kinase (NEB) according to the manufacturer's directions.

Construction of pREB Plasmid: pREB was constructed from pSB2K3-1 (http://parts.mit.edu/), a chimera of pSCANS-5 (gift of John Dunn, Brookhaven National Laboratory) and pSB1A3-1 (http://parts.mit.edu/). The multiple cloning site of pSB2K3-1 was replaced by a PstI-BstBI-BclI multiple cloning site by primer annealing and cloning. Primer duplexes were prepared using the following steps: the reaction mixture, 100pM each primer, 2uL restriction buffer (NEB) and distilled water to 20uL was

incubated as follows: 95°C for 4 minutes, 0.1°C/s ramp to 80°C, 80°C for 4 minutes, 0.1°C/s ramp to 70°C, 70°C for 4 minutes, 0.1°C/s ramp to 60°C, 60°C for 4 minutes, 0.1°C/s ramp to 50°C, 50°C for 4 minutes, 0.1°C/s ramp to 22°C, 22°C for 10 minutes. The annealed duplexes were 5' phosphorylated using Polynucleotide Kinase (NEB). pREB was then cleaned of restriction sites using QuickChange Multi Site-Directed Mutagenesis Kit (Stratagene) and screened by digestion.

Construction and Cloning of the Beta Scaffold: The beta scaffold was constructed by annealing two partially overlapping primers as described above. The overhangs were then filled in using Klenow fragment (NEB) extension according to the manufacturer's directions. The extension product was digested with BstBI and cloned into pREB.

Assembly of Section Fragments in *E. coli*: Parts were cloned into the scaffold using the same cloning method as above with the addition of treating the purified cut vector with Antarctic Phosphatase (NEB) according to the manufacturer's directions and using a molar ratio of 6-10:1 insert:vector in the ligation reaction. Screening was again performed by colony PCR but included a second PCR to verify directionality via an internal primer.

Assembly of Section Fragments in vitro: Fragments were assembled *in vitro* using both PCR ligation and traditional T4 DNA ligation. T4 DNA ligation products were subsequently selected by PCR. All amplification was carried out as described above using either Taq polymerase, Vent Polymerase or a 99:1 Taq:PfuTurbo (Stratagene) enzyme mixture. Selection of ligation products was carried out using various serial dilutions of the ligation products as template.

Ligation of 2 or 3 DNA Fragments for Phage Transfection: 4E9 molecules of each DNA fragment was ligated using T4 DNA ligase (NEB) and incubated at 16°C overnight. In certain cases, up to 4E10 molecules of a particular DNA fragment was added to drive the reaction towards the desired outcome.

Preparation of Competent Cells for Phage Transfection: Competent cells were prepared according to Garcia's protocol (Garcia, 1996) and allowed to rest at 4°C for 20-24 hours prior to transfection. Garcia's protocol is reproduced here:

1. Grow 20 ml of cells to a density of 5E8 cells/ml in L broth at the desired temperature.
2. Pellet the cells in a centrifuge at 5,000 rpm for 5 min. Remove the supernatant.
3. Resuspend the cell pellet in 10 ml of ice-cold 50mM CaCl2 (half volume of starting culture).
4. Incubate the cells on ice for 30 min.
5. Pellet the cells in a centrifuge at 5,000 rpm for 5 min. Remove the supernatant.
6. Resuspend the cell pellet in 2 ml of ice-cold 50 mM CaCl2 (one-tenth volume of the starting culture). The cells are ready to take up DNA.

Transfection of the Ligation Products: All pipette tips were pre-chilled at -20°C, molten 0.7% T-agar was kept at 46°C and 1.5% T-agar plates were equilibrated to room temperature. The ligation mixture was added to 200uL of cold (4°C) competent cells and

incubated in an ice bath for 30 minutes. The mixture was then added to 2.5mL of molten (46°C) 0.7% T-agar, gently mixed for 10 seconds by manual agitation and poured onto a 1.5% T-agar plate. The plates were then incubated at 37°C for 3-5 hours.

Plating of T7: T7 was plated by adding various dilutions of a phage stock to 200uL of saturated BL21 culture and 3mL of molten (46°C) 0.7% T-agar and pouring the mixed contents onto 1.5% T-agar plates. Plaques appeared after 3-5 hours of incubation at 37°C.

Isolation of T7 Genomic DNA From Crude Cell Lysates: T7 genomic DNA was isolated according to Garcia's protocol (Garcia, 1996):

1. Grow 40 ml of permissive cells to a density of 1E8-1E9 cells/ml at 37°C in a rotary shaking water bath. Inoculate the cells with a drop from a master phage stock. Continue to shake cells in the water bath at 37°C until the culture clarifies.
2. Add NaCl to a final concentration of 1 molar. Centrifuge the lysate at 10,000 rpm for 10 min. Discard the cellular debris, and centrifuge the lysate at 24,000 rpm for 90 min in a SW28 rotor (Beckman).
3. Discard the supernatant, and add 1 ml of T7 buffer or TES buffer to the phage pellet.
4. Let the pellet site at 4°C for at least 5 hours. Resuspend the pellet, and quickly spin down the cellular debris. Discard the pellet.
5. To the supernatant, add 0.5 ml of 50 mM Tris-HCl, pH 8 saturated phenol and gently mix the sample until an emulsion forms. Quickly microfuge the sample to separate the layers. Carefully remove the aqueous layer without disturbing the organic layer. Phenol extract with 0.5 ml 50 mM Tris-HCl, pH 8 saturated phenol one more time.
6. To the aqueous layer, add 0.5 ml of 50mM Tris-HCl, pH 8 saturated phenol: chloroform: isoamyl alcohol (25:34:1 by volume) mixture, and gently mix the sample until an emulsion forms. Quickly microfuge the sample to separate layers. Carefully remove the aqueous layer without disturbing the organic layer. Phenol: chloroform: isoamyl alcohol and extract the aqueous layer one more time.
7. Add 3 times volume of 95% ethanol alcohol to the sample. A fibrous precipitate should form. Spin down the precipitate, remove the supernatant, and wash the pellet with ethanol. Dry the pellet. Dissolve the pellet in 500 uL of water or TES buffer. There should be about 1E9 molecules of phage DNA/uL. Store the DNA at -20°C.

Purification of T7 Particles via CsCl-gradient Centrifugation: T7 particles were purified by cesium chloride gradient centrifugation according to Garcia's protocol (Garcia, 1996):

1. Grow 100ml of permissive cells to a density of 1E8 to 1E9 cells/ml at 37°C in a rotary shaking water bath. Inoculate the cells with a drop from a master phage stock. Continue to shake cells in the water bath at 37°C until culture clarifies. [NOTE – As a standard laboratory protocol, T7 stocks have always been propagated at 30°C; however, at this temperature cultures infected with (A1, A2, A3)- T7 mutants take longer to clarify that those infected with (A1, A2, A3)+ phages or with mutants that eject their DNA faster. At 37°C the differences in lysis periods are not as pronounced. Stocks of (A1, A2, A3)- T7 mutants are propagated at 37°C to decrease the growth disadvantage of spontaneous arising mutants that eject their DNA faster. For constancy (A1, A2, A3)+ phages are also grown at this higher temperature.]
2. Add NaCl to the lysate to make the final concentration 1 molar. Centrifuge the lysate at 10,000 rpm for 10 min, Discard the cellular debris, and add 10 grams polyethylene glycol (PEG) m.w. 8000 (10% w/v) to the supernatant. Gently stir the mixture until the PEG has totally dissolved. Keep lysate on ice for 1 hour.

3. Pellet the phage at 5,000 rpm for 15 min. Decant the supernatant, and very gently resuspend the pellet in 3.5 ml of T7 buffer. Centrifuge the lysate at 5,000 rpm for 10 min, and keep the supernatant.
4. Pour a cesium chloride step gradient: ass .5 ml of cesium chloride with a density of 1.6 to the bottom of a centrifuge tube that fits in a SW 40.1 rotor. Gently layer 0.5 ml of cesium chloride ρ=1.5 onto the ρ=1.6 layer. Finally ass 0.5 ml of cesium chloride ρ=1.4 onto the ρ=1.5 layer.
5. Gently layer the phage supernatant onto the cesium chloride step gradient. Centrifuge the phage in a SW 50.1 rotor at 30,000 rpm for 2 to 3 hours. The phage will band at the ρ=1.5 layer.
6. Remove the phage band from the side of the tube with a syringe.
7. Remove the cesium chloride by dialysis against 0.5 to 1 liter of T7 buffer at 4°C.

Purification of T7 Genomic DNA From CsCl-gradient Purified Phage Particles: T7 particles were purified by cesium chloride gradient centrifugation (above). The DNA was then purified by subsequent rounds of phenol and phenol:chloroform extraction as follows: pH7.8 phenol (Sambrook & Russell, 2001) was added in a 1:1 volume ratio to the sample and the tube was inverted to mix the aqueous and organic phases. The mixture was centrifuged for 10 minutes at 13,000g. The aqueous layer was removed and subjected to an additional round of phenol extraction. This resulting aqueous layer was added in a 1:1 volume ratio to pH7.8 phenol:chloroform:isoamyl alcohol (25:24:1, Sambrook and Russell:A1.23), mixed and centrifuged for 5 minutes. This extraction step was repeated again. The DNA was precipitated by adding a 10% sample volume of 3M sodium acetate and 2-5 sample volumes of cold (4°C) absolute ethanol. The samples were mixed and incubated at -80°C for 1 hour. The DNA was then pelleted by centrifugation for 30 minutes at 13,000g and at 4°C. The DNA pellet was washed once with 80% ethanol, dried and resuspended in TE buffer.

Purification of Restriction Enzyme Digested Fragments: All restriction enzyme digestions were carried out according to the manufacturer's directions. All fragments smaller than 10kb were purified using Qiaquick gel extraction kit (Qiagen). All fragments larger than 10kb were purified by electro-elution as follows: 20ug of digested product was preincubated with 1uL of a 1000x solution of SYBR Gold (Molecular probes) for 15 minutes. 200ng of DNA was loaded into each well of a 0.5% TAE agarose gel and electrophoreised at 1-1.5V/cm and at 4°C for 16-20 hours. Agarose blocks containing desired restriction fragments were excised under UV transillumination and loaded into a dialysis bag (3500 MWCO Snakeskin dialysis tubing, Pierce) containing 1X TAE. Fragments were electro-eluted from the agarose blocks for 1-5 hours at 5V/cm. After the completion of electro-elution was confirmed by UV visualization, the electric field was reversed for 1 minute to aid in elution. The liquid contents of the bag were then subjected to one round of phenol extraction to remove trace amounts of agarose and the DNA was ethanol precipitated and resuspended in TE buffer.

Plating of Phage for Comparative Plaque Analysis: Stocks of cesium chloride purified phage were serially diluted to an appropriate titer. 50, 100 or 200uL of that dilution was mixed with 200uL of saturated BL21 culture, added to 12mL of molten (50°C) 0.7% T-agar and plated directly on Petri dishes. Plaques were allowed to grow for 5-48 hours at 30°C or 37°C.

Measuring Phage Lysis Curves: 1mL containing 2E8 cells of BL21 was infected at a MOI of 5 and 200uL of the resulting mixture was loaded per well into a 96 well ViewPlate (Packard) at 30°C. Mineral oil was layered into each well and the OD was monitored at 30°C with agitation by a Wallac Victor2 plate reader (Perkin-Elmer). The half-lysis time was taken at the time when the absorbance of a culture equals the average of its absorbance at (i) the time of infection and (ii) the end of lysis.

Template preparation of phage genomic DNA for sequencing: Only full length packaged genomic DNA (preparation described above) was used as template.

Template preparation of cloned parts for sequencing: The preparation of sequencing template for cloned parts was done using Qiaprep spin Miniprep Kit (Qiagen). When the quantity of purified plasmid was insufficient for sequencing, a subsequent TempliPhi (Amersham) reaction was used to amplify the sequencing template.

Template preparation of in-vitro constructs for sequencing: *In-vitro* constructs were amplified using PCR, gel purified and used as template in a sequencing reaction.

Sequencing of DNA: All sequencing was performed by the MIT Biopolymers Laboratory using a Perkin Elmer Applied Biosystems Division model 377 DNA sequencer. When long regions of DNA were sequenced, primers were designed at 500-800bp intervals to both sense and antisense strands. All reported sequence represents at least two separate sequence runs with no intervening ambiguities.

Sequencing Analysis and Contig Assembly: Sequence analysis and contig assembly was done with AlignX and Contig Express (InforMax).

Strains & Media

Escherichia coli:
BL21: B *hsdS Gal-*
BR3: B *rpoC-E2258K*
D1210: HB101 *lacIq*
DH5alpha: ϕ80*lacZΔM15* Δ*(lacZYA-argF) U169 endA1 recA1 hsdR17 (rk-, mk+) thi-1 gyrA96 relA1 phoA*
DH10B: *mcrA* Δ(*mrr-hsdRMS-mcrBC*) ϕ80*lacZ*ΔM15 Δ*lacX74 deoR recA1 araD139* Δ(*ara leu*)7697 *galU galK rpsL endA1 nupG*
IJ1126: *E. coli* K-12 *recB21 recC22 sbcA5 endA gal thi* Su+ Δ(*mcrC-mrr*)*102::*Tn*10*
IJ1127: IJ1126 *lacUV5 lacZ::*T7 *gene1*-Knr

Bacteriophage:
$T7^+$, wild-type bacteriophage T7 (gift of Ian J. Molineux). The T7 stock used in this study descends from a stock maintained by the following labs: M. Delbrück, R. L. Sinsheimer, F. W. Studier, and I. J. Molineux.

Media:

L-broth or LB Medium (Luria-Bertani Medium) (Sambrook & Russell, 2001)

10 g Bacto-tryptone
5 g yeast extract
10 g NaCl
distilled water up to 1 L

1.5% T-agar (Garcia, 1996)

10 g Bacto-Tryptone
5 g NaCl
15 g Bacto-agar
distilled water up to 1 L

0.7% T-agar (Garcia, 1996)

10 g Bacto-Tryptone
5 g NaCl
7 g Bacto-agar
distilled water up to 1 L

50X TAE Electrophoresis Buffer (Sambrook & Russell, 2001)

242 g Tris base
57.1 ml glacial acetic acid
100 ml 0.5 EDTA pH 8.0
distilled water up to 1 L

T7 Buffer (Garcia, 1996)

0.1 M Tris-HCl pH 7.5
1 M NaCl
1 mM EDTA pH 7.5

TES Buffer (Garcia, 1996)

50 mM NaCl
50 mM Tris-HCl pH 7.5
5mM EDTA pH 7.5

ρ=1.43 cesium chloride (Garcia, 1996)

33 g cesium chloride
50 ml 10 mM Tris-HCL pH 7.5, 10mM MgCl-2

ρ=1.53 cesium chloride (Garcia, 1996)

41 g cesium chloride
50 ml 10 mM Tris-HCL pH 7.5, 10mM MgCl-2

ρ=1.62 cesium chloride (Garcia, 1996)

50 g cesium chloride
50 ml 10 mM Tris-HCL pH 7.5, 10mM MgCl-2

TE (Sambrook & Russell, 2001)

10mM Tris-Cl pH 8.0
1mM EDTA pH 8.0

Supplementary References

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Sambrook J, Russell DW (2001) Molecular Cloning: A Laboratory Manual, 3rd ed. Cold Spring Harbor Laboratory Press.

Vincze T, Posfai J, Roberts RJ (2003) NEBcutter: A program to cleave DNA with restriction enzymes. Nucleic Acids Res. 31: 3688-91.