

Supplementary Table 1: APE gene assembly oligomers

Oligomer Name	Sequence (5' to 3')
Oligo1Finger3 (O1F3)	ttgcgacatctgcggtcgtaaattcgctXXXXXXXXXXXXXXXXXXXXXcacacccaaaatccacctgcgctcaga
Oligo2Linker3-2 (O2link3-2)	cacatccgtacccacaccggtgaaaaaccggttcgcttgcgacatctgcggtcgtaaatttc
Oligo3Finger2 (O3F2)	CCAatgtagaatttgtatgagaaatttctctXXXXXXXXXXXXXXXXXXXXXcacatccgtacccacaccggtgaaa
Oligo4Linker2-1 (O4link2-1)	catattagaattcatactggacaaaAACCATTCCAatgtagaatttgtatgagaaatttc
Oligo5Finger1 (O5F1)	tgaatcttgcgaccgctgctttctctXXXXXXXXXXXXXXXXXXXXXcatattagaattcatactggacaaa
Biotinylated seed oligo	biotin-tctgacgcaggtggattttggtgtg
Assembly check-f (5'chk3-fin1)	tgaatcttgcgaccgctgctttctct
Assembly check-r (zif268-3'chk)	tctgacgcaggtggattttggtgtg

Where xxxxx indicates location of 21 nt coding for 7 amino acids in positions -1 to 6 of recognition helix.

Supplementary Table 2: Oligomers for generation of linear expression template with GFP fusion

Oligomer Name	Sequence (5' to 3')
yEmCitrine-R1-f	CCTCTAGAAATAATTTTGTAACTTAAGAAGGAGGAAAAAAAAatgtctaaagtggaagaattattcac
yEmCitrine-r	GTAGCAGCCTGAGTCGTTATTATTTGTACAATTCATCCATACCATGG
Proline-linker-EGFP-f	cacacccaaaatccacctgcgctcagaaagaccagcgcagccatctaaagtggaagaattattcac
EGFP-His6-r	GTAGCAGCCTGAGTCGTTATTATgatgatgatgatgatgagaaacccccTTTGTACAATTCATCCATACCATGG
Genespecific-f	AGAAATAATTTTGTAACTtaagaaggaggaaaaaaaaaattggaacgctcgtacgcttgcggtggaatcttgcgaccgctgctttctct
Extension-f	gatcttaaggctagagtacTAATACGACTCACTATAGGGAGACCACAACGGTTTCCTCTAGAAATAATTTTGTAACTtaagaagga
Extension-r	CAAAAAACCCCTCAAGACCCGTTTAGAGGCCCAAGGGGTTATGCTAGGTAGCAGCCTGAGTCG
5'final_highTm	Cy3-gatcttaaggctagagtacTAATACGACTCACTATAGGG
3'final_highTm	CAAAAAACCCCTCAAGACCCGTTTAGAG

Supplementary Table 3: Error rate analysis for APE synthesis

ZF synthesis method (polymerase used for extension)	# of full-length sequence reads (out of 32)	Total kb of interest sequence	# deletion events	# insertion events	# substitution events	Total error events	Error/kb
Phusion -gel stab	32	7.648	4	2	0	6	0.785
Phusion +gel stab	32	7.648	5	0	1	6	0.785
Klenow +gel stab	28	6.692	15	1	1	17	2.54

Supplementary Table 4: Klenow extension, Cy5-labelled target oligomer design

Oligomer Name	Sequence (5' to 3')
5'CompCy5	Cy5-GTCATACCGCCGGA
Target design	GGCCAAT X XXX XXX XXX X TTTCCGGCGGTATGAC

Where xxxx indicates location of target sequence (11 nt target = 9 nt binding site with flanking nt on each end)

Supplementary Table 5: Microarray robotic printing routine

Step	Printing reagent	Stamps /spot	Stamps /ink	Humidity	Wash frequency
1 (prespot)	0.5% BSA in water	1	4	45-55%	No same-sample wash, 1 source well per linear template
2	Linear template printing (6 µL final PCR product + 64 µL 2% BSA in water)	2	4	45-55%	Wash after 32 inks
3 (overspot)	0.5% BSA in water	1	4	45-55%	No same-sample wash, 1 source well per linear template
4	Cy5-DNA target printing (10 µL Klenow product + 60 µL 2% BSA in water)	3	3	45-55%	Wash after 32 inks

Supplementary Table 6: Gene synthesis technique comparison (for Figure 1d)

year	Previously reported error rates (publication reference)	Assembly technique	Before
2004	PA Carr et al, Protein mediated error correction for de novo DNA synth, NAR 2004	PCR assembly	1.8 error/kb
2004	Xiong A-S et al, A simple, rapid, high fidelity and cost-effective PCR-based 2step DNA synthesis method for long gene sequences, NAR 2004	PCR assembly PTDS	0.84-6.72 error/kb
2005	Binkowski BF et al, Correcting errors in synthetic DNA through consensus shuffling, NAR 2005	PCR assembly	0.98-1.3 errors/kb
2005	Fuhrmann M et al, Removal of mismatched bases from synthetic genes by enzymatic mismatch cleavage, NAR 2005	ligation	5.8-7.2 error/kb
2006	Xiong A-S et al, PCR-based accurate synthesis of long DNA sequences, NatureProtocols 2006	PCR assembly	<1 error/kb
2007	Kong DS et al, Parallel gene synthesis in a microfluidic device, NAR 2007	PCR	1.8-2.2 error/kb
2007	Mamedov TG et al, Rational denovo gene synth by rapid PCA and expression of endothelial protein-c and thrombin receptor genes, J Biotechnol 2007	PCR, PCA, fastPCA	0.53-1.1 error/kb
2008	Marsic D et al, PCR-based gene synthesis to produce recombinant proteins for crystallization, BMC Biotech 2008	SeqTBIO	1-3 error/kb
2009	Ye H et al, Experimental analysis of gene assembly with TopDown one-step real-time gene synth, NAR 2009	PCA assembly	no consideration of error rate
2010	Matzas M et al, High-fidelity gene synthesis by retrieval of sequence-verified DNA identified using HT pyrosequencing, Nature Biotech 2010	PCR assembly	25 error/kb (starting oligomers)
2010	Kosuri S et al, Scalable gene synth by selective amplification of DNA pools from highfidelity microchips, Nat Biotech 2010	PCR assembly	0.67-0.88 errors/kb
2010	Borovkov AY et al, High-quality gene assembly directly from unpurified mixtures of microarray-synthesized oligos, NAR 2010	Ligation, Overlapping PCR	2.7-2.9 errors/kb
2011	Quan J et al, Parallel onchip gene synth and application to optimization of protein expression, Nat Biotech 2011	PCA	1.9 error/kb
2012	Saaem I et al, Error correction of microchip synthesized genes using Surveyor nuclease, NAR 2012	nSDA-PCA, PCR	1.9 error/kb
2012	Schwartz JJ et al, Accurate gene snthesis with tag-directed retrieval of sequence-verified DNA molecules, Nat Methods, 2012	Dialout PCR	
2012	Ma S et al, Error Correction in gene synthesis technology, Trends Biotechnol, 2012	various	
2013	Dormitzer PR et al, Synthetic generation of influenza vaccine viruses for rapid response to pandemics, Science translation med, 2013	Ligation+PCA	0.64-0.75errors/kb
2014	Wan W et al, Error removal in microchip-synthesized DNA using immobilized MutS, NAR 2014	PCR	11.44-14.25/kb
2014	Currin A, et al, SpeedyGenes: an imporoved gene syntehsis method for efficient production of error-corrected, synthetic protein libraries for directed evolution, Protein Engineering, Design & Selection 2014	PCR	functional colony output, no seq data
2015	APE solid phase gene synthesis (this publication)	APE	0.78error/kb (with Phusion polymerase)

Supplementary Table 7: DNA target sequences for ZF combinatorics (Figure 1f)

Oligomer Name	Sequence (5' to 3')
target_BAA	GGC CAA TTT GAG TGG GCG TTT TCC GGC GGT ATG AC
target_CAA	GGC CAA TTA GAT TGG GCG TTT TCC GGC GGT ATG AC
target_DAA	GGC CAA TTT GTA TGG GCG TTT TCC GGC GGT ATG AC
target_ABA	GGC CAA TTA GCG GAC GCG TTT TCC GGC GGT ATG AC
target_ACA	GGC CAA TTA GCG GTA GCG TTT TCC GGC GGT ATG AC
target_ADA	GGC CAA TTA GCG GAT GCG TTT TCC GGC GGT ATG AC
target_AAB	GGC CAA TTA GCG TGG GTG TTT TCC GGC GGT ATG AC
target_AAC	GGC CAA TTA GCG TGG GCC TTT TCC GGC GGT ATG AC
target_AAD	GGC CAA TTA GCG TGG GGA GTT TCC GGC GGT ATG AC
target_ABB	GGC CAA TTA GCG GAC GTG TTT TCC GGC GGT ATG AC
target_CBB	GGC CAA TTA GAT GAC GTG TTT TCC GGC GGT ATG AC
target_DBB	GGC CAA TTT GTA GAC GTG TTT TCC GGC GGT ATG AC
target_BAB	GGC CAA TTT GAG TGG GTG TTT TCC GGC GGT ATG AC
target_BCB	GGC CAA TTT GAG GTA GTG TTT TCC GGC GGT ATG AC
target_BDB	GGC CAA TTT GAG GAT GTG TTT TCC GGC GGT ATG AC
target_BBA	GGC CAA TTT GAG GAC GCG TTT TCC GGC GGT ATG AC
target_BBC	GGC CAA TTT GAG GAC GCC TTT TCC GGC GGT ATG AC
target_BBD	GGC CAA TTT GAG GAC GGA GTT TCC GGC GGT ATG AC
target_ACC	GGC CAA TTA GCG GTA GCC TTT TCC GGC GGT ATG AC
target_BCC	GGC CAA TTT GAG GTA GCC TTT TCC GGC GGT ATG AC
target_DCC	GGC CAA TTT GTA GTA GCC TTT TCC GGC GGT ATG AC
target_CAC	GGC CAA TTA GAT TGG GCC TTT TCC GGC GGT ATG AC
target_CBC	GGC CAA TTA GAT GAC GCC TTT TCC GGC GGT ATG AC
target_CDC	GGC CAA TTA GAT GAT GCC TTT TCC GGC GGT ATG AC
target_CCA	GGC CAA TTA GAT GTA GCG TTT TCC GGC GGT ATG AC
target_CCB	GGC CAA TTA GAT GTA GTG TTT TCC GGC GGT ATG AC
target_CCD	GGC CAA TTA GAT GTA GGA GTT TCC GGC GGT ATG AC
target_ADD	GGC CAA TTA GCG GAT GGA GTT TCC GGC GGT ATG AC
target_BDD	GGC CAA TTT GAG GAT GGA GTT TCC GGC GGT ATG AC
target_CDD	GGC CAA TTA GAT GAT GGA GTT TCC GGC GGT ATG AC
target_DAD	GGC CAA TTT GTA TGG GGA GTT TCC GGC GGT ATG AC
target_DBD	GGC CAA TTT GTA GAC GGA GTT TCC GGC GGT ATG AC
target_DCD	GGC CAA TTT GTA GTA GGA GTT TCC GGC GGT ATG AC
target_DDA	GGC CAA TTT GTA GAT GCG TTT TCC GGC GGT ATG AC
target_DDB	GGC CAA TTT GTA GAT GTG TTT TCC GGC GGT ATG AC
target_DDC	GGC CAA TTT GTA GAT GCC TTT TCC GGC GGT ATG AC
target_BCA	GGC CAA TTT GAG GTA GCG TTT TCC GGC GGT ATG AC
target_BDA	GGC CAA TTT GAG GAT GCG TTT TCC GGC GGT ATG AC
target_CBA	GGC CAA TTA GAT GAC GCG TTT TCC GGC GGT ATG AC
target_CDA	GGC CAA TTA GAT GAT GCG TTT TCC GGC GGT ATG AC
target_DBA	GGC CAA TTT GTA GAC GCG TTT TCC GGC GGT ATG AC
target_DCA	GGC CAA TTT GTA GTA GCG TTT TCC GGC GGT ATG AC
target_ACB	GGC CAA TTA GCG GTA GTG TTT TCC GGC GGT ATG AC
target_ADB	GGC CAA TTA GCG GAT GTG TTT TCC GGC GGT ATG AC
target_CAB	GGC CAA TTA GAT TGG GTG TTT TCC GGC GGT ATG AC
target_CDB	GGC CAA TTA GAT GAT GTG TTT TCC GGC GGT ATG AC

target_DAB	GGC CAA TTT GTA TGG GTG TTT TCC GGC GGT ATG AC
target_DCB	GGC CAA TTT GTA GTA GTG TTT TCC GGC GGT ATG AC
target_ABC	GGC CAA TTA GCG GAC GCC TTT TCC GGC GGT ATG AC
target_ADC	GGC CAA TTA GCG GAT GCC TTT TCC GGC GGT ATG AC
target_BAC	GGC CAA TTT GAG TGG GCC TTT TCC GGC GGT ATG AC
target_BDC	GGC CAA TTT GAG GAT GCC TTT TCC GGC GGT ATG AC
target_DAC	GGC CAA TTT GTA TGG GCC TTT TCC GGC GGT ATG AC
target_DBC	GGC CAA TTT GTA GAC GCC TTT TCC GGC GGT ATG AC
target_ABD	GGC CAA TTA GCG GAC GGA GTT TCC GGC GGT ATG AC
target_ACD	GGC CAA TTA GCG GTA GGA GTT TCC GGC GGT ATG AC
target_BAD	GGC CAA TTT GAG TGG GGA GTT TCC GGC GGT ATG AC
target_BCD	GGC CAA TTT GAG GTA GGA GTT TCC GGC GGT ATG AC
target_CAD	GGC CAA TTA GAT TGG GGA GTT TCC GGC GGT ATG AC
target_CBD	GGC CAA TTA GAT GAC GGA GTT TCC GGC GGT ATG AC
target_268wt	GGC CAA TTA GCG TGG GCG TTT TCC GGC GGT ATG AC
target_37-12	GGC CAA TTT GAG GAC GTG TTT TCC GGC GGT ATG AC
target_92-1	GGC CAA TTA GAT GTA GCC TTT TCC GGC GGT ATG AC
target_158-2	GGC CAA TTT GTA GAT GGA GTT TCC GGC GGT ATG AC

Supplementary Table 8: APE synthesis oligomers ZF combinatorics (Figure 1f)

Oligomer Name	Sequence (5' to 3')
Zif268_Oligo1fin3	ttgcgacatctgcggtcgtaaattcgctcgttctgacgaacgtaaacgtcacacccaaaatccacctgcgtcaga
Zif268_Oligo3fin2	CCAatgtagaatttgatgagaaatttctctcgttctgaccacctgaccacccacatccgtaccacaccggtgaaa
Zif268_Oligo5fin1	tgaatcttgcgaccgctggtttctctcgttctgacgaactgaccctcatattagaattcactactggacaaa
37-12Oligo1fin3	ttgcgacatctgcggtcgtaaattcgct CGTCACGACCAGCTGACCCGT cacacccaaaatccacctgcgtcaga
37-12Oligo3fin2	CCAatgtagaatttgatgagaaatttctct GACCGTGCTAACCTGCGTCGT cacatccgtaccacaccggtgaaa
37-12Oligo5fin1	tgaatcttgcgaccgctggtttctct CGTAACTTCATCCTGCAGCGT catattagaattcactactggacaaa
92-1Oligo1fin3	ttgcgacatctgcggtcgtaaattcgct GAACGTGGTAACCTGACCCGT cacacccaaaatccacctgcgtcaga
92-1Oligo3fin2	CCAatgtagaatttgatgagaaatttctct CAGCGTTCTTCTCTGGTTCGT cacatccgtaccacaccggtgaaa
92-1Oligo5fin1	tgaatcttgcgaccgctggtttctct GACTCTCCGACCCGTCGT catattagaattcactactggacaaa
158-2Oligo1fin3	ttgcgacatctgcggtcgtaaattcgct CAGTCTACCTCTCTGCAGCGT cacacccaaaatccacctgcgtcaga
158-2Oligo3fin2	CCAatgtagaatttgatgagaaatttctct GTTTCGTACAACCTGACCCGT cacatccgtaccacaccggtgaaa
158-2Oligo5fin1	tgaatcttgcgaccgctggtttctct GACAAAACCAAACCTGCGTGT catattagaattcactactggacaaa