SUPPLEMENTARY INFORMATION

pϕ29 Construction

A) PCR of DNAP

1.5 ul p29_DNAP_GGA_FWD
1.5 ul p29_DNAP_GGA_REV
1 ng template gBlock DNAP
25 ul Q5 2X MasterMix
21 ul water

Cycling Conditions

1. 98 °C for 1 min
2. 98 °C for 10 sec
3. 64 °C for 20 sec
4. 72 °C for 20 sec
   Go to step 2, 30X
5. 72 °C for 5 min
   12 °C forever

B) PCR of TPBP

1.5 ul p29_TPBP_GGA_FWD
1.5 ul p29_TPBP_GGA_REV
1 ng template gBlock TPBP
25 ul Q5 2X MasterMix
21 ul water

Cycling Conditions

1. 98 °C for 1 min
2. 98 °C for 10 sec
3. 66 °C for 20 sec
4. 72 °C for 20 sec
   Go to step 2, 30X
5. 72 °C for 5 min
   12 °C forever

C) PCR of pSEVA224

1.5 ul pSEVA224_FWD
1.5 ul pSEVA224_REV
0.5 ng template pSEVA224
25 ul Q5 2X MasterMix
21 ul water

Cycling Conditions

1. 98 °C for 1 min
2. 98 °C for 10 sec
3. 69 °C for 20 sec
4. 72 °C for 60 sec
   Go to step 2, 30X
5. 72 °C for 5 min
   12 °C foreve

D) BsaI Digestion of Insert Sequences: DNAP and TPBP

37°C for 1 hour
5 ul NEB CutSmart buffer
1 ul BsaI
14 ul water
20 ul DNA (0.48 pMol DNAP or 1 pMol TPBP – in separate reactions)

E) Ligation of Insert Sequences: DNAP and TPBP

25°C for 1 hour
15 ul 2X T7 Ligase Buffer
0.087 pmol TPBP
0.087 pmol DNAP
18.72 ul water

1 ul T7 ligase

F) BsaI/rSAP pSEVA224 Backbone Digestion
37°C for 1 hour
1000 ng SEVA224
5 ul NEB CutSmart buffer
1 ul BsaI
35 ul H_2O
1 ul rSAP, added 30 minutes into the digestion

G) pϕ29 Golden Gate Assembly (GGA)
0.06 pmol pSEVA224-rSAP
0.12 pmol TPBP-DNAP
2.5 ul T4 ligase buffer
0.25 ul T4 ligase
0.75 BsaI-HFv2
7.87 ul water

Cycling Conditions
1. 37 °C for 5 min
2. 16 °C for 5 min
   Go to step 1, 30x
3. 80 °C for 20 min
4. 12 °C forever

H) PCR to amplify DNAP-TPBP insert off pϕ29 GGA product
1.5 ul PTRC5_FWD
1.5 ul M13_R24_REV
0.5 ng template pϕ29
25 ul Q5 2X MasterMix
2.5 ul water

Cycling Conditions
1. 98 °C for 1 min
2. 98 °C for 10 sec
3. 72 °C for 20 sec
4. 72 °C for 35 sec
   Go to step 2, 30X
5. 72 °C for 5 min
   12 °C forever

I) Colony PCR to amplify DNAP-TPBP insert off transformed colonies
11 ul ApexRed MasterMix
8 ul water
0.5 ul PTRC5_FWD
0.5 ul M13_R24_REV
2 ul colony, resuspended in 100 ul water

Cycling Conditions
1. 95 °C for 5 min
2. 95 °C for 30 sec
3. 58 °C for 30 sec
4. 72 °C for 3 min, 30 sec
   Go to step 2, 30X
5. 72 °C for 5 min
   12 °C forever

J) Colony PCR to Sequence DNAP-TPBP insert from transformed colonies
11 ul ApexRed MasterMix
8 ul water
0.5 ul FWD primer
0.5 ul REV primer
2 ul colony, resuspended in 100 ul water

Cycling Conditions
1. 95 °C for 5 min
2. 95 °C for 30 sec
3. 55-60 °C for 30 sec
4. 72 °C for 3 min, 30 sec
   Go to step 2, 30X
5. 72 °C for 5 min
   12 °C forever

Primer Pairs: PTRC5_FWD/ P29_DNAP1_REV, P29_DNAP2_FWD/P29_TPBP2_REV, P29_TPBP3_FWD/ P29_SSBP3_REV, P29_DSBP4_FWD/M13_R24_REV

K) PCR to Insert Missing Sequence

1 ul template
1.5 ul DNAP_Fix_REV
1.5 ul TPBP_Fix_FWD
25 ul Q5 2X MasterMix
21 ul H_2O

Cycling

1. 98 °C for 1 min
2. 98 °C for 10 sec
3. 67 °C for 20 sec
4. 72 °C for 2 min 50 sec
5. Go to step 2, 30X
6. 72 °C for 5 min, 12 °C forever

L) Digestion of Fixed pϕ29

37 °C for 1 hour
1000 ng pHelper_fix
5 ul CutSmart Buffer
1 ul BsaI-HFv2
29.5 ul water
1 DpnI

M) Ligation of Fixed pϕ29

20 °C for 1 hour
2 ul 10X T4 buffer
15 ul (500 ng) pHelper_fix
1 ul T4 ligase
2 ul water

N) Digestion to Validate pHelper

37 °C for 1 hour
1 ug DNA (pHelper_fix, or pHelper, not fixed control)
5 ul 10X CutSmart Buffer
1 ul MlyI
Water to 50 ul

pL Construction

O) PCR of DHFR_R67

1 ul DHFR_R67 template
1.5 ul P29_MIDDLE_FWD
1.5 ul P29_MIDDLE_REV
25 ul Q5 2X MasterMix
21 ul H_2O

Cycling

1. 98 °C for 1 min
2. 98 °C for 10 sec
3. 67 °C for 20 sec
4. 72 °C for 20 sec
5. Go to step 2, 30X
6. 72 °C for 5 min, 12 °C forever

P) PCR of ORI_L

1 ul P29_ORIL template
1.5 ul P29_ORIL_FWD
1.5 ul P29_ORIL_REV
25 ul Q5 2X MasterMix
21 ul H_2O

Cycling

1. 98 °C for 1 min
2. 98 °C for 10 sec
3. 67 °C for 20 sec
4. 72 °C for 20 sec
5. Go to step 2, 30X
6. 72 °C for 5 min, 12 °C forever

Q) PCR of ORI_R

1 ul P29_ORIL template
1.5 ul P29_ORIL_FWD
1.5 ul P29_ORIL_REV
25 ul Q5 2X MasterMix
21 ul H_2O

Cycling

1. 98 °C for 1 min
2. 98 °C for 10 sec
3. 64 °C for 20 sec
4. 72 °C for 20 sec
5. Go to step 2, 30X
6. 72 °C for 5 min, 12 °C forever

R) BspQI Digest ORI_R/L

Incubate at 50 °C for 1 hr
4.6 pmols (400 ng) DNA: ORI_R or ORI_L
5 ul 10X NEB3.1 Buffer
1 ul BspQI
Water to 50 ul

S) BspQI Digest DHFR_R67

Incubate at 50 °C for 1 hr
4.6 pmols (1000 ng) DHFR_R67
5 ul 10X NEB3.1 Buffer
1 ul BspQI
Water to 50 ul

T) Ligation of ORI_R, ORI_L, and DHFR_R67

Incubate at 25 °C for 1 hour
15 ul 2X T7 DNA Ligase Buffer
Equimolar quantities: ORI_L, ORI_R, DHFR_R67
1 ul T7 DNA Ligase
Water to 30 ul

U) Colony PCR to verify presence of pL

11 ul ApexRed MasterMix
8 ul water
0.5 ul DHFR_R67 FWD
0.5 ul DHFR_R67 REV
2 ul colony, resuspended in 100 ul water

Cycling Conditions

1. 95 °C for 5 min
2. 95 °C for 30 sec
3. 58 °C for 30 sec
4. 72 °C for 30 sec
   Go to step 2, 30X
5. 72 °C for 5 min, 12 °C forever

V) PCR to amplify pGS21a

1 ul template (miniprepped pGS21a)
1.5 ul pGS-21a his6-GST-Phi29DNAP FWD
1.5 ul pGS-21a his6-GST-Phi29DNAP REV
25 ul Q5 2X MasterMix
21 ul H_2O

Cycling

1. 98 °C for 1 min
2. 98 °C for 10 sec
3. 64 °C for 20 sec
4. 72 °C for 1 min 20 sec
5. Go to step 2, 30X
6. 72 °C for 5 min, 12 °C forever

W) PCR to amplify TPBP from pϕ29

1 ul template (miniprepped pHelper)
1.5 ul Phi29_TPBP_fix FWD
1.5 ul Phi29_TPBP_fix REV
25 ul Q5 2X MasterMix
21 ul H_2O

Cycling

1. 98 °C for 1 min
2. 98 °C for 10 sec
3. 65 °C for 20 sec
4. 72 °C for 20 sec
5. Go to step 2, 30X
6. 72 °C for 5 min, 12 °C forever

X) PaqCI GGA

37°C for 1 hour
75 ng (0.014 pmol) pGS21a backbone
29 ng (0.028 pmol) TPBP insert
2 ul T4 DNA ligase buffer (10X)
1.5 ul PaqCI
0.5 ul PaqCI activator
2 ul T4 ligase
Water to 20 ul

Y) PCR to amplify TPBP insert off pGS21a-ϕ29 GGA product

1.5 ul GST_DNAP_HIS1_FWD
1.5 ul T7_TERM_REV
0.5 ng template pGS21a-ϕ29
25 ul Q5 2X MasterMix
Water to 50 ul

Cycling Conditions

1. 98 °C for 1 min
2. 98 °C for 10 sec
3. 72 °C for 20 sec
4. 72 °C for 20 sec
   Go to step 2, 30X
5. 72 °C for 5 min, 12 °C forever

Z) Colony PCR to verify pGS21a-ϕ29 GGA

11 ul ApexRed MasterMix
8 ul water
0.5 ul GST_DNAP_HIS1_FWD
0.5 ul T7_TERM_REV
2 ul colony, resuspended in 100 ul water

Cycling Conditions

1. 95 °C for 5 min
2. 95 °C for 30 sec
3. 58 °C for 30 sec
4. 72 °C for 2 min
   Go to step 2, 30X
5. 72 °C for 5 min, 12 °C forever

AA) PCR of DHFR_R67 for SEVA224
1 ul DHFR_R67 template
1.5 ul DHFR_FWD
1.5 ul DHFR_REV
25 ul Q5 2X MasterMix
21 ul H_2O

Cycling
1. 98 °C for 1 min
2. 98 °C for 10 sec
3. 67 °C for 20 sec
4. 72 °C for 20 sec
5. Go to step 2, 30X
6. 72 °C for 5 min, 12 °C forever

Water to 20 ul

AB) PCR of SEVA224 for DHFR_R67
1 ul SEVA224 template
1.5 ul SEVA_FWD
1.5 ul SEVA_REV
25 ul Q5 2X MasterMix
21 ul H_2O

Cycling
1. 98 °C for 1 min
2. 98 °C for 10 sec
3. 67 °C for 20 sec
4. 72 °C for 20 sec
5. Go to step 2, 30X
6. 72 °C for 5 min, 12 °C forever

AC) DpnI Digest SEVA224
Incubate at 37 °C for 1 hr
1200 ng SEVA224
5 ul Cutsmart Buffer
1 ul DpnI
Water to 50 ul

AD) BsaI Digest DHFR_R67
Incubate at 37 °C for 1 hr
1600 ng DHFR_R67
5 ul Cutsmart Buffer
1 ul BsaI
Water to 50 ul

AE) BsaI Digest SEVA224
Incubate at 37 °C for 1 hr
740 ng SEVA224
5 ul Cutsmart Buffer
1 ul BsaI
Water to 50 ul
1 ul rSAP, added at 30 min

AF) Ligation of DHFR_R67 and SEVA224
0.04 pmols DHFR_R67
0.12 pmols SEVA224
2 ul DNA Ligase Buffer
1 T4 ligase

Cycling
1. 16 °C for 1 hour
2. 65 °C for 10 min
3. 10 °C forever

BA) PCR of p1

1 ng p1 DNA
1.5 ul p1_FWD
1.5 ul p1_REV
25 ul QuickStart Mastermix
21 ul water

Cycling

1. 98 °C for 30 sec
2. 98 °C for 10 sec
3. 72 °C for 15 sec
4. 72 °C for 3 min
5. Go to step 2, 30X
6. 72 °C for 10 min, 12 °C forever

BB) PCR of pGSϕ29

0.5 ul pGSϕ29 DNA
1.5 ul pGSϕ29_p1_FWD primer
1.5 ul pGSϕ29_p1_REV primer
25 ul Phusion Mastermix
21.5 ul H_2O

Cycling

1. 98 °C for 1 min
2. 98 °C for 10 sec
3. 67 °C for 20 sec
4. 72 °C for 20 sec
5. Go to step 2, 30X
6. 72 °C for 10 min, 12 °C forever

CA) EP PCR of all ϕ29 proteins off pGSϕ29

10 ul Taq PCR Buffer
2 ul dNTP mix
55 mM $MgCl_2$
0.01 mM or 0.15 mM $MnCl_2$
5 ng pGSϕ29 DNA
3 ul Whole_insert_FWD
3 ul Whole_insert_REV
1 ul Taq Polymerase
65 or 51 uL H_2O

Cycling

1. 95 °C for 1 min
2. 94 °C for 30 sec
3. 58 °C for 30 sec
4. 72 °C for 4 min
5. Go to step 2, 20X
6. 72 °C for 10 min, 4 °C forever

CB) EP PCR of ϕ29 TP + DNAP off pGSϕ29

10 ul Taq PCR Buffer
2 ul dNTP mix
55 mM $MgCl_2$
0.01 mM or 0.15 mM $MnCl_2$
5 ng pGSϕ29 DNA
3 ul DNAPTP_FWD
3 ul DNAPTP_REV
1 ul Taq Polymerase
65 or 51 uL H_2O

Cycling

1. 95 °C for 1 min
2. 94 °C for 30 sec
3. 55 °C for 30 sec
4. 72 °C for 2 min
5. Go to step 2, 20X
6. 72 °C for 10 min, 4 °C forever

CC) PCR protocol for Mutagenesis of ϕ29 TP using Genemorph kit

41.5 ul H2O
5 ul Mutazyme II rxn buffer
40 mM dNTP mix
0.25 ul FWD primer
0.25 ul REV primer
1 ul Mutazyme II DNAP
1 ul DNA

Cycling

1. 95 °C for 2 min
2. 95 °C for 10 sec
3. 66 °C for 30 sec
4. 72 °C for 1 min
5. Go to step 2, 30X
6. 72 °C for 10 min, 4 °C forever

Sequence Information

Phi29 Linear Plasmid (pL) Primers

Name	Sequence
P29_ORIL_FWD	AAAGTAAGCCCCCACCCTCACA
P29_ORIL_FWD_THIO	A*A*AGTAAGCCCCCACCCTCACA
P29_ORIL_REV	GCCGTAAAGCATCAGAAGAGC
P29_ORIR_FWD	TGATGCTTTACGCGAGAAGAGC
P29_ORIR_REV	AAAGTAGGGTACAGCGACAACA
P29_ORIR_REV_THIO	A*A*AGTAGGGTACAGCGACAACA
P29_MIDDLE_FWD	GATCGAGATCGATAGAAGAGCT
P29_MIDDLE_REV	AAAGCGCGCAGAAGAGCAGATA

Phi29 Linear Plasmid g-blocks

Name	Sequence
P29_ORI_L	AAAGTAAGCCCCCACCCTCACATGATACCATTCTCCTAATATCGACATAATCCGTCGATCCTCGGCATACCATGATCAGGGAGGG AAACTACTACTTAATATATCAATCTATAGACCTACTAGATAGGTTTGTCAATGAACAACATAAAACGACACAGAATCCCACGTTTT AGCGCTTCGTCTGTGTCGCAGCTCTTCTGATGCTTTACGGC
P29_ORI_R	TGATGCTTTACGCGAGAAGAGCCCTCCTATGATTGGTTGTCTTATTACCTTACTTCTATTATAGTATAACATGTTAAACGATAGTTT GTCTACCCTTTTCGACAAATTGATGATAATAAATAGTATAGGTATATAGTCGTGATTTAGTTGTTAGATTCTTGTCGAAGATAGTC GGTCAATGGGGAAATGGTGTATGTTGTCGCTGTACCCTACTTT
P29_MIDDLE	GATCGAGATCGATAGAAGAGCTGATGCTTTACGGCTAGCTCAGTCCTAGGTATAGTGCTAGCTACTAGAGAAAGAGGAGAAATA CTAGATGGAACGAAGTAGCAATGAAGTCAGTAATCCAGTTGCTGGCAATTTTGTATTCCCATCGAACGCCACGTTTGGTATGGGA GATCGCGTGCGCAAGAAATCCGGCGCCGCCTGGCAAGGTCAGATTGTCGGGTGGTACTGCACAAATTTGACCCCCGAAGGCTAC GCCGTCGAGTCTGAGGCTCACCCAGGCTCAGTACAGATTTATCCTGTTGCGGCGCTTGAACGCATCAACTGAGTATCTGCTCTTCT GCGCGCTTT

Phi29 Helper Plasmid Golden Gate (pϕ29) Primers

Name	Sequence
SEVA_phi29_GGA_FWD	CCTAAGGGTCTCGAAGGGATCCTCTAGAGTCGACC
SEVA_phi29_GGA_REV	CCTAAGGGTCTCGGCACGGGTACCGAGCTCGAAT
p29_DNAP_GGA_FWD	CCTAAGGGTCTCGGTGCGAGTCCGTAGTAAGGAGC
p29_DNAP_GGA_REV	CCTAAGGGTCTCGTACGATCTACCAGTACAACACC
p29_TPBP_GGA_FWD	CCTAAGGGTCTCCCGTAACAAAGCCGAATACGCTC
p29_TPBP_GGA_REV	CCTAAGGGTCTCCCCTTTTACAGGGACAGCTGTAAG

pϕ29: https://benchling.com/s/seq-Kcrnbh4PZuUCnRfSlw6L

Colony PCR to sequence and validate TPBP-DNAP Insert Primers

Name	Sequence
PTRC5_FWD	CACTGCATAATTCGTGTCGCTCAAGGCG
P29_DNAP1_REV	ACCAGAGGTCCGCAATTTCGCC
P29_DNAP2_FWD	CGCGGTGGTTTTACGTGGCTGA
P29_TPBP2_REV	GAAGCCACCACCCCGTATGCAT
P29_TPBP3_FWD	TACCAAAGCCAAGATCGCGCGC

P29_SSBP3_REV	TCGCGTCCCCGTATGTATCGCT
P29_DSBP4_FWD	GCCCCAGAAGAACAGGTCGCTG
M13_R24_REV	CGCCAGGGTTTTCCCAGTCACGAC

Phi29 Helper Plasmid Missing Sequence Insertion (pϕ29) Primers

Name	Sequence
DNAP_Fix_REV	CTGGTCTCGTTGCAGTCGTCCTTATGAGTCGTG CTTACTTGATCGTGAATGTGTCATCTACCAGTA CAACACCGCC
TPBP_Fix_FWD	CTGGTCTCGGCAAATGGCGCGGAGCCCAAG AATCCGCATTAAAGATAATGACAAAGCCGA ATACGCTCGT

pGS21a-Phi29 Plasmid Primers

Name	Sequence
pGS-21a his6-GST-WTPhi29DNAP_FWD	GGAACTCACCTGCCACTAACTGCTAACAAAGCCCGAA
pGS-21a his6-GST-WTPhi29DNAP_REV	GGAACTCACCTGCCACTTGCCCGGATCTCAGTGGTGGT
Phi29_Plasmid_Fix_FWD	GGAACTCACCTGCACCTGGCACGACTCATAAGGACG
Phi29_Plasmid_Fix_REV	GGAACTCACCTGCACCTAGTTACAGGGACAGCTGTAAG

pGS21a-ϕ29: https://benchling.com/s/seq-pSqC9fjRFhvl5Rw8RFIG

Colony PCR to Validate pGS21a-Phi29 Plasmid Primers

Name	Sequence
GST_DNAP_HIS1_FWD	CCGAAACCGGTGCAAGTTCCG
T7_TERM_REV	TTGCTCAGCGGTGGCAGCAG

DHFR_R67 Primers for insertion in SEVA224

Name	Sequence
DHFR_FWD	GGGAAAGGTCTCGATCTTTACGGCTAGCTCAGTCCTAGGTATAGT
DHFR_REV	GGGAAAGGTCTCGATGAACTCAGTTGATGCGTTCAAGCG

SEVA224 Primers for DHFR_R67 insertion

Name	Sequence
SEVA_FWD	GGGAAAGGTCTCCTCATCACACCCTAGGCCGCGGCC
SEVA_REV	GGGAAAGGTCTCCAGATTCACCACCCTGAATTGACTCTCTTCC

P1 Primers

Name	Sequence
P1_FWD	CCAAAGCACCTGCCATGtgTACTGTAGGGTAAAAAGAGGC
P1_REV	CCAAACCACCTGCGATGgcTTATTTAGCACCGTGCGG

P1: https://benchling.com/calinplesa/f/lib_EWzgbIm4-akp1/seq_JlVtOZ14-akp1-9371-9656/edit

pGSϕ29 Primers for p1 insertion: site 1 (between DSBP and SSBP)

Name	Sequence
pGSϕ29_p1_FWD_1	CCAAACCACCTGCCTAGAAGCCTACAGACCTTAAGGAGGAACTACGAT
pGSϕ29_p1_FWD_1	CCAAACCACCTGCCTAGTACATTATTCAGCGACCTGTTCTTCTGGGG

pGSϕ29 Primers for p1 insertion: site 1 (between TP and DSBP)

Name	Sequence
pGSϕ29_p1_FWD_2	CCAAACCACCTGCCTAGAAGCACTCGAGACAGGGAGGAACCA
pGSϕ29_p1_FWD_2	CCAAACCACCTGCCTAGTACAAGAAGCCTTTCAGGCTTAAATCAAAGTCGC

Primers for Error-Prone PCR of all four ϕ29 proteins off pGSϕ29

Name	Sequence
Whole_insert_FWD	CCTTGACACCTGCAACCtgtgcaacccactcgacttcttttgga
Whole_insert_REV	CCTTGACACCTGCAACCtcagcttcctttcgggctttgttagca

Primers for PCR of pGSϕ29 backbone for insertion of mutagenized ϕ29 proteins

Name	Sequence
pGS_for_Whole_insert_FWD	CCTTGACACCTGCGCAActgagttggctgctgccaccg
pGS_for_Whole_insert_REV	CCTTGACACCTGCGCAAcacaaggcccttaattttccaataacctagtataggg

Primers for Error-Prone PCR of ϕ29 TP+DNAP off pGSϕ29

Name	Sequence
DNAPTP_FWD	GCACATCACCTGCGGAAtggcctttgcagggctggcaa
DNAPTP_REV	GCACATCACCTGCGGAACCTTTCAGGCTTAAATCAAAGTCGCCG

Primers for PCR of pGSϕ29 backbone for insertion of mutagenized ϕ29 TP+DNAP

Name	Sequence
pGS_for_DNAPTP_FWD	CCTTGACACCTGCGCAActgagttggctgctgccaccg
pGS_for_DNAPTP_REV	CCTTGACACCTGCGCAAcacaaggcccttaattttccaataacctagtataggg

Primers for Error-Prone PCR of ϕ29 TP off pGSϕ29

Name	Sequence
TP_FWD	GACCATCACCTGCCGTTTGACAAAGCCGAATACGCTCGTTTGG
TP_REV	GACCATCACCTGCCGTTAGTTAGAAGCCTTTCAGGCTTAAATCAAAGTCGC

Primers for PCR of pGSϕ29 backbone for insertion of mutagenized ϕ29 TP

Name	Sequence
pGS_for_TP_FWD	GACCATCACCTGCACTGAACTGACTCGAGACAGGGAGGAACCA
pGS_for_TP_REV	GACCATCACCTGCACTGGTCATTATCTTTAATGCGGATTCTTGGGCT