NOTEBOOK

ENTRY: [P01]

1. Into a PCR tube, add:

	Sample 1
RCA linear template (v1) (100 µM stock)	2 µl
Linear scaffold DNA (v1) (100 µM stock)	2 µl
ddH_2O	13 µl
TOTAL	**17 µl**

2. Incubate the reaction mixture at 95°C for 5 minutes, set the thermocycler to gradually cool down to 25°C.
3. Into the same PCR tube, add:

	Sample 1
Reaction mixture from step 1	17 µl
10X T4 Ligase Buffer	2 µl
T4 Ligase	1 µl
TOTAL	**20 µl**

4. Incubate at 22°C for 15 minutes.
5. Perform oligonucleotide purification protocol to the reaction mixture, and elute with 17 µl ddH_2O preheated at 65°C.
6. Into a new PCR tube, add:

	Sample 1
Oligonucleotide purification product	17 µl
10X Exonuclease I reaction buffer	2 µl
Exonuclease I	1 µl
TOTAL	**20 µl**

7. Incubate at 37°C for 2 hours.
8. Perform oligonucleotide purification protocol to the reaction mixture, and elute with 20 µl ddH2O preheated at 65°C.
9. Measure the sample concentration and dilute to 1 ng/µl.

ENTRY: [P02]

1. Into the PCR tubes, add:

	(1) (+) With IDT primer	**(2) (-) Without primer**
ddH_2O	12 µl	14 µl
rCutSmart Buffer	2 µl	2 µl

dNTP mix (10 mM each, stock)	2 µl	2 µl
RCA template (v1) (1 ng/µl stock)	1 µl	1 µl
RCA primer (10 µM stock)	2 µl	-
TOTAL	**19 µl**	**19 µl**

2. Incubate at 95°C for 5 minutes, and then return to ice.
3. Add 1 µl of phi29 DNA polymerase to each tube.
4. Incubate at 30°C for 2 hours.
5. Perform heat inactivation at 65°C for 10 minutes.
6. Run 10 µl of each sample to gel electrophoresis using 0.8% Agarose/TAE gel with RedSafe.

ENTRY: [P03]

1. Into each PCR tubes, add:

	(1)	**(2)**	**(-) control**	**(+) control**
10X G-Quadruplex buffer	12.5 µl	12.5 µl	12.5 µl	12.5 µl
G-Quadruplex sample	10 µl from (1) (+) With IDT primer	10 µl from (2) (-) Without primer	10 µl from failed RCA	6.25 µl from IDT G-Quad 8 µM stock
Hemin, in DMSO (1 mM stock)	1 µl	1 µl	1 µl	1 µl
ddH_2O	98.5 µl	98.5 µl	98.5 µl	102.25 µl
TMB (50 mM stock in DMSO)	1.5 µl	1.5 µl	1.5 µl	1.5 µl
H_2O_2	1.5 µl	1.5 µl	1.5 µl	1.5 µl
TOTAL	**125 µl**	**125 µl**	**125 µl**	**125 µl**

ENTRY: [P04]

1. Into a PCR tube, add:

	Sample 1
RCA linear template (v2) (100 µM stock)	2 µl
Linear scaffold DNA (v2) (100 µM stock)	2 µl
ddH_2O	13 µl
TOTAL	**17 µl**

2. Incubate the reaction mixture at 95°C for 5 minutes, set the thermocycler to gradually cool down to 25°C.
3. Into the same PCR tube, add:

	Sample 1

Reaction mixture from step 1	17 µl
10X T4 Ligase Buffer	2 µl
T4 Ligase	1 µl
TOTAL	**20 µl**

4. Incubate at 22°C for 15 minutes.
5. Perform oligonucleotide purification protocol to the reaction mixture, and elute with 17 µl ddH_2O preheated at 65°C.
6. Into a new PCR tube, add:

	Sample 1
Oligonucleotide purification product	17 µl
10X Exonuclease I reaction buffer	2 µl
Exonuclease I	1 µl
TOTAL	**20 µl**

7. Incubate at 37°C for 2 hours.
8. Perform oligonucleotide purification protocol to the reaction mixture, and elute with 20 µl ddH2O preheated at 65°C.
9. Measure the sample concentration and dilute to 1 ng/µl.

ENTRY: [P05]

1. Into the PCR tubes, add:

	(1) (+) With IDT primer	**(2) (-) Without primer**
ddH_2O	12 µl	14 µl
rCutSmart Buffer	2 µl	2 µl
dNTP mix (10 mM each, stock)	2 µl	2 µl
RCA template (v2) (1 ng/µl stock)	1 µl	1 µl
RCA primer (10 µM stock)	2 µl	-
TOTAL	**19 µl**	**19 µl**

2. Incubate at 95°C for 5 minutes, and then return to ice.
3. Add 1 µl of phi29 DNA polymerase to each tube.
4. Incubate at 30°C for 2 hours.
5. Perform heat inactivation at 65°C for 10 minutes.
6. Run 10 µl of each sample to gel electrophoresis using 0.8% Agarose/TAE gel with RedSafe.

ENTRY: [P06]

1. Into a PCR tube, add:

	Sample 1
RCA linear template (v3) (100 µM stock)	2 µl
Linear scaffold DNA (v3) (100 µM stock)	2 µl
ddH_2O	13 µl
TOTAL	**17 µl**

2. Incubate the reaction mixture at 95°C for 5 minutes, set the thermocycler to gradually cool down to 25°C.
3. Into the same PCR tube, add:

	Sample 1
Reaction mixture from step 1	17 µl
10X T4 Ligase Buffer	2 µl
T4 Ligase	1 µl
TOTAL	**20 µl**

4. Incubate at 22°C for 15 minutes.
5. Perform oligonucleotide purification protocol to the reaction mixture, and elute with 17 µl ddH_2O preheated at 65°C.
6. Into a new PCR tube, add:

	Sample 1
Oligonucleotide purification product	16.8 µl
10X Exonuclease I reaction buffer	2 µl
Exonuclease I	1 µl
Triton X-100	0.2 µl
TOTAL	**20 µl**

7. Incubate at 37°C for 2 hours.
8. Perform oligonucleotide purification protocol to the reaction mixture, and elute with 20 µl ddH2O preheated at 65°C.
9. Measure the sample concentration and dilute to 1 ng/µl.

ENTRY: [P07]

1. Into the PCR tubes, add:

	(1) (+) Triton & ExoI-treated, with IDT primer	**(2) (-) Triton & ExoI-treated, without primer**	**(3) (+) ExoI-treated, with IDT primer**	**(4) (-) ExoI-treated, without primer**
ddH_2O	12 µl	14 µl	12 µl	14 µl
rCutSmart Buffer	2 µl	2 µl	2 µl	2 µl

dNTP mix (10 mM each, stock)	2 µl	2 µl	2 µl	2 µl
RCA template (v3) (1 ng/µl stock)	1 µl (Triton-100 and ExoI-treated)	1 µl (Triton-100 and ExoI-treated)	1 µl (ExoI-treated)	1 µl (ExoI-treated)
RCA primer (10 µM stock)	2 µl	-	2 µl	-
TOTAL	**19 µl**	**19 µl**	**19 µl**	**19 µl**

2. Incubate at 95°C for 5 minutes, and then return to ice.
3. Add 1 µl of phi29 DNA polymerase to each tube.
4. Incubate at 30°C for 2 hours.
5. Perform heat inactivation at 65°C for 10 minutes.
6. Run 10 µl of each sample to gel electrophoresis using 0.8% Agarose/TAE gel with GelGreen.

ENTRY: [P08]

1. Into a PCR tube, add:

	Sample 1
RCA linear template (v3) (100 µM stock)	2 µl
Linear scaffold DNA (v3) (100 µM stock)	2 µl
ddH_2O	13 µl
TOTAL	**17 µl**

2. Incubate the reaction mixture at 95°C for 5 minutes, set the thermocycler to gradually cool down to 25°C.
3. Into the same PCR tube, add:

	Sample 1
Reaction mixture from step 1	17 µl
10X T4 Ligase Buffer	2 µl
T4 Ligase	1 µl
TOTAL	**20 µl**

4. Incubate at 22°C for 15 minutes.
5. Perform oligonucleotide purification protocol to the reaction mixture, and elute with 18 µl ddH_2O preheated at 65°C.
6. Into a new PCR tube, add:

	Sample 1
ddH_2O	70.5 µl
Oligonucleotide purification product	18 µl
10X Exonuclease III buffer	10 µl
Exonuclease III (TaKaRa Bio)	0.5 µl

Exonuclease I (Thermo Fisher Scientific)	1 µl
TOTAL	**100 µl**

7. Incubate at 37°C for 1 hour.
8. Perform oligonucleotide purification protocol to the reaction mixture, and elute with 20 µl ddH2O preheated at 65°C.
9. Measure the sample concentration and dilute to 1 ng/µl.

ENTRY: [P09]

1. Into the PCR tubes, add:

	(1) (+) ExoI & ExoIII-treated, with IDT primer, cut with Nt.BsmAI	**(2) (+) ExoI & ExoIII-treated, with IDT primer**	**(3) (-) ExoI & ExoIII-treated, without primer**
ddH_2O	11.5 µl	12 µl	14 µl
rCutSmart Buffer	2 µl	2 µl	2 µl
dNTP mix (2.5 mM each, stock)	2 µl	2 µl	2 µl
RCA template (v3) (1 ng/µl stock)	1 µl	1 µl	1 µl
RCA primer (10 µM stock)	2 µl	2 µl	-
TOTAL	**18.5 µl**	**19 µl**	**19 µl**

2. Incubate at 95°C for 5 minutes, and then return to ice.
3. Into the same PCR tubes, add:

	(1) (+) ExoI & ExoIII-treated, with IDT primer, cut with Nt.BsmAI	**(2) (+) ExoI & ExoIII-treated, with IDT primer**	**(3) (-) ExoI & ExoIII-treated, without primer**
Product from previous steps	18.5 µl	19 µl	19 µl
phi29 DNA polymerase	1 µl	1 µl	1 µl
Nt.BsmAI	0.5 µl	-	-
TOTAL	**20 µl**	**20 µl**	**20 µl**

4. Incubate at 37°C for 2 hours.
5. Perform heat inactivation at 65°C for 20 minutes.
6. Run 10 µl of each sample to gel electrophoresis using 0.8% Agarose/TAE gel with GelGreen.

ENTRY: [P10]

1. Into each PCR tubes, add:

	(1) (+) ExoI &	**(2) (+) ExoI &**	**(3) (-) ExoI &**	**(+) control,**

	ExoIII-treated, with IDT primer, cut with Nt.BsmAI	**ExoIII-treated, with IDT primer**	**ExoIII-treated, without primer**	**from IDT G-Quad 8 µM stock**
10X G-Quadruplex buffer	12.5 µl	12.5 µl	12.5 µl	12.5 µl
G-Quadruplex sample	10 µl	10 µl	10 µl	6.25 µl
Hemin, in DMSO (1 mM stock)	1 µl	1 µl	1 µl	1 µl
ddH_2O	98.5 µl	98.5 µl	98.5 µl	102.25 µl
TMB (50 mM stock in DMSO)	1.5 µl	1.5 µl	1.5 µl	1.5 µl
H_2O_2	1.5 µl	1.5 µl	1.5 µl	1.5 µl
TOTAL	**125 µl**	**125 µl**	**125 µl**	**125 µl**

ENTRY: [P11]

1. Into each centrifuge tube, add:

	Sample 1
DNA-cleaving DNAzymes 1 and 2	2 µM each
10X DNA-cleaving DNAzyme buffer	5 µl
ddH_2O	top up until (50 µl - test fragment)
TOTAL	**(50 µl - test fragment)**

2. Incubate at 95°C for 5 minutes, followed by gradual cool down to 25°C over 15 minutes of time.
3. Into the centrifuge tube, add:

	Sample 1
Products from step 2	(50 µl - test fragment)
Test fragment (simulating RCA products)	5 nM
TOTAL	**50 µl**

4. Incubate at room temperature for 40 minutes.
5. Run the sample to gel electrophoresis using 5% Agarose/TAE gel with GelGreen.

ENTRY: [P12]

1. Into a PCR tube, add:

	Sample 1
RCA linear template (v1) (100 µM stock)	2 µl
Linear scaffold DNA (v1) (100 µM stock)	2 µl

ddH_2O	13 µl
TOTAL	**17 µl**

2. Incubate the reaction mixture at 95°C for 5 minutes, set the thermocycler to gradually cool down to 25°C.
3. Into the same PCR tube, add:

	Sample 1
Reaction mixture from step 1	17 µl
10X T4 Ligase Buffer	2 µl
T4 Ligase	1 µl
TOTAL	**20 µl**

4. Incubate at 22°C for 15 minutes.
5. Perform oligonucleotide purification protocol to the reaction mixture, and elute with 17 µl ddH_2O preheated at 65°C.
6. Into a new PCR tube, add:

	Sample 1
Oligonucleotide purification product	17 µl
10X Exonuclease I reaction buffer	2 µl
Exonuclease I	1 µl
TOTAL	**20 µl**

7. Incubate at 37°C for 2 hours.
8. Perform oligonucleotide purification protocol to the reaction mixture, and elute with 20 µl ddH2O preheated at 65°C.
9. Measure the sample concentration and dilute to 1 ng/µl.

ENTRY: [P13]

1. Into the PCR tube, add:

	Sample 1
ddH_2O	11.4 µl
rCutSmart Buffer	2 µl
dNTP mix (10 mM each, stock)	2 µl
RCA template (v1) (1 ng/µl stock)	1 µl
RCA primer (10 µM stock)	2 µl
DTT (0.1 M stock)	0.6 µl
TOTAL	**19 µl**

2. Incubate at 95°C for 5 minutes, and then return to ice.
3. Add 1 µl of phi29 DNA polymerase to each tube.
4. Incubate at 30°C for 2 hours.
5. Perform heat inactivation at 65°C for 10 minutes.

6. Run 10 µl of the sample to gel electrophoresis using 0.8% Agarose/TAE gel with GelGreen.

ENTRY: [P14]

1. Into each PCR tubes, add:

	(+) RCA-generated G-quadruplex	**(+) IDT-synthesized G-quadruplex with addition of DTT**	**(+) IDT-synthesized G-quadruplex**
10X G-Quadruplex buffer	25 µl	25 µl	25 µl
G-Quadruplex sample	10 µl	12.5 µl	12.5 µl
DTT (0.1 M stock)	-	0.6 µl	-
Hemin, in DMSO (1 mM stock)	2 µl	2 µl	2 µl
ddH_2O	207 µl	203.9 µl	204.5 µl
TMB (50 mM stock in DMSO)	3 µl	3 µl	3 µl
H_2O_2	3 µl	3 µl	3 µl
TOTAL	**250 µl**	**250 µl**	**250 µl**

ENTRY: [P15]

1. Into a PCR tube, add:

	Sample 1
RCA linear template (v1) (100 µM stock)	2 µl
Linear scaffold DNA (v1) (100 µM stock)	2 µl
ddH_2O	13 µl
TOTAL	**17 µl**

2. Incubate the reaction mixture at 95°C for 5 minutes, set the thermocycler to gradually cool down to 25°C.
3. Into the same PCR tube, add:

	Sample 1
Reaction mixture from step 1	17 µl
10X T4 Ligase Buffer	2 µl
T4 Ligase	1 µl
TOTAL	**20 µl**

4. Incubate at 22°C for 15 minutes.
5. Perform oligonucleotide purification protocol to the reaction mixture, and elute with 17 µl ddH_2O preheated at 65°C.
6. Into a new PCR tube, add:

	Sample 1
Oligonucleotide purification product	17 µl
10X Exonuclease I reaction buffer	2 µl
Exonuclease I	1 µl
TOTAL	**20 µl**

7. Incubate at 37°C for 2 hours.
8. Perform oligonucleotide purification protocol to the reaction mixture, and elute with 20 µl ddH2O preheated at 65°C.
9. Measure the sample concentration and dilute to 1 ng/µl.

ENTRY: [P16]

1. Into the PCR tubes, add:

	(1) (+) With IDT primer	**(2) (-) Without primer**
ddH_2O	12 µl	14 µl
rCutSmart Buffer	2 µl	2 µl
dNTP mix (10 mM each, stock)	2 µl	2 µl
RCA template (v1) (1 ng/µl stock)	1 µl	1 µl
RCA primer (10 µM stock)	2 µl	-
TOTAL	**19 µl**	**19 µl**

2. Incubate at 95°C for 5 minutes, and then return to ice.
3. Add 1 µl of phi29 DNA polymerase to each tube.
4. Incubate at 30°C for 2 hours.
5. Perform heat inactivation at 65°C for 10 minutes.
6. Run 10 µl of each sample to gel electrophoresis using 0.8% Agarose/TAE gel with GelGreen.

ENTRY: [P17]

1. Into each PCR tubes, add:

	(1) (+) RCA-generated G-quadruplex	**(2) (-) false positive RCA result**
10X G-Quadruplex buffer	12.5 µl	12.5 µl
G-Quadruplex sample	10 µl from (1) (+) With IDT primer	10 µl from (2) (-) Without primer
Hemin, in DMSO (1 mM stock)	1 µl	1 µl
ddH_2O	98.5 µl	98.5 µl
TMB (50 mM stock in DMSO)	1.5 µl	1.5 µl

H_2O_2	1.5 µl	1.5 µl
TOTAL	**125 µl**	**125 µl**

ENTRY: [P18]

1. Into each PCR tubes, add:

	(1)	**(2)**	**(3) control**	**(4)**
10X G-Quadruplex buffer	12.5 µl	12.5 µl	12.5 µl	12.5 µl
G-Quadruplex sample (from IDT G-Quad 8 µM stock)	18.75 µl	12.5 µl	6.25 µl	0.625 µl
Hemin, in DMSO (1 mM stock)	1 µl	1 µl	1 µl	1 µl
ddH_2O	89.75 µl	96 µl	102.25 µl	107.875 µl
TMB (50 mM stock in DMSO)	1.5 µl	1.5 µl	1.5 µl	1.5 µl
H_2O_2	1.5 µl	1.5 µl	1.5 µl	1.5 µl
TOTAL	**125 µl**	**125 µl**	**125 µl**	**125 µl**

ENTRY: [P19]

1. Into a PCR tube, add:

	Sample 1
RCA linear template (v3) (100 µM stock)	2 µl
Linear scaffold DNA (v3) (100 µM stock)	2 µl
ddH_2O	13 µl
TOTAL	**17 µl**

2. Incubate the reaction mixture at 95°C for 5 minutes, set the thermocycler to gradually cool down to 25°C.
3. Into the same PCR tube, add:

	Sample 1
Reaction mixture from step 1	17 µl
10X T4 Ligase Buffer	2 µl
T4 Ligase	1 µl

TOTAL	**20 µl**

4. Incubate at 22°C for 15 minutes.
5. Perform oligonucleotide purification protocol to the reaction mixture, and elute with 20 µl ddH_2O preheated at 65°C.

ENTRY: [P20]

1. Into the PCR tubes, add:

	(1) RCA (v3) cut with Nt.BsmAI	**(2) RCA (v3) uncut**
ddH_2O	11.5 µl	12 µl
rCutSmart Buffer	2 µl	2 µl
dNTP mix (2.5 mM each, stock)	2 µl	2 µl
RCA template (v3) (1 ng/µl stock)	1 µl	1 µl
RCA primer (10 µM stock)	2 µl	2 µl
TOTAL	**18.5 µl**	**19 µl**

2. Incubate at 95°C for 5 minutes, and then return to ice.
3. Into the same PCR tubes, add:

	(1) RCA (v3) cut with Nt.BsmAI	**(2) RCA (v3) uncut**
Product from previous steps	18.5 µl	19 µl
phi29 DNA polymerase	1 µl	1 µl
Nt.BsmAI	0.5 µl	-
TOTAL	**20 µl**	**20 µl**

4. Incubate at 37°C for 2 hours.
5. Perform heat inactivation at 65°C for 20 minutes.
6. Run 10 µl of each sample to gel electrophoresis using 0.8% Agarose/TAE gel with GelGreen.

ENTRY: [P21]

1. Into each PCR tubes, add:

	(1) RCA(v3)/Nt.BsmAI-GQuad	**(2) RCA(v3)-GQuad**	**(+) control**
10X G-Quadruplex buffer	12.5 µl	12.5 µl	12.5 µl
G-Quadruplex sample	10 µl	10 µl	6.25 µl
Hemin, in DMSO (1 mM stock)	1 µl	1 µl	1 µl

ddH_2O	98.5 µl	98.5 µl	102.25 µl
TMB (50 mM stock in DMSO)	1.5 µl	1.5 µl	1.5 µl
H_2O_2	1.5 µl	1.5 µl	1.5 µl
TOTAL	**125 µl**	**125 µl**	**125 µl**

ENTRY: [P22]

10. Into a PCR tube, add:

	Sample 1
RCA linear template (v3) (100 µM stock)	2 µl
Linear scaffold DNA (v3) (100 µM stock)	2 µl
ddH_2O	13 µl
TOTAL	**17 µl**

11. Incubate the reaction mixture at 95°C for 5 minutes, set the thermocycler to gradually cool down to 25°C.
12. Into the same PCR tube, add:

	Sample 1
Reaction mixture from step 1	17 µl
10X T4 Ligase Buffer	2 µl
T4 Ligase	1 µl
TOTAL	**20 µl**

13. Incubate at 22°C for 15 minutes.
14. Perform oligonucleotide purification protocol to the reaction mixture, and elute with 18 µl ddH_2O preheated at 65°C.
15. Into a new PCR tube, add:

	Sample 1
ddH_2O	70.5 µl
Oligonucleotide purification product	18 µl
10X Exonuclease III buffer	10 µl
Exonuclease III (TaKaRa Bio)	0.5 µl
Exonuclease I (Thermo Fisher Scientific)	1 µl
TOTAL	**100 µl**

16. Incubate at 37°C for 1 hour.
17. Perform oligonucleotide purification protocol to the reaction mixture, and elute with 20 µl ddH2O preheated at 65°C.
18. Measure the sample concentration and dilute to 1 ng/µl.

ENTRY: [P23]

1. Into the PCR tubes, add:

	(1) 1 ng	**(2) 2 ng**	**(3) 4 ng**	**(4) 8 ng**	**(5) uncut**	**(6) (-)**
ddH_2O	11.5 µl	10.5 µl	8.5 µl	4.5 µl	12 µl	14 µl
rCutSmart Buffer	2 µl	2 µl	2 µl	2 µl	2 µl	2 µl
dNTP mix (10 mM each, stock)	2 µl	2 µl	2 µl	2 µl	2 µl	2 µl
RCA template (v3) (1 ng/µl stock)	1 µl	2 µl	4 µl	8 µl	1 µl	1 µl
RCA primer (10 µM stock)	2 µl	2 µl	2 µl	2 µl	2 µl	-
TOTAL	**18.5 µl**	**18.5 µl**	**18.5 µl**	**18.5 µl**	**19 µl**	**19 µl**

2. Incubate at 95°C for 5 minutes, and then return to ice.
3. Into the same PCR tubes, add:

	(1) 1 ng	**(2) 2 ng**	**(3) 4 ng**	**(4) 8 ng**	**(5) uncut**	**(6) (-)**
Product from previous steps	18.5 µl	18.5 µl	18.5 µl	18.5 µl	19 µl	19 µl
phi29 DNA polymerase	1 µl	1 µl	1 µl	1 µl	1 µl	1 µl
Nt.BsmAI	0.5 µl	0.5 µl	0.5 µl	0.5 µl	-	-
TOTAL	**20 µl**	**20 µl**	**20 µl**	**20 µl**	**20 µl**	**20 µl**

4. Incubate at 37°C for 2 hours.
5. Perform heat inactivation at 65°C for 20 minutes.
6. Run 10 µl of each sample to gel electrophoresis using 0.8% Agarose/TAE gel with GelGreen.

ENTRY: [P24]

1. Into each PCR tubes, add:

	(1) 1 ng	**(2) 2 ng**	**(3) 4 ng**	**(4) 8 ng**	**(5) uncut**	**(6) (-)**	**(7) (+) control**
10X G-Quadruplex buffer	12.5 µl	12.5 µl	12.5 µl	12.5 µl	12.5 µl	12.5 µl	12.5 µl
G-Quadruplex sample	10 µl	10 µl	10 µl	10 µl	10 µl	10 µl	6.25 µl
Hemin, in DMSO (1 mM stock)	1 µl	1 µl	1 µl	1 µl	1 µl	1 µl	1 µl
ddH_2O	98.5 µl	98.5 µl	98.5 µl	98.5 µl	98.5 µl	98.5 µl	102.25 µl
TMB (50 mM stock in DMSO)	1.5 µl	1.5 µl	1.5 µl	1.5 µl	1.5 µl	1.5 µl	1.5 µl

H_2O_2	1.5 µl	1.5 µl	1.5 µl	1.5 µl	1.5 µl	1.5 µl	1.5 µl
TOTAL	**125 µl**	**125 µl**	**125 µl**	**125 µl**	**125 µl**	**125 µl**	**125 µl**

ENTRY: [P25]

1. Into each PCR tubes, add:

	SDS-Treated	**Control (Untreated)**
Cell culture	50 µl	50 µl
1X SDS	50 µl	-
1X Neutralisation buffer	75 µl	-
ddH_2O	-	125 µl
TOTAL	**175 µl**	**175 µl**

2. Mix all tubes by flicking.
3. Spin down in a microcentrifuge for 2 minutes.
4. Mix 50 µl of each sample of cell culture with 50 µl of glycerol.
5. Add 3 µl of each sample from step (4) on a microscope slide.
6. Secure the cover slip on top of the microscope slide and observe the cells under the microscope.

ENTRY: [P26]

1. Into each PCR tubes, add:

	Nt.BsmAI & HhaI	**HhaI**
ddH_2O	0.32 µl	0.66 µl
rCutSmart Buffer	2 µl	2 µl
Nt.BsmAI	0.34 µl	-
HhaI	0.34 µl	0.34 µl
pSB1C3-KPC-2 (68.4 ng/µl stock)	14 µl	14 µl
TOTAL	**17 µl**	**17 µl**

2. Incubate at 37°C for 45 minutes.
3. Into the same PCR tubes, add:

	Nt.BsmAI & HhaI	**HhaI**
Sample from step (2)	17 µl	17 µl
phi29 DNA polymerase	1 µl	1 µl
dNTP	2 µl	2 µl
TOTAL	**20 µl**	**20 µl**

4. Incubate at 30°C for 15 minutes.
5. Perform heat inactivation at 65°C for 20 minutes.

6. Run 10 µl of each sample to gel electrophoresis using 5% Agarose/TAE gel with GelGreen.

ENTRY: [P27]

1. Into a PCR tube, add:

	Sample 1
RCA linear template (v1) (100 µM stock)	2 µl
Linear scaffold DNA (v1) (100 µM stock)	2 µl
ddH_2O	13 µl
TOTAL	**17 µl**

2. Incubate the reaction mixture at 95°C for 5 minutes, set the thermocycler to gradually cool down to 25°C.
3. Into the same PCR tube, add:

	Sample 1
Reaction mixture from step 1	17 µl
10X T4 Ligase Buffer	2 µl
T4 Ligase	1 µl
TOTAL	**20 µl**

4. Incubate at 22°C for 15 minutes.
5. Perform oligonucleotide purification protocol to the reaction mixture, and elute with 17 µl ddH_2O preheated at 65°C.
6. Into a new PCR tube, add:

	Sample 1
Oligonucleotide purification product	17 µl
10X Exonuclease I reaction buffer	2 µl
Exonuclease I	1 µl
TOTAL	**20 µl**

7. Incubate at 37°C for 2 hours.
8. Perform oligonucleotide purification protocol to the reaction mixture, and elute with 20 µl ddH2O preheated at 65°C.
9. Measure the sample concentration and dilute to 1 ng/µl.

ENTRY: [P28]

1. Into the PCR tubes, add:

	10X Reaction Buffer	**rCutSmart Buffer**
ddH_2O	11.4 µl	13.4 µl
Buffer	2 µl	2 µl
dNTP mix (10 mM each, stock)	2 µl	2 µl

RCA template (v1) (1 ng/µl stock)	1 µl	1 µl
RCA primer (10 µM stock)	2 µl	-
DTT (0.1 M stock)	0.6 µl	0.6 µl
TOTAL	**19 µl**	**19 µl**

2. Incubate at 95°C for 5 minutes, and then return to ice.
3. Add 1 µl of phi29 DNA polymerase to each tube.
4. Incubate at 30°C for 2 hours.
5. Perform heat inactivation at 65°C for 10 minutes.
6. Run 10 µl of each sample to gel electrophoresis using 0.8% Agarose/TAE gel with GelGreen.

BUFFER PREPARATION PROTOCOLS:

G-Quad Buffer Preparation (10X) (in 1L)

Required Materials:

Tris	0.1 M (100 mL of 1M Tris)
Triton X-100	0.5% (5 mL of 1M Triton X-100)
DMSO	10% (100 mL of 1M DMSO)
KCl	1 M (500 mL of 2M KCl)
ddH_2O	Variable
HCl	Variable

1. Mix the first 4 components as per the final concentrations listed in the table.
2. Top up to 800.0 ml using ddH_2O.
3. Adjust to pH 7.5 with HCl.
4. Top up to 1000 ml using ddH_2O.

13PD1 DNAzyme Buffer (1X) (in 1L)

Required Materials:

ZnCl2	0.01 M (10 mL of 1 M $ZnCl_2$)
MnCl2	0.2 M (200 mL of 1 M $MnCl_2$)
MgCl2	0.1 M (100 mL of 1 M $MgCl_2$)
NaCl	0.375 M (75 mL of 5 M NaCl)
MOPS	0.1 M (100 mL of 1 M MOPS)

ddH2O	Variable
NaOH	Variable

1. Mix the first 5 components as per the final concentrations listed in the table.
2. Top up to 800.0 ml using ddH_2O.
3. Adjust to pH 7.5 with NaOH.