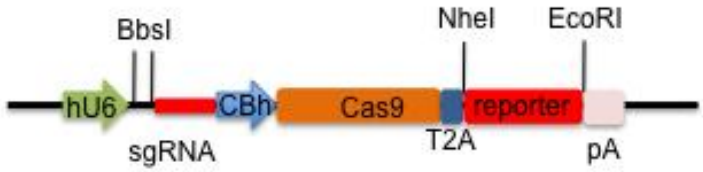
I. Cloning of sgRNAs into CRISPR/Cas9-T2A-Reporter vectors

pX330-T2A-Reporter (mCherry/BFP)



5′-**CACC**G**NNNNNNNNNNNNNNNNNNN** -3′
3′- **C**NNNNNNNNNNNNNNNNNNN**CAAA**-5′

1. Digest pX330-T2A-mCherry/BFP

Reagents	**Volume**
pX330-T2A-Reporter	1μg
Fastdigest BbsI (fermentas)	1μl
FastAP (Fermentas)	1μl
10x FastDigest buffer	2μl
H_20	Up to 20μl
Digest for 60 min at 37^{0}C 70^{0}C for 5min	

2. Gel purify digested pX330-T2A-mCherry/BFP using QIAquick gel extraction kit and elute in H_2O

3. Phosphorylate and anneal each pair of oligos

Reagents	**Volume**
Oligo 1 (stock 100μM)	1μl (10μM)
Oligo 1 (stock 100μM)	1μl (10μM)
10x T4 ligation buffer (NEB)	1μl
H_20	6.5μl
T4 PNK (NEB)	0.5μl
Total	**10μl**

Anneal parameters	
37^{0}C	30 min
95^{0}C	5 min and then ramp down to 25^{0}C at 0.1^{0}C/second, hold 4^{0}C

Annealed oligo duplex (10μM) will be diluted 1:20 to get 500nM stock analysed with 4% agarose gel (used 5μl). From 500nM stock will be further diluted 1:10 get 50nM oligo duplex for cloning. Oligo duplex are stored at -20^{0}C

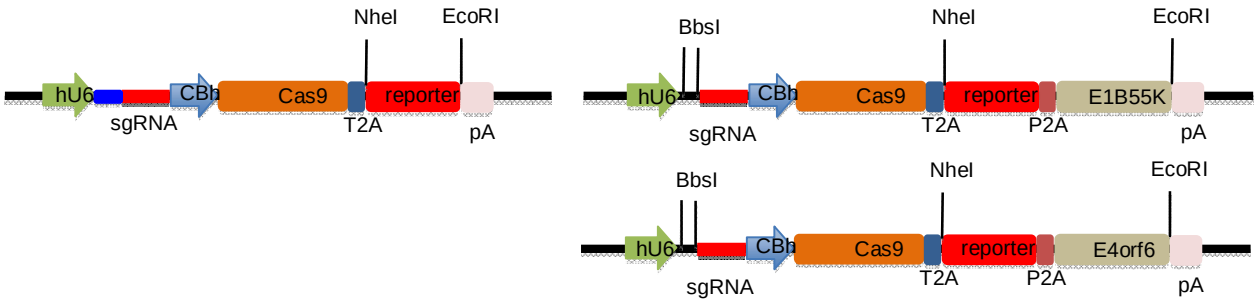
4. Ligation reaction

Reagents	**Volume**
BbsI digested pX330-T2A-mC/BFP	Final 50ng
Phosphorylated and annealed duplex (1:200	1.5μl

dilution) (**step 3**)	
10X T4 ligation buffer (NEB)	1µl
T4 Ligase (NEB, M)	0.5µl
H20	Up to 10µl
Incubate at 22^{0}C for 15 min	

5. Transformation
6. Plasmid isolation
7. Sequencing to confirm the insert

II. Cloning of new inserts into sgRNA-pX330-T2A-Reporter vectors



1. Digest pX330-sgRNA-T2A-mCherry/BFP

Reagents	**Volume**
pX330-sgRNA-T2A-Reporter	1µg
Fastdigest NheI (fermentas)	1µl
Fastdigest EcoRI (fermentas)	1µl
FastAP (Fermentas)	1µl
10x FastDigest buffer	2µl
H20	Up to 20µl
Digest for 60min at 37^{0}C 70^{0}C for 5min	

2. Gel purify digested pX330-sgRNA-T2A-mCherry/BFP using QIAquick gel extraction kit and elute in H_2O
3. Digest pX330-T2A-Reporter-P2A-Ad4 E1B55K/E4orf6 vector

Reagents	**Volume**
The vector	1µg
Fastdigest NheI (fermentas)	1µl
Fastdigest EcoRI (fermentas)	1µl
10x FastDigest buffer	2µl
H20	Up to 20µl
Digest for 60min at 37^{0}C 70^{0}C for 5min	

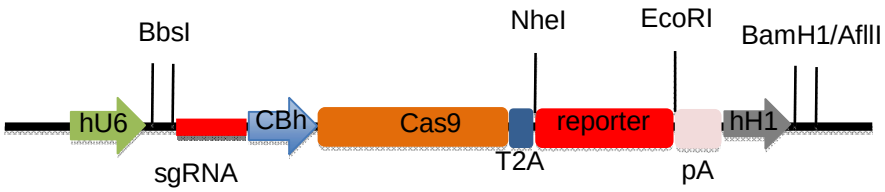
4. Gel purify digested Reporter-P2A-Ad4 E1B55K/Ad4 E4orf6 fragments using QIAquick gel extraction kit and elute in H_2O

5. Ligation reaction

Reagents	**Volume**
NheI/EcoRI digested vector	Up to 50ng
NheI/EcoRI digested fragment	Up to 30ng
10X T4 ligation buffer (NEB)	1μl
T4 Ligase (NEB, M)	0.5μl
H20	Up to 10μl
Incubate at 22^{0}C for 15 min	

6. Transformation
7. Plasmid isolation
8. Sequencing to confirm the insert

III. Cloning of shRNAs into CRISPR/Cas9-T2A-reporter-hH1-shRNA vectors



shRNA oligos

Sense Loop Antisense

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5′-gatccNNNNNNNNNNNNNNNNNNNNTCAAGAGNNNNNNNNNNNNNNNNNNNNttttttc    -3′
3′-    gNNNNNNNNNNNNNNNNNNNNNNAGTTCTCNNNNNNNNNNNNNNNNNNNNaaaaagaatt-5′
```

1. Digest pX330-T2A-mCherry/BFP-hH1 vector

Reagents	**Volume**
pX330-T2A-mCherry/BFP-hH1 vector	1μg
Fastdigest BamH1 (fermentas)	1μl
Fastdigest AflII (fermentas)	1μl
FastAP (Fermentas)	1μl
10x FastDigest buffer	2μl
H20	Up to 20μl
Digest for 60min at 37^{0}C 70^{0}C for 5min	

2. Gel purify digested pX330-T2A-mCherry/BFP-hH1 using QIAquick gel extraction kit and elute in H_2O

3. Phosphorylate and anneal each pair of oligos

Reagents	**Volume**
Oligo 1 (stock 100μM)	1μl (10μM)
Oligo 1 (stock 100μM)	1μl (10μM)
10x T4 ligation buffer (NEB)	1μl
H20	6.5μl
T4 PNK (NEB)	0.5μl
Total	**10μl**

Anneal parameters	
37^{0}C	30 min
95^{0}C	5 min and then ramp down to 25^{0}C at 0.1^{0}C/second, hold 4^{0}C

Annealed oligo duplex (10μM) will be diluted 1:20 to get 500nM stock analysed with 4% agarose gel (used 5μl). From 500nM stock will be further diluted 1:10 get 50nM oligo duplex for cloning. Oligo duplex are stored at -20^{0}C

4. Ligation reaction

Reagents	**Volume**
pX330-T2A-mCherry/BFP-hH1 vector	Final 50ng
Phosphorylated and annealed duplex (1:200 dilution) (**step 3**)	1.5μl
10X T4 ligation buffer (NEB)	1μl
T4 Ligase (NEB, M)	0.5μl
H20	Up to 10μl
Incubate at 22^{0}C for 15 min	

5. Transformation
6. Plasmid isolation
7. Sequencing to confirm the insert