Step by step protocol for blue-white selection RNAi T-vector cloning

Transformation protocol 1

1. Gene X one-tube PCR/ RT-PCR

0.5-1 hours at last elongation step 72°C, more A overhangs

2. Ligation of gene X RT-PCR products with pYZT vectors.

In 1.5 ml eppendorf tube, add:

3 µl	*gene X* RT-PCR /PCR purified products
0.5 µl	pYZ101 T -vector (Mini-prep)
4.5 µl	2 X T4 DNA RAPID ligation buffer
1 µl	T4 DNA ligase (Promega)

Gently mix, then incubate at RT for 1-2 hours

Note: To reduce background, 1 unit *Xcm* I could be included in ligation mix if the insert without *Xcm* I restriction site)

3. Transformation

1) Preparation of HT115 eletrophoration competent cells:
   a) Shake HT115 cells at 37°C overnight
   b) Transfer 0.5 ml and dilute to 8 ml
   c) Shake at 37°C for 3-4 hrs
   d) Centrifuge at 5000rpm for 5 min at 4 °C
   e) Wash once with 1 ml ice-cold water and repeat another twice
   f) Keep 40-50µl water rest
2) 2µl the above rapid ligation mixture is used for electroporation of HT115 competent cells
3) Shake at 37°C over night

4. Colony PCR 2.5 Hrs

1. Preparation of PCR mixture:

10 µl	10 x PCR buffer
7 µl	dNTPs (each 2.5 mM)
4 µl	MgCl2 (25 -40mM)
0.3 µl	LacZ L primer (100 µM)
0.3 µl	LacZ R primer (100 µM)

1-2 µl	Taq polymerase (5 unit / µl)
X µl	Mili-Q water
100 µl	

2. Pick individual white colonies using tooth-picks, and put them into 5 µl miliQ water. Stir the tooth-picks.
3. Transfer 2.5 µl of each sample 14 ml Falcon tubes containing LB and Ampicillin for standard HB10b culture and shake at 37°C over night.
4. Add 20 µl PCR reaction mixture in the remaining 2.5 µl of each sample. After PCR amplification (5 min 95°C for the first denaturation step; 20 sec, 94°C for denaturation; 30 sec at 57°C for annealing, 1min 30sec at 72°C for extension, totally 32 cycles; 30min at 72°C for last extension. As pimers, used LacZ.L (5'CGTTGTAAAACGACGGCCA3') and LacZ.R (5'AGCGGATAACAATTTCACACAGG3').
5. Run the PCR products on a 1.2 % Agarose gel, visualized by EtBr and photographed using a Bio-rad Gel–doc instrument.
6. Keep clones with an insert >250bp (i.e.considered to be positive).

Transformations protocol 2

1. **Gene X one-tube RT-PCR**
2. **Ligation of gene X RT-PCR products with pYZT vector**

In 1.5 ml eppendorf tube, add:

7 µl	*gene X* RT-PCR purified products
1 µl	pYZ101 T -vector (Mini-prep)
1 µl	10 X T4 DNA ligation buffer
1 µl	T4 DNA ligase (Promega)

Gently mix, then incubate at 15°C for 16 hours

or, in 1.5 ml eppendorf tube, add:

3 µl	*gene X* RT-PCR /PCR purified products
0.5 µl	pYZ101 T -vector (Mini-prep)
4.5 µl	2 X T4 DNA RAPID ligation buffer
1 µl	T4 DNA ligase (Promega)

Gently mix, then incubate at RT for 1-2 hours

3. Optional -Transformation

1µl of the above mixture is used for electroporation of *E.coli* HB10b cells. Shake at 37°C over night.

4. Colony PCR

1. Preparation of PCR mixture:

10 µl	10 x PCR buffer
7 µl	dNTPs (each 2.5 mM)
4 µl	MgCl2 (25 -40mM)
0.3 µl	LacZ L primer (100 µM)
0.3 µl	LacZ R primer (100 µM)
1-2 µl	Taq polymerase (5 unit /µl)
X µl	Mili-Q water
100 µl	

2. Pick individual white colonies using tooth-picks, and put them into 5 µl miliQ water. Stir the tooth-picks.
3. Transfer 2.5 µl of each sample 14 ml Falcon tubes containing LB and Ampicillin for standard HB10b culture and shake at 37°C over night.
4. Add 20 µl PCR reaction mixture in the remaining 2.5 µl of each sample. After PCR amplification (5 min 95°C for the first denaturation step; 20 sec, 94°C for denaturation; 30 sec at 57°C for annealing, 1min 30sec at 72°C for extension, totally 32 cycles; 30min at 72°C for last extension. As pimers, used LacZ.L (5'CGTTGTAAAACGACGGCCA3') and LacZ.R (5'AGCGGATAACAATTTCACACAGG3').
5. Run the PCR products on a 1.2 % Agarose gel, visualized by EtBr and photographed using a Bio-rad Gel–doc instrument.
6. Keep clones with an insert >250bp (i.e.considered to be positive).
7. Mini preparation of plasmid DNA using kit.
8. Re-transformation

   0.2µl of the above mini-prep plasmid DNA is used for heat shock CaCl2 chemical or electroporation transformation of *E.coli* HT115 competent cells.

9. Shake at 37°C over night.