Protocol： Direct conversion from MEF to neuron

Lentiviral *shRNAs* against mouse *PTBP1*：

*sh*1.TRCN0000295168 KnockDown Level: 0.97

Sequence:CCGGCACTATGGTTAACTACTATACCTCGAGGTATAGTAGTTAACCATAGTGTTTTTG

AATTCAAAAACACTATGGTTAACTACTATACCTCGAGGTATAGTAGTTAACCATAGTG

sh2.TRCN0000287704 0.97

Sequence:CCGGCCAAAGCCTCTTTATTCTCTTCTCGAGAAGAGAATAAAGAGGCTTTGGTTTTTG

AATTCAAAAACCAAAGCCTCTTTATTCTCTTCTCGAGAAGAGAATAAAGAGGCTTTGG

sh3.TRCN0000287703 0.94

Sequence:CCGGCTCAATGTCAAGTACAACAATCTCGAGATTGTTGTACTTGACATTGAGTTTTTG

AATTCAAAAACTCAATGTCAAGTACAACAATCTCGAGATTGTTGTACTTGACATTGAG

sh4.TRCN0000295113 0.91

Sequence:CCGGGACCTTACAGACCAGAGATTTCTCGAGAAATCTCTGGTCTGTAAGGTCTTTTTG

AATTCAAAAAGACCTTACAGACCAGAGATTTCTCGAGAAATCTCTGGTCTGTAAGGTC

sh5.TRCN0000109272 0.88

Sequence:CCGGCTCAATGTCAAGTACAACAATCTCGAGATTGTTGTACTTGACATTGAGTTTTTG

AATTCAAAAACTCAATGTCAAGTACAACAATCTCGAGATTGTTGTACTTGACATTGAG

were cloned in the pLKO.1 vector.

(A)

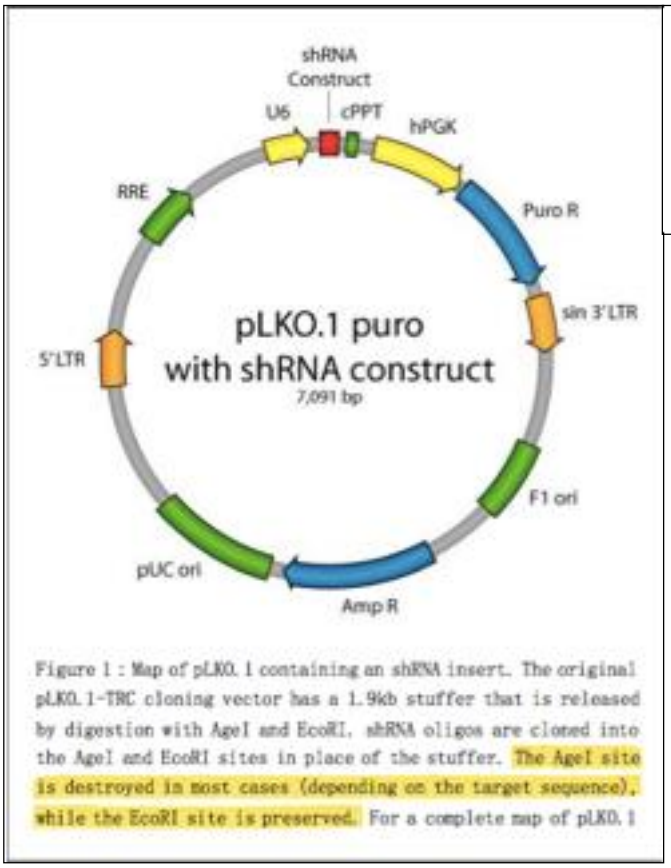


Figure 1 : Map of pLKO.1 containing an shRNA insert. The original pLKO.1-TRC cloning vector has a 1.9kb stuffer that is released by digestion with AgeI and EcoRI. shRNA oligos are cloned into the AgeI and EcoRI sites in place of the stuffer. The AgeI site is destroyed in most cases (depending on the target sequence), while the EcoRI site is preserved. For a complete map of pLKO.1

(B)

Forward oligo:

5' CCGG—21bp sense—CTCGAG—21bp antisense—TTTTTG 3'

Reverse oligo:

5' AATTCAAAAA—21bp sense—CTCGAG—21bp antisense 3'

(C)

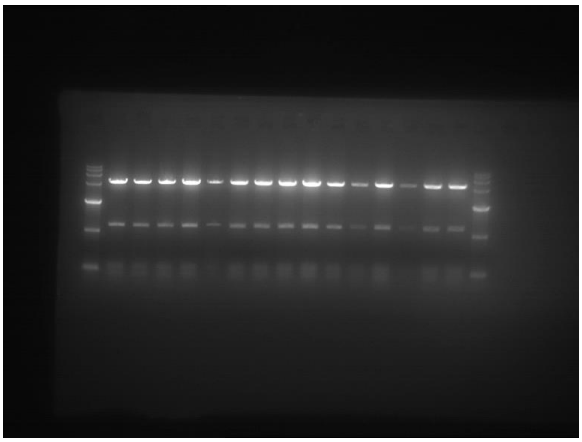


Figure 1.

(A) pLKO.1 vector，puro positive.

(B) The design of the oligos of shRNA.

(C) We use enzyme digestion to prove that the 5 shDNA(sh1~sh5) of mPTB are indeed on the pLKO.1vector. Each shDNA has 3 parallel tests and all are positive.。

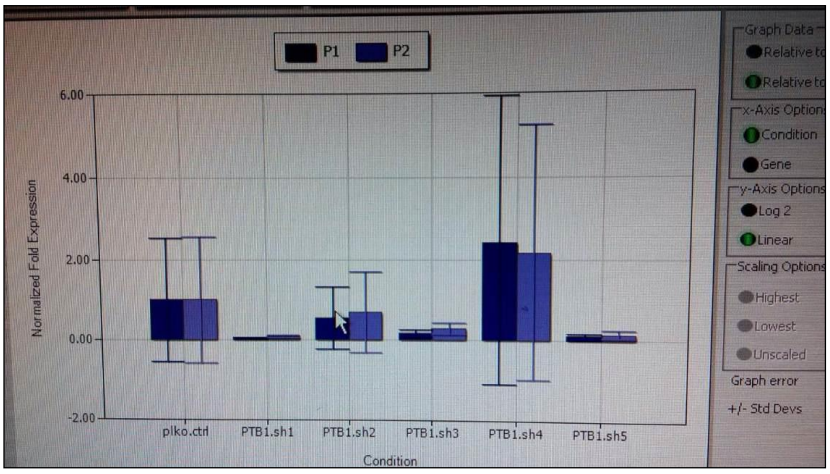


Figure 2 qPCR for checking the effect of knocking down PTB in mES. sh 1 and sh 5 had good effect and were selected to infect MEF.

Individual *shRNAs* were packaged into replication-incompetent lentiviral particles in HEK293T cells by co-transfecting individual pLKO plasmids with the packaging mix (Sigma). Viral particles were collected twice 48 hrs and 72 hrs post-transfection. We checked the virus in mES, and then we selected virus sh1 and sh5 for their good effect in knocking down PTB in mES(Figure 2).

MEF cells were infected with individual lentiviral particles for 16 hrs followed by selection with 2 g/ml Puromycin for 48 hrs. Then transfer the cells to Poly-D-Lysine/Laminin-coated Tissue Cultureware.

Steps of making Poly-D-Lysine/Laminin-coated Tissue Cultureware：

Preparation of 100 µg/mL Poly-D-Lysine Stock Solution

1. To prepare poly-D-lysine, dissolve 5 mg poly-D-lysine (Sigma Catalog #P7280) in 50 mL sterile water.

2. Aliquot solution in polypropylene vials and store at 2 - 8 ℃.

Preparation of 10 µg/mL Laminin Stock Solution

1. Thaw laminin (Sigma Catalog #L2020) at 2 - 8 ℃, to prevent laminin from gelling.

2. Prepare a 10 µg/mL working solution of laminin by diluting the laminin in sterile PBS or sterile water (the amount prepared should correspond to the amount needed for immediate use).

3. Store the remaining laminin (which has not been diluted) in appropriately sized working aliquots at -20 ℃.

Preparation of Poly-D-Lysine/Laminin-coated Tissue Cultureware

1. Dispense the appropriate volume of 100 µg/mL PDL stock solution for the chosen tissue culture vessel as indicated in Table 4.

2. Incubate for 2 hours at 37 ℃ or overnight (~20 hours) at 2 - 8 ℃.

3. Wash each well/flask with sterile PBS according to the recommended volumes in Table 5. Remove as much of the PBS as possible.

4. Dispense 10 µg/mL laminin stock solution at the volume indicated in Table 4.

5. Incubate for 2 hours at 37°C or overnight at 2 - 8°C.

6. Wash each well/flask with sterile PBS according to the recommended volumes indicated in Table 5.
   Only remove the PBS when ready to plate the cells. Do not let the coated plates completely dry.

7. The substrate-coated tissue cultureware are ready for use and should be used within the same day.

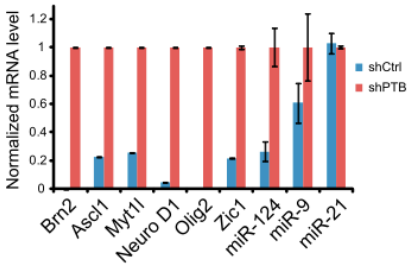
8. Proceed to Section 5.0 for plating cells for adherent monolayer cultures.

we add neural medium(Neurobasal medium: DMEM/F12 = 3:1 (36ml:12ml), N2: 0.5ml, B27: 1ml, Glu: 0.5ml, P/S: 0.5ml, BDNF: 30ng/ml). At day2, we took the photo of the cells(Figure 3).
Then we checked the marked genes of neural development by IF with Anti-MAP2 at Day15 (Figure 4).

The ideal datas are like these:
qPCR with primers:

Ascl1 F	CATCTCCCCCAACTACTCCA	Zic1 F	TTTCCTGGCTGCGGCAAGGTTT
Ascl1 R	CCAGCAGCTCTTGTTCCTCT	Zic1 R	ACGTGCATGTGCTTCTTGCGGT
Brn2 F	GCGGATCAAACTCGGATTTA	Olig2 F	ATGCACGACCTCAACATCGCCA
Brn2 R	TCTGCCTCTTCCAACCACTT	Olig2 R	ACCAGTCGCTTCATCTCCTCCA
Myt1l F	ATCAAGCCATGGAAACTTGG		
Myt1l R	TCCACCTCTGACAAGCTCCT		

Oligos of marked genes for qPCR.



and do IF like this: