



Description:

pT3-sgRNA is a sgRNA-expressing vector for *in vitro* transcription:

1. pT3-sgRNA was derived from pGEM-T easy by ligation of a 2.0 kb DNA fragment containing a sgRNA expression cassette (T3 promoter-1.9kb Stuffer-sgRNA scaffold);
2. The expression of sgRNA is controlled by T3 promoter;
3. The plasmid can be digested by *BsmBI*, which will remove the 1.9 kb stuffer and generate sticky ends for cloning of sgRNA oligos (please see [Protocol for sgRNA construction](#));
4. Linearize the plasmid by *XhoI* digestion before *in vitro* transcription.

Location of Features:

- SP6 promoter : nt 1-20
- T3 promoter : nt 100-117
- Stuffer : nt 118-2000
- sgRNA scaffold : nt 2001-2086
- T7 promoter (reversed) : nt 2169-2150
- Phage f1 ori (reversed) : nt 2788-2333
- Amp resistant gene : nt 2971-3831

Note:

T3 promoter: 5'- AATTAACCCTCACTAAAG -3'.