Synthesis of mouse Kdm4d mRNA for microinjection

MATERIALS

- Plasmid
  - pcDNA-Flag-mKdm4d-polyA
  - pcDNA-Flag-mKdm4d(H189A)-polyA
- Restriction Enzyme
  - XbaI (NEB)
- mRNA synthesis
  - mMESSAGE mMACHINE® T7 ULTRA Transcription Kit (AM1345, Life Technologies)
  - GlycoBlue (15mg/ml, AM9516, Life Technologies)
  - NaAce (3 M Sodium Acetate Solution pH 5.2, R1181, Thermo Scientific)
  - RNA-ladder (Millennium™ RNA Markers, AM7150, Life Technologies)

PROCEDURES

1. Purify plasmids from LB
2. One-cut by XbaI (5ug plasmid/50ul reaction)
   - Mix 44ul of plasmid(5ug)+water, 5ul CutSmart, keep 1ul as uncut
   - Add XbaI 1ul, 37C o/n
   - Run 1ul of cut/uncut products in 1% gel (cut: 7.2 kb)
3. Purify the one-cut product by Phenol-Chloroform-IAA
   - 48ul of XbaI cut product
   - Add 152ul water to increase volume up to 200ul
   - Add 200ul PCI, voltex
   - 15000RPM 4C 15min
   - 180ul upper aqueous phase + 180ul Chloroform, voltex
   - 15000 RPM 4C 15min
   - ~150ul upper phase + 1ul Glycoblue + 15ul 3M NaAce + 375ul 100%EtOH
   - Keep 4C > 20min
   - 15000 RPM 4C 15min
   - Discard Sup
   - Wash pellet by 500ul of 70%EtOH
   - 15000 RPM 4C 15min
   - Discard 70%EtOH
   - Dry up at RT

- Dissolve in 7 ul of water
- Nano-drop (300~400 ng/ul)

4. In vitro transcription by T7 ULTRA kit (1ug one-cut plasmid DNA/20ul reaction)
   - Mix 6ul one-cut plasmid(1ug)+water, 10ul 2xNTP, 2ul 10x-buffer, 2ul T7 enzyme
   - Incubate 37C 120min
   - Add 1ul DNase in T7 ULTRA kit
   - Incubate 37C 15min
   - Add 80ul water, transfer to 1.5ml tube
   - Add 60ul LiCl in T7 ULTRA kit
   - Keep at -20C > 30min
   - 15000 RPM 4C 20min
   - Discard Sup
   - Wash pellet by 500ul of 70%EtOH
   - 15000 RPM 4C 15min
   - Discard 70%EtOH
   - Dry up 15min at RT
   - Dissolve in 20 ul water at 4C o/n
   - Nano-drop (1500~2000 ng/ul)
   - Run 200ng RNA in 1% fresh gel with RNA-ladder
     2ul RNA(200ng)+water, 4ul 2xLoading Buffer, 2ul 1/100-diluted EtBr
     =8ul total, denature 75C 10min, then RUN. ~1800 bp
   - Make aliquot (1ul each) and freeze at -80C

Example of a dish for microinjection of mRNA