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in vitro Transcription

REAGENTS

- T3 RNA polymerase (P2083, Promega)
- UltraPure DNase/RNase-Free Distilled Water (10977, GIBCO)
- 10 mM NTP (Invitrogen)
  ATP: 18330-019, GTP: 18332-015, CTP: 18331-017, UTP: 18333-013
- $m^7G(5')ppp(5')G$ RNA Capping Analog (15619-018, Invitrogen)
- Recombinant RNase Inhibitor (2311A, TaKaRa)
- TURBO DNase (AM2238, Ambion)

REAGENT SETUP

1) Dilution of Capping Analog to 10 mM by UltraPure Water
2) Preparation of NTP mix

   NTP mix for CAS9 mRNA synthesis (NTP with CAP analog)

   ATP:CTP:UTP:GTP:GTP capping analog = 4:4:4:1:3 (molar ratio)

   NTP mix for guideRNA synthesis (NTP without CAP analog)

   ATP:CTP:UTP:GTP = 1:1:1:1 (molar ratio)

PROCEDURE

1) Incubate following mix at 37°C for 2 hr.

	(μl)
5x buffer	8
NTP mix	6.4
0.1 M DTT	4
Linearlized Template DNA	3000 ng of CAS9 template, or 1000 ng of gRNA template
RNase inhibitor	2
Polymerase	4
UltraPureWater	up to 40
Total	40 μl

2) Add 2 μl of DNase, and mix gently by pipetting.
3) Incubate at 37°C for 30 min.
4) Add 60 μl of UltraPure Water and 100 μl RNase-free PCI, and mix by vortexing.
5) Centrgifuge at 20000 x g for 5 min, and transfer the supernatant to a fresh tube.
6) Add 40 μl of 10 M of ammonium acetate and 370 μl of ethanol. Mix by vortexing.
7) Keep at -80°C for 15 min.
8) Centrgifuge at 20000 x g for 30 min. Discurd sup and add 200 μl of 70% EtOH.
9) Centrgifuge at 20000 x g for 5 min. Discurd sup.
10) Dilute RNA to 200 μg/ml by 10-30 μl of UltraPure Water. Aliquot and store at -80°C.