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# X-region PolyU/UC mutagenesis

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**Protocol status:** Working
**We use this protocol and it's working**

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## Abstract

Protocol to PCR and in vitro transcribe the HCV X region w/out mutagenesis

## Materials

**STAR METHOD**

| REAGENT | SOURCE | IDENTIFIER |
|---|---|---|
| Platinum® *Taq* DNA Polymerase kit | Invitrogen | Cat#10966-018 |
| Set of dATP, dCTP, dGTP, dTTP | Promega | Cat# U1420 |
| MEGAscript® T7 Transcription Kit | Ambion<br>Thermo Fisher Technologies | Cat#1334 |
| HCV polyU/UC plasmid | Gift from Michael Gale lab | NA |
| HCV X-region plasmid | Gift from Michael Gale lab | NA |
| UltraPure Water | Invitrogen | Cat#10977-015 |

**Material:**

Primer: X-region F/ X-region R ( [M] 10 micromolar (µM) )

PolyU/UC F and R ( [M] 10 micromolar (µM) )

dNTPs ( [M] 10 millimolar (mM) ) Fermentas –For PCR

HCV PolyU/UC X-region plasmid

# HCV X-region PolyU/UC PCR - T7 in vitro transcription

1 PCR:

1) Thaw component and cDNA, spin down before use

2) Assemble reaction: Master Mix: 10X (each reaction assemble 3 tubes)

| Reagent | Volume (ul) |
|---|---|
| H2O | 26 |
| 10X Buffer | 5 |
| MgCl2 | 5 |
| dNTP (10uM) | 2 |
| Forward Primer (10uM) | 5 |
| Reverse Primer (10uM) | 5 |
| Taq pol. (5unit/ul) | 0.25 |
| Plasmid (10ng/ul) | 1.5 |

Total: 50 µL

3) Run PCR

| Program | Temperature (C) | Time | |
|---|---|---|---|
| Denaturation | 95 | 3 min | Hold |
| Denaturation | 95 | 30 sec | 35 cycles |
| Annealing | 55 | 30 sec | |
| Extension | 72 | 90 sec | |
| Final extension | 72 | 5 min | Hold |
| | 4 | ∞ | Hold |

## II. In vitro transcription:

15m

2 15m

- Spin-down all reagents
- Keep the 10X Reaction Buffer at room temperature while assembling the reaction.
- Vortex all solutions until they are completely in solution.
- Assemble dNTP mix (2µL ATP+ 2µL CTP+ 2µL GTP+ 2µL TTP/reaction).
- Store the ribonucleotides on ice!

Assemble the reaction in an appropriate RNase free PCR tube.

1) 1 µg DNA plasmid (digested)
2) 8 µL NTP Mix
3) 2 µL 10X Reaction Buffer
4) 0.5-1 µL RNaseOUT (RNase Inhibitor)
5) 2 µL T7 enzyme mix
6) RNase Free water up to 20 µL

- Pipette the mixture up and down gently and microfuge
- Incubate 3-6hrs at 37 °C using PCR machine. The shorter the ivtRNA, the longer the reaction time needed.
- (Optional but do most times) Template plasmid Digestion: 1µLTurbo DNase and mix well, 00:15:00 at 37 °C

## IV. RNA precipitation: Transfer to an 1.5ml RNase free vial

35m

3 If you expect very low yields of RNA, do not dilute the transcription reaction with water prior to adding the LiCl. 35m

- Add 30 µL $H_2O$
  30 µL LiCl
- Mix well, incubate 00:30:00 at -20 °C
- Centrifuge at 4°C/20 min/max
- Remove supernatant
- Add 1 mL 70% COLD ethanol
- Centrifuge at 4°C/5 min/max
- Remove supernatant and allow the pellet to dry
- Resuspend in 15-20 µL RNase-free $H_2O$
- Place the tube 00:05:00 at 65 °C water bath
- Dilute 1 µL in 19 µL $H_2O$ to measure concentration

## Protocol references

Jie Xu (Lopez Lab)