Oct 03, 2024

X-region PolyU/UC mutagenesis

DOI
https://dx.doi.org/10.17504/protocols.io.8epv5rxy6g1b/v1

Carolina Lopez[1]

[1]Washington University

Cecilia Escudero
wustl

DOI: https://dx.doi.org/10.17504/protocols.io.8epv5rxy6g1b/v1

Protocol Citation: Carolina Lopez 2024. X-region PolyU/UC mutagenesis. **protocols.io** https://dx.doi.org/10.17504/protocols.io.8epv5rxy6g1b/v1

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

Created: May 28, 2024

Last Modified: October 03, 2024

Protocol Integer ID: 100753

Keywords: uc mutagenesis protocol to pcr, uc mutagenesis protocol, pcr

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 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)