LAMP-Seq Starter Kit

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NOTE: This protocol has not been validated with clinical samples. To facilitate collaborations with interested parties to jointly advance the fight against the current coronavirus pandemic, we have set up a public forum on www.LAMP-Seq.org.

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Purpose of this LAMP-Seq Starter Kit

This kit provides detailed instructions for establishing a LAMP-Seq reaction. The purpose of this kit is setting up the method in other labs for further development and optimization.

This protocol has not been validated on clinical samples.

Materials

- 1.5 ml reaction tubes
- PCR strip tubes and caps
- Thermocycler
- Agarose gel equipment
- Ultrapure water
- WarmStart® LAMP Kit (NEB, E1700S)
- NEBNext® High-Fidelity 2X PCR Master Mix (NEB, M0541S)
- 2019-nCoV_N_Positive Control plasmid (IDT, 10006625)
- HiScribe™ T7 High Yield RNA Synthesis Kit (NEB, E2040S)
- Unrelated plasmid (e.g. pX330-U6-Chimeric_BB-CBh-hSpCas9, Addgene #42230)
- Primer C-IVT-fwd (IDT

  AAGCTAATACGACTCACTATAGCATACAATGTAACACAAGCTTTCGG
- Primer C-IVT-rev (IDT)

  CTTGATCTTTGAAATTTGGATCTTTGTC

- Primer C-F3 (IDT)

  AACACAAGCTTTCGGCAG

- Primer C-B3 (IDT)

  GAAATTTGGATCTTTGTCATCC

- Primer C-FIP-AGCTTACACT (IDT)

  TGCGGCCAATGTTTGTAATCAG AGCTTACACT CCAAGGAAATTTTGGGGAC

- Primer C-BIP (IDT)

  CGCATTGGCATGGAAGTCAC TTTGATGGCACCTGTGTAG

- Primer C-LF (IDT)

  TTCCTTGTCTGATTAGTTC

- Primer C-LB (IDT)

  ACCTTCGGGAACGTGGTT

- Primer PCR-C-fwd-10 (IDT)

  ACACTCTTTCCCTACACGACGCTCTTCCGATCTAACGCTGAAGCGCTGGGGGC

  AAA

- Primer PCR-C-rev-11 (IDT)

  TGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTTTGTAATCAGTTCCTTGTCTG

Protocol

1. Generate template RNA by IVT
    a. Perform a NEBNext PCR

i. Primers: C-IVT-fwd and C-IVT-rev

ii. Template: 2019-nCoV_N_Positive Control plasmid

b. Column-purify and elute in water,

c. perform an IVT reaction using HiScribe™ T7 High Yield RNA Synthesis Kit for 12 hours,

d. column-purify and elute in water,

e. measure concentration using NanoDrop.

2. Make the following plasmid pre-dilutions:

a. Make a stock dilution of an unrelated plasmid in water at 5 ng/μl:

i. 5 μl plasmid (1 μg/μl) + 995 μl water.

b. Create a dilution series:

i. Using the plasmid dilution, sequentially dilute template RNA down to four molecules / μl. Use large pipetting volumes if possible (e.g. 2 μl + 998 μl).

3. Prepare a 10x LAMP primer mix:

a. 16 μl C-FIP-AGCTTACACT (100 μM),

b. 16 μl C-BIP (100 μM),

c. 2 μl C-F3 (100 μM),

d. 2 μl C-B3 (100 μM),

e. 4 μl C-LF (100 μM),

f. 4 μl C-LB (100 μM),

g. 56 μl water,

h. mix by pipetting.

4. Perform barcoded LAMP reactions:

a. Prepare a reaction mix:
    i. 312.5 μl 2x LAMP Master Mix (NEB),
    ii. 62.5 μl 10x LAMP primer mix,
    iii. 125 μl water,
    iv. 125 μl template RNA (four molecules / μl RNA in 5 ng/μl plasmid DNA),
    v. mix by pipetting.
b. Aliquot 25 μl reaction mix into each of 24 tubes in PCR strips,
c. incubate the LAMP reactions in a thermocycler for 40 minutes at 65 °C, and subsequently for 10 minutes at 95 °C.

5. Dilute the LAMP reactions 100-fold in PCR strips:
    a. 2 μl LAMP reaction + 198 μl water.
6. Perform PCR reactions:
    a. Prepare a reaction mix:
        i. 150 μl NEBNext 2x Master Mix (NEB),
        ii. 1.5 μl PCR-C-fwd-10 (100 μM),
        iii. 1.5 μl PCR-C-rev-11 (100 μM),
        iv. 147 μl water,
        v. mix by pipetting.
    b. Aliquot 9 μl reaction mix into each of 24 tubes in PCR strips,
    c. to each reaction, add 1 μl of diluted LAMP reaction,
    d. mix by pipetting,
    e. run the PCR reaction in a thermocycler with the following protocol:
        i. 98 °C 3 minutes,

ii. 98 °C 20 seconds (15 cycles),

iii. 65 °C 20 seconds (15 cycles),

iv. 72 °C 30 seconds (15 cycles),

v. 72 °C 3 minutes,

vi. 4 °C hold.

7. Run 5 μl of each PCR reaction on a 1% agarose gel.

Expected outcome

Positive reactions are expected to yield a single strong band at an estimated size of 184 bp. At 20 molecules per reaction, around 10 out of 24 reactions are expected to be detected as positive.