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.
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)
  
  AAGCTAATACGACTCACTATAGCATACAATGTAACACAAGCTTTTCGG
- Primer C-IVT-rev (IDT)
  
  CTTGATCTTTTGAAATTTGGATCTTTGTC
- Primer C-F3 (IDT)
  AACACAAGCTTTTGCCAG

- Primer C-B3 (IDT)
  GAAATTTGGATCTTTGTCCATCC

- Primer C-FIP-AGCTTACACT (IDT)
  TGCGGCAATGGTGGTCAATCAG AGCTTACACT CCAAGGAATTTTGAGGGAC

- Primer C-BIP (IDT)
  CGCATGGGCATGGAGTCAC TTTGATGGCACCCTGTGTAAG

- Primer C-LF (IDT)
  TTCCTGTCTGATTAGTTC

- Primer C-LB (IDT)
  ACCTTCGGGAACGTGGTT

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

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

**Protocol**

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