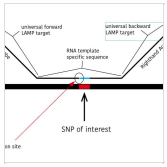

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Variant (E484K) ALERT - Ligation-Dependent Loop-Mediated Isothermal Amplification

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ABSTRACT

A 2-hour, 2-temperature protocol, using RNA templated DNA ligation, for the visual detection of the E484K mutation of concern pertaining to SARS-CoV-2.

N.B. This is the first version of this protocol, stay tuned for increased sensitivity and multiplexing (A.B.)

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PROTOCOL CITATION

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Ligation Primers

1 Oligonucleotides used for RNA templated DNA ligation using

SplintR Ligase - 1,250 units **New England Biolabs Catalog #M0375S**

All sequences shown 5' to 3. Underlined segments are complementary to the target and bold nucleotide is specific to the G>A substitution at position 23012 resulting in the E484K amino acid change.

E484K-Donor
/5Phos/AACACCATTACAAGCTACGTACATGACATAATCCAAACTCATAAATTCTCGCATTTTAGATCCGTCTCCTTACAGGACACATCATCC

E484-Acceptor
CATCTCTAACTCTACTAAGACTTCCATTTATCAACAATAGCTGACATGTTCTAGCAGCGACAGGACACACGACAGGAGGAAAGTAACAATTAAAACCTT**T**

Ligation

2 ▪ Prepare ligation mix to use with previously isolated RNA. A standard Trizol/Chloroform method or column purification method for RNA isolation works well.

X1 Ligation mix (multiply for number of samples processed)

- **2 µl** E484K-Donor (100nM)
- **2 µl** E484K-Acceptor (100nM)
- **1 µl** SplintR Buffer (NEB)
- SplintR Ligase - 1,250 units **New England Biolabs Catalog #M0375S** (NEB)
- **1.8 µl** molecular biology grade water

▪ add **3 µl** RNA per **7 µl** of reaction mix for a **10 µl** reaction volume

3 Incubate at **37 °C** for **00:20:00** followed by a **00:20:00** at **65 °C** inactivation step. 40m

4 Place ligation reactions on ice.

LAMP Primers

5 Oligonucleotides used for Loop-Mediated Isothermal Amplification
all sequences shown 5' to 3'

FIP
CTTTCCTCCTGTCGTGTGTCCTCCATTTATCAACAATAGCTGAC

BIP
ACCTTTAACACCATTACAAGCTACGACGGATCTAAAATGCGAGAA

F3
CATCTCTAACTCTACTAAGACT

B3
GGATGATGTGTCCTGTAAGG

LAMP 50m

6 ▪ Prepare LAMP mix.

X1 LAMP mix (multiply for number of samples processed)

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2.5 µl Isothermal Buffer 1 (NEB)

Magnesium Sulfate (MgSO4) Solution - 6.0 ml **New England**

0.5 µl **Biolabs Catalog #B1003S** (100mM)

3.5 µl dNTPs (10µM)

0.8 µl FIP (50µM)

0.8 µl BIP (50µM)

0.5 µl F3 (10µM)

0.5 µl B3 (10µM)

SYTO 9 fluorescent nucleic acid stain **Life**

0.1 µl **Technologies Catalog #S-34854** (100µM)

Bst 2.0 WarmStart DNA Polymerase - 8,000 units **New England**

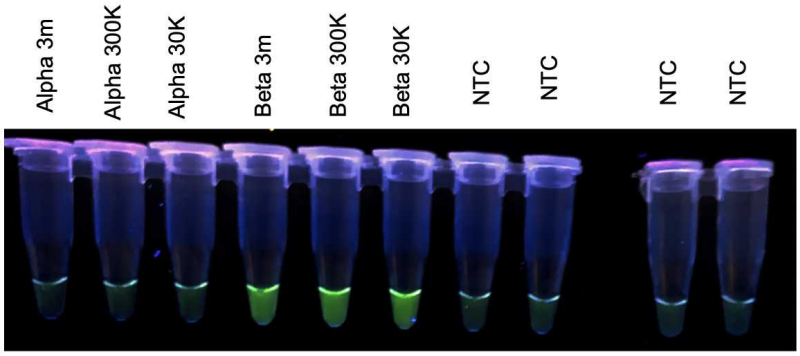
1 µl **Biolabs Catalog #M0538L**

11.8 µl Molecular Biology Grade water

- add **3 µl** of the ligation product from the Ligation step to **22 µl** of LAMP mix for a **25 µl** reaction.

7 Incubate at **65 °C** for **00:50:00** 50m

8 Visualize reaction tubes over a UV transilluminator. Samples containing a G>A mutation at position 23012 will exhibit fluorescence.



RNA samples are synthetic controls from Twist Biosciences, at various (approximate) copy numbers

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