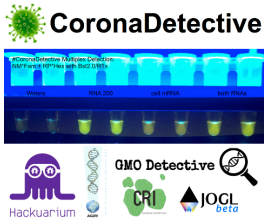
Nov 19, 2020 Version 2

Freeze-drying (Lyophilization) and manufacturing of Corona Detective assay V.2



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We use this protocol and it's working

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Abstract

A protocol for freeze-drying (Lyophilization) of CoronaDetective tests,
and more generally any QUASR RT-LAMP reaction.

The amounts here are for one 96 well standard PCR plate and for 20 µL reactions

This can be scaled for any amount and size.

Guidelines

The amounts here are for one standard 96 well PCR plate (12 experiments), and for 20 µL reactions

This can be scaled for any amount of plates at any reaction volume.

Materials

MATERIALS

- Isothermal Amplification Buffer - 6.0 ml **New England Biolabs Catalog #**B0537S
- Magnesium Sulfate (MgSO4) Solution - 6.0 ml **New England Biolabs Catalog #**B1003S
- Recombinant RNasin(R) RNase Inhibitor, 10,000u **Promega Catalog #**N2515
- Deoxynucleotide (dNTP) Solution Mix **New England Biolabs Catalog #**N0447S
- 2019-nCoV_N_Positive Control **Integrated DNA Technologies Catalog #**10006625
- Bst 2.0 Warm Start DNA Polymerase Glycerol-free **New England Biolabs Catalog #**M0402B
- D-( )-Trehalose dihydrate **Sigma Aldrich Catalog #**T5251
- WarmStart® RTx (Glycerol-Free) **New England Biolabs Catalog #**M0439B-HC1

Safety warnings

There is no biohazard risk from producing these freeze-dried tubes, but care must be taken to avoid any potential contamination with target sequences or RNAse.

Best practices should be followed (appropriate PPE, RNaseAway, etc.).

Before start

Make sure to have all needed primers and reagents in sufficient quantities.

Fluorescence-tagged primers and complementary quencher sequences are essential for QUASR detection.

Standard Enzymes usually come in 50% Glycerol, so as to be stable in the -20C freezer. The glycerol interferes with freeze-drying.

Make sure your enzymes are Glycerol-free and stored at -80C.

Prepare the Harvest Right Lyophilizer system (or similar) for its freeze-drying run (Clean and Make sure vacuum is pulling)

Other systems are possible, and robotics are useful for scaling.

1

Thaw components (dNTPs, 10X Primer mixes (need to be made), $MgSO_4$, Isothermal amplification buffer, and Enzymes)

Vortex and quickly spin tubes down before opening for dispensing.

This protocol is for one standard 96 well PCR plate and can be scaled as needed.

1.1 **10X Primer mix:** assuming your primer stocks are at [M] 100 millimolar (mM) for 200 µL add together

Primer sequences taken from: https://www.nature.com/articles/s41587-020-0513-4 (Supplementary Data 2)
and adapted for QUASR detection according to
https://www.nature.com/articles/srep44778

For the **NM SARS CoV 2** primer set:

Fam-FIP/BIP [M] 16 micromolar (µM) - 32 µL each
LB/LF [M] 8 micromolar (µM) - 16 µL each
F3/B3 [M] 2 micromolar (µM) - 4 µL each
Anti-FIP-Q [M] 24 micromolar (µM) - 48 µL
48 µL DNAse/RNAse free water

For the **RNAseP human internal control** primer set:

FIP/BIP [M] 16 micromolar (µM) - 32 µL each
LB/Hex-LF [M] 8 micromolar (µM) - 16 µL each
F3/B3 [M] 2 micromolar (µM) - 4 µL each
Anti-LF-Q [M] 12 micromolar (µM) 24 µL
72 µL DNAse/RNAse free water

2 **FD QUASR RT-LAMP Mastermix:** In a 2ml tube mix together
280 µL dNTPs [M] 10 millimolar (mM) each dNTP +
200 µL of each 10x Primer Mix (NM and RNAseP From step **1.1**)+
5.33 µL Glycerol free Bst 2.0 WarmStart® DNA Polymerase [M] 120000 U/ml +

8 µL Glycerol Free WarmStart® RTx Reverse Transcriptase [M] 75000 U/ml +
400 µL Trehalose [M] 50 Mass Percent (for a 50% trehalose solution, mix 200mg trehalose with 400uL H_20 and filter or autoclave) +
506.67 µL DNAse/RNAse free water

Vortex all or mix by pipetting up and down and then spindown.

2.1 **Rehydration Buffer:** Either now or at a later time make the rehydration buffer. For a whole plate, mix
200 µL 10X Isothermal Amplification Buffer +
100 µL Magnesium Sulfate (MgSO4) Solution [M] 100 millimolar (mM) +
1300 µL DNAse/RNAse free water ,
store in a cool dark place or in a fridge (4 °C) (stable for shipment)

3 **Dispensing:** In each well of a PCR plate place 16 µL of the **Mastermix** from step **2**

A digital dispenser or liquid handling robot is useful here for larger scales

3.1 ***Controls:*** *Optionally, add* 4 µL *of Internal controls (Such as IDT* **2019-nCoV_N_Positive Control***) to selected tubes and mark them as such.*

4 **Sealing and piercing:** Seal the plate(s) with either foil or parafilm and then make a small puncture in the seal of each tube.

This is done when reactions are freeze-dried in order to prevent the small pellets from "jumping" out of the tubes under vacuum.
A 96-Pin Replicator is perfect for this but a multi-pipette, toothpick, tip, scalpel, or scissors will do.

5 **Freeze-drying:** Depending on your freeze drier, you might need to now freeze the tubes, and make sure they remain frozen (such as by placing in a frozen metal rack or touching a frozen metal block).
Otherwise, simply place in a freeze-drier and run overnight or until done.
We start by freezing to ~ -40 °C for a few hours
then turn on the vacuum (aiming for 500mtorr) for another couple of hours,

then slowly heating by 10 °C every hour with the vacuum still on.

Equipment

Scientific Freeze Dryer	NAME
lyophilizer	TYPE
Harvest Right	BRAND
HRFD-Med-Sci-EU	SKU
https://harvestright.com/	LINK

6 **Storage:** Make sure each tube has a similarly sized dried pellet and reseal the plate with either film, foil, or caps.
Store in a dark and dry place preferably in a sealed bag with a desiccant. (stable for shipment)