FLASH amp

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1 Works for me dx.doi.org/10.17504/protocols.io.bk2hkyb6

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ABSTRACT

FLASH amp is a method that allows for room temperature detection and amplification of a viral RNA sequence of interest submitted as part of the Xprize competition. The main components of the reaction are not included here and are given generic names such as enzyme X as they are currently not protected under IP. The steps outlined with tubes labeled with the generic names would allow any lab to reproduce the method.

Version 1 of this method outlines the protocol for a fluorescence-based readout that can be used with a qPCR machine or TECAN style plate reader.

Version 2 (to be released shortly) will be a colorimetric readout that can be assessed by eye.

The outlined protocol assumes the user performing this in a laboratory setting will setup reactions in a clean room and analyze results in a seperate post-amplification room. In addition, since our amplification is rapid and at room temperature, care but be taken to add ligand to the master mix quickly and the reactions be sealed.

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PROTOCOL CITATION

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KEYWORDS

Xprize

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STEPS MATERIALS

NAME	CATALOG #	VENDOR
Pyrophosphatase, Inorganic (E.coli) - 50 units	M0361L	New England Biolabs
Corning® Low Volume 384-well Black Flat Bottom Polystyrene	3821BC	

EQUIPMENT

NAME	CATALOG #	VENDOR
SPARK	SPARK	

DISCLAIMER:

This method is currently used for research and development, not for diagnostic purposes.

Sample collection

1 Sample should be collected 1 hour after any food is consumed.
10 minutes before collection rinse mouth well with water.
Collect passive drool or pooled salive below tongue into collection tube.

Set-up

2 Resuspend freeze dried master mix 1 with **7.04 µl** of water

3 Resuspend freeze dried master mix 2 in **1 µl** water and add to above

4 Add **0.3 µl**

Pyrophosphatase, Inorganic (E.coli) - 50 units
by New England Biolabs
Catalog #: M0361L

5 Add **0.5 µl**

Undisclosed enzyme Z

6 Add **0.5 µl**
Undisclosed Enzyme Y

7 Add **0.13 µl**
Undisclosed Enzyme X

8 Put mix in

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Corning® Low Volume 384-well Black Flat Bottom Polystyrene
Catalog #: 3821BC

Add **0.5 µl** of sample and seal plate.

A large master mix of the above components can be mixed and aliquoted into wells.

Data collection

9 Place plate in TECAN plate reader.

SPARK
Microwell plate reader
TECAN SPARK

Set gain to 120, excitation 480 and emission 550.
A qPCR machine can also be used.
Collect data at T= 30 min.
Data can also be collected every 1s for 30 min to monitor kinetics of reaction in the particular plate reader being used if not TECAN SPARK reader.

Fluorescence values of >4000 are positive and <4000 are negative.

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