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Filter-paper based PURExpress detection of ZikV and CoV2 RNA by enhancer toehold (TacToe) sensors

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Tanvi Kale¹, Chaitanya Anil Athale¹

¹IISER Pune

Chaitanya Anil Athale: Supervisor, Conceptualization, Funds acquisition.



Chaitanya Anil Athale

IISER Pune

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Protocol status: Working

We use this protocol and it's working

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Disclaimer

This method is a scientific protocol that has not been validated on clinical samples so far.

Abstract

This method details the approach to using in vitro transcription-translation (TxTr) systems based on PURExpress adsorbed on a paper disc. These to run a toehold detection reaction for COVID RNA. The work is related to a manuscript titled "Translational Enhancer Based Amplification of Toehold Sensors (TacToe) for Improved Sensitivity and Speed of Viral RNA Detection" to appear in ACS Synthetic Biology.

Attachments



[dnaSequences-](#)

[Toehold...](#)

234KB



Materials

Whatman Filter Paper discs
Biopsy Punch (3 mm) with metal caps
PureXpress CFE
Toehold plasmid DNA
384 well plate (Corning Costar)

Before start

Before you start you need the DNA sequences that are provided in the attached files.

Reaction Kinetics using PURExpress

40m

1 BSA treated paper disk preparation:

- 1) Make 5% BSA solution in ultrapure water
- 2) Soak filter paper disc (Whatmann filter paper 42, ashless, dm 42.5) in BSA solution, keep it at room temperature for overnight in a beaker
- 3) Rinse the filter paper using ultrapure water (repeat the process for 5 times)
- 4) Let filter paper dry at room temperature (overnight) or at 65 °C in hybridizer (~1-1.5h)
- 5) Use sterile 2mm/3mm biopsy punch to create small paper discs
- 6) Store the discs in sterile petri plate

2 PCR amplification of the sensor and the trigger from purified plasmids

The Sensors and triggers are amplified using the following primers:

	A	B	C	D	E
	Primer	Sequence	Length (bp)	T _m (°C)	Description
	27B_LacZ_F	AACGCTGCTC TGGGCTAAC	19	64.4	Forward Primers for sensor amplification
	27B_LacZ_R	CGTGTGCTTC TCAAATGCC	19	64.5	Reverse Primers for sensor amplification
	trig_F	TTTAGAGGCC CCAAGG	16	58.7	Forward primer for trigger amplification
	trig_R	GTTGCGCTAA TACGACTCAC TA	22	60.9	Reverse primer for trigger amplification

Following are the lengths of the amplicons (linearised sensor/trigger):

A	B	C
Amplicon	Amplicon length (bp)	Primers used to amplify
ZIKV 27B	3305	27B_LacZ_F and 27B_LacZ_R
ZIKV TacToe	3326	
COV TacToe	3327	
ZIKV Trigger	400	trig_F and trig_R
COV Trigger	233	



- 3 Prepare the following reaction mix (Master-mix):

30m

DNA working concentration:**ZIKV:**

Toehold: 24nM

Trigger: 54nM

COV:

Toehold: 0.3nM

Trigger: 8nM

Components	Working Conc.	Blank(μl)	Test(μl)
Buffer A*		2.01	2.01
Buffer B*		1.5	1.5
CPRG (20mg/ml)	0.6ug/ml	0.21	0.21
Rnase inhibitor		0.5	0.5
Toehold DNA	(Adjust depending on the yield and the reaction requirements)		
Trigger DNA	(Adjust depending on the yield and the reaction requirements)		
Water		2.78	-
Total Volume		7	7

*Buffer A and B are the two mixes from NEB's PURExpress

Buffer B	1.5	1.5 on ice while preparing the
master-mix		

- 4 From the 7 μl master-mix, pipette out 2μl on each paper-disk placed in a 384 well plate to get triplicates of each reaction (2μl x 3).

10m

- 5 Measure absorbance at 570nm over 100 minutes at a 1 minute interval in a plate reader incubated at 37°C

1h 40m

- 6 **Freeze drying disks with CFE (Home-made or PURExpress)+ CPRG**

1. Cell free reaction (Mastermix + CFE) applied on paper discs (3mm)(Mastermix 1.02ul +CFE 0.44ul)
2. Air dry



3. Flash freeze in liquid Nitrogen
4. Put the disc in PCR tubes (1disc/tube). Make 4 small holes on capusing sterile syringe.
5. Put the tubes in lyophilizer @-75 °C, 4 hour , 0.04 mbar pressure
6. Samples store @Room Temperature in sterile falcon with silica inside
7. Rehydration: Plasmid (~24nM) + water (0.54ul)

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