May 29, 2025

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

DOI
dx.doi.org/10.17504/protocols.io.q26g7p3p1gwz/v1

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CFE-calibration

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OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.q26g7p3p1gwz/v1

Protocol Citation: Tanvi Kale, Chaitanya Anil Athale 2025. Filter-paper based PURExpress detection of ZikV and CoV2 RNA by enhancer toehold (TacToe) sensors . **protocols.io https://dx.doi.org/10.17504/protocols.io.q26g7p3p1gwz/v1**

Manuscript citation:
Tanvi Kale, Rudvi Pednekar, Séverine Marianne Cazaux, Valentina Ferrando Letelier, Justin R. J. Vigar, Fernán Federici, Keith Pardee, and Chaitanya A. Athale (accepted) Translational Enhancer Based Amplification of Toehold Sensors (TacToe) for Improved Sensitivity and Speed of Viral RNA Detection. ACS Synthetic Biology
https://doi.org/10.1021/acssynbio.4c00861

Protocol status: Working
We use this protocol and it's working

Created: October 17, 2023

Last Modified: May 29, 2025

Protocol Integer ID: 89431

Keywords: toehold, sensor, point of care, covid, zika, RNA, diagnostics, point-of-care, filter-paper, lacZ

Funders Acknowledgements:
Shastri Indo-Canadian Institute (SICI)
Grant ID: SCPRG
Canada's International Development Research Centre (IDRC)
Grant ID: 109434-001

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 Ampli cation of Toehold Sensors (TacToe) for Improved Sensitivity and Speed of Viral RNA Detection" to appear in ACS Synthetic Biology.

Attachments

ZIP

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 ºc 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)**	**Tm (℃)**	**Description**
	27B_LacZ_F	AACGCTGCTCT GGGCTAAC	19	64.4	Forward Primers for sensor amplification
	27B_LacZ_R	CGTGTGCTTCT CAAATGCC	19	64.5	Reverse Primers for sensor amplification
	trig_F	TTTAGAGGCCC CAAGG	16	58.7	Forward primer for trigger amplification
	trig_R	GTTGCGCTAAT ACGACTCACTA	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 ºC, 4 hour , 0.04 mbar pressure
6. Samples store @Room Temperature in sterile falcon with silica inside
7. Rehydration: Plasmid (~24nM) + water (0.54ul)

Acknowledgements

The work was supported by funding from the Shastri Indo-Canadian Institute (SICI) Shastri Covid-19 Pandemic Response Grant (SCPRG) and Canada's International Development Research Centre (IDRC) (109434-001) through the Canadian 2019 Novel Coronavirus (COVID-19) Rapid Research Funding Opportunity and the National Agency for Research and Development (ANID). ZIKV Sensor27B 772 LacZ and ZIKV NASBA 27B were a gift from James Collins & 773 Alexander Green (Addgene plasmids #75010 and #75006).