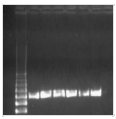


Nov 27, 2019

Respiratory picornavirus genotyping conventional nested RT-PCR ("Wisdom VP42 assay") V.2

Forked from Respiratory picornavirus genotyping conventional nested RT-PCR ("Wisdom VP42 assay")

Ian Mackay[1], Judy Northill[1]

[1]Public Health Virology, Forensic and Scientific Services

1 Works for me dx.doi.org/10.17504/protocols.io.9tyh6pw

Ian M. Mackay
Public Health Virology, Forensic and Scientific Services

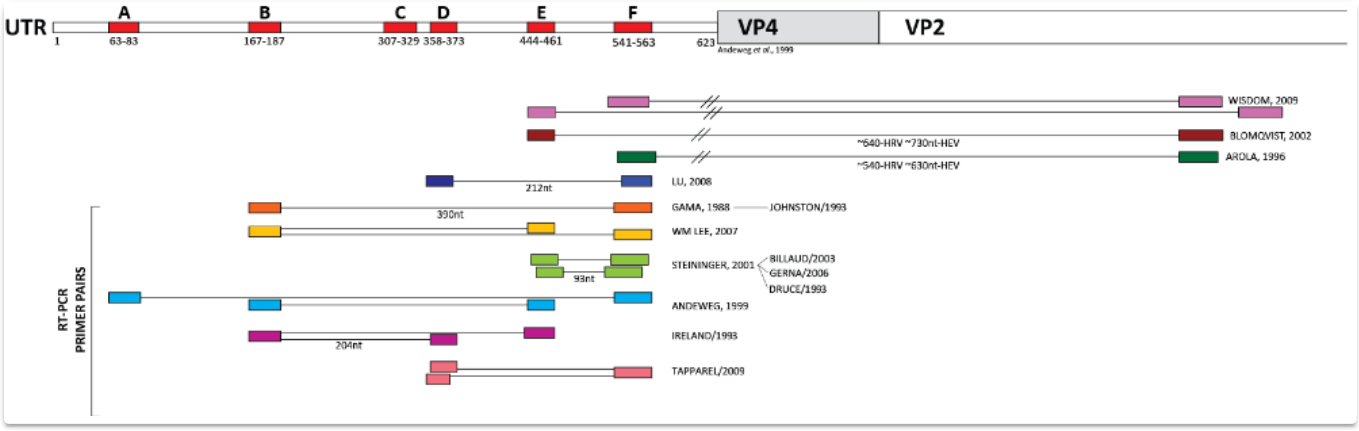
ABSTRACT

This is my preferred, previously published [Ref 1], rhinovirus (RV) and enterovirus (EV) genotyping assay when seeking to identify the genotype of a respiratory picornavirus detected in a clinical sample extract. It is employed after use of a screening real-time RT-PCR has identified a respiratory picornavirus.

I have not confirmed that it can detect every single RV genotype but I do know that it detects *many* from each of the three RV species (*Human rhinovirus A*, *Human rhinovirus B* and *Human rhinovirus C*) as well as at least some *Human enterovirus* (EV) genotypes.

The assay picks up EVs due to the shared genetic similarities in the 5'UTR target region. EVs can be discriminated using subgenomic sequencing (see VP42 typing assay protocol), or simply described as 'respiratory EVs' since there is no specific-specific vaccine or treatment available anyway.

This is a robust primary subgenomic sequencing assay. It is more sensitive than any VP1 protocols because it targets more conserved primer target sites. It produces a more reliable typing result than does the 5'UTR region alone.



EXTERNAL LINK

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2786677/

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Reference 1. Screening Respiratory Samples for Detection of Human Rhinoviruses (HRVs) and Enteroviruses: Comprehensive VP4-VP2 Typing Reveals High Incidence and Genetic Diversity of HRV Species C.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2786677/

MATERIALS

NAME	CATALOG #	VENDOR

NAME	CATALOG #	VENDOR
MyFi Mix	BIO-25049	Bioline
SuperScript™ III One-Step RT-PCR System with Platinum™ Taq DNA Polymerase	12574026	Thermo Fisher

STEPS MATERIALS

NAME	CATALOG #	VENDOR
SuperScript III One-Step RT-PCR System with Platinum Taq	12457-026	Invitrogen - Thermo Fisher
MyFi Mix	BIO-25049	Bioline

Oligonucleotide sequences...

1

	Name	Sequence (5'-3')
RT-PCR (Round 1)	HRV_HEV VP42 OS	CCGGCCCCTGAATG**Y**GGCTAA
	HRV_HEV VP42 OAS	ACAT**R**TT**Y**T**SN**CCAAA**N**A**YD**CCCAT
PCR (Round 1)	HRV_HEV VP42 IS	ACC**R**ACTACTTTGGGTGTCCGTG
	HRV_HEV VP42 IAS	TC**W**GG**H**A**RY**TTCCA**M**CACCA**N**CC

1. Expected amplicon sizes: Round 1: ~380 base pairs; Round 2:
2. The naming used here is my in-house adaptation (FYI: 01 - forward / sense; 02 - reverse / antisense; .x - version of the design of this particular named oligonucleotide). If you prefer to be true to the original publication, please see Ref 1

Reagents

2

SuperScript III One-Step RT-PCR System with Platinum Taq
by Invitrogen - Thermo Fisher
Catalog #: 12457-026

MyFi Mix
by Bioline
Catalog #: BIO-25049

Reaction set-up

3 ROUND 1 REACTION MIX

- Ideally, set up more reaction mixes than you'll need for both rounds at the same time
- Strips of 8x 0.2ml tubes are good for this use
- Freeze in a frost-free freezer at -20°C until needed

Reagent	Vol (µl) 1x	Final reaction concentration
Nuclease-free water	4.08	N/A
Reaction Mix (2X)	10.00	1X
HRV_HEV VP42 OS 200pmol/µl	0.06	600nM
HRV_HEV VP42 OAS 200pmol/µl	0.06	600nM
SuperScript® III RT/ Platinum® Taq Enzyme Mix	0.20	Unknown
Template	5.00	N/A

1. Dispense 15µL to each reaction tube
2. Total reaction volume will be 20µl

Reagent	Vol (µl) 1x	Final reaction concentration
Nuclease-free water	7.92	N/A
MyTaq Reaction Buffer (2X)	10.00	1X
HRV_HEV VP42 IS 200pmol/µl	0.038	380nM
HRV_HEV VP42 IAS 200pmol/µl	0.038	4.75mM
1st round amplicon	2.00	N/A

1. Dispense 18µL into each reaction tube
2. Total reaction volume will be 20µl

Amplification: ROUND 1, RT-PCR

4

- This reaction has been run using a SimpliAmp (Applied Biosystems) thermal cycler
- Transfer 5µl of nucleic acid extract (extracted RNA, controls or NTC [nuclease-free water]) to defrosted reaction tubes and cycle

55°C	20 min	1X
94°C	2 min	1X
94°C	30 sec	|
50°C	45 sec	| 4X
68°C	50 sec	|
94°C	30 sec	|
40°C	45 sec	| 25X
68°C	50 sec	|
68°C	5 min	1X
15°C	∞	

Amplification: ROUND 2, PCR

5

- This reaction has been run using a SimpliAmp (Applied Biosystems) thermal cycler
- Transfer 2µl of 1:100 pre-diluted Round 1 amplicon into defrosted 0.2ml reaction tubes and cycle

95°C	1 min	1X
95°C	30 sec	|
50°C	45 sec	| 4X
72°C	50 sec	|
95°C	30 sec	|
40°C	45 sec	| 25X
72°C	50 sec	|
72°C	5 min	1X
15°C	∞	

Amplicon visualisation

6

- electrophorese 2-5µl amplicon on a 1.5% agarose gel in 0.5X TBE buffer