



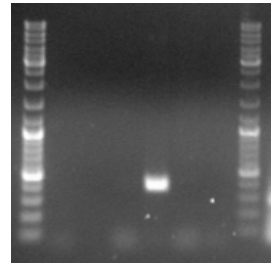
Mar 30, 2018

Version 1

🌐 Respiratory picornavirus genotyping conventional nested RT-PCR ("Wisdom VP42 assay") V.1

DOI

<https://dx.doi.org/10.17504/protocols.io.nz7df9n>



Ian Mackay¹, Claire Y. T. Wang¹, Katherine E Arden¹

¹The University of Queensland



Ian M Mackay

The University of Queensland

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Manuscript citation:

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/>

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Protocol status: Working

We use this protocol and it's working

Created: March 25, 2018

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Protocol Integer ID: 11039

Keywords: s, genotypes of respiratory picornavirus, respiratory picornavirus, human rhinovirus, rhinovirus, human enterovirus, respiratory ev, enterovirus, genotyping assay, single rv genotype, genotyping conventional nested rt, using subgenomic sequencing, wisdom vp42 assay, sequencing assay, subgenomic sequencing, specific vaccine, genotype, rv species, pcr, typing assay protocol, conventional nested rt, assay protocol

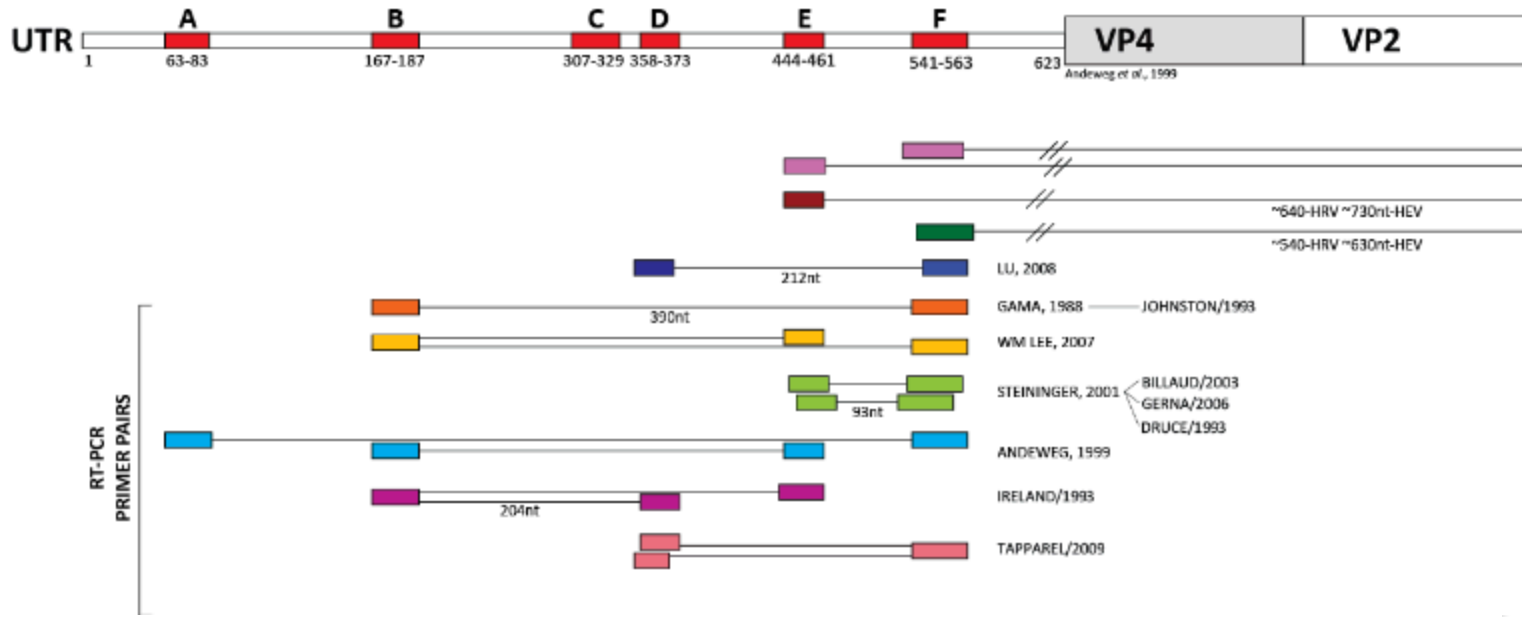
Abstract

This is my preferred, previously published [Ref 1], rhinovirus (RV) and enterovirus (EV) genotyping assay when seeking to characterize the genotypes of respiratory picornavirus identified after use of a screening real-time RT-PCR to test nucleic acid extracts from clinical samples.

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.





Materials

STEP MATERIALS

⊗ SensiFAST Probe no ROX one-step kit **Bioline Catalog #BIO-76005**

⊗ MyTaq HS DNA Polymerase **Bioline Catalog #BIO-21113**

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Protocol materials

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Oligonucleotide sequences...

1

	Name	Sequence (5'-3')
RT-PCR (Round 1)	HRV_HEV VP42 OS	CCGGCCCCTGAATGYGGCTAA
	HRV_HEV VP42 OAS	ACATRTTYTSNCCAAANAYDCCCAT
PCR (Round 1)	HRV_HEV VP42 IS	ACCRACACTTTGGGTGTCCGTG
	HRV_HEV VP42 IAS	TCWGGHARYTTCCAMCACCANCC

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

 SensiFAST Probe no ROX one-step kit **Bioline Catalog #BIO-76005** MyTaq HS DNA Polymerase **Bioline Catalog #BIO-21113**

Reaction set-up

3

Reagent	Vol (μl) 1x	Final reaction concentration
Nuclease-free water	1.4	N/A
SensiFAST no ROX One-Step Mix (2X)	10	1X
Primers (μM) ¹	6	600nM
MgCl ₂	0	3mM
RNase inhibitor	0.4	Unknown
RT/Taq (?U/μl)	0.2	Unknown
Template	2	N/A

1. Both mixed to this final concentration
2. Dispense 18μL to each reaction tube.
3. Add 2μL of template (extracted RNA, controls or NTC [nuclease-free water])



4. Total reaction volume is 20µL

Reagent	Vol (µl) 1x	Final reaction concentration
Nuclease-free water	8.7	N/A
MyTaq Reaction Buffer (5X)	4	1X
Primers (µM) ¹	3.8	380nM
MgCl ₂	1.4	4.75mM
MyTaq HS DNA Polymerase (5U/µL)	0.2	Unknown
1st round amplicon	2	N/A

1. Both mixed to this final concentration
2. Dispense 18µL into each reaction tube
3. NB: a 1:100 pre-dilution can be made first
4. Total reaction volume is 20µL

Amplification

4

45°C	20 min	1X
94°C	2 min	1X
94°C	18 sec	
50°C	21 sec	35X
72°C	90 sec	
72°C	7 min	1X
4°C	∞	

95° C	1 min	1X
94° C	18 sec	



	50° C	21 sec	35X
	72° C	90 sec	
	4°C	∞	

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