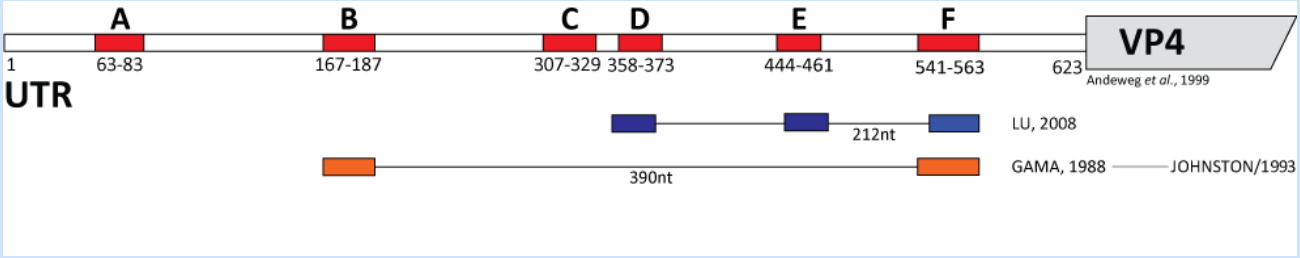
Human rhinovirus screening conventional RT-PCR ("Gama assay") Version 4

Ian Mackay,Katherine E. Arden

Abstract

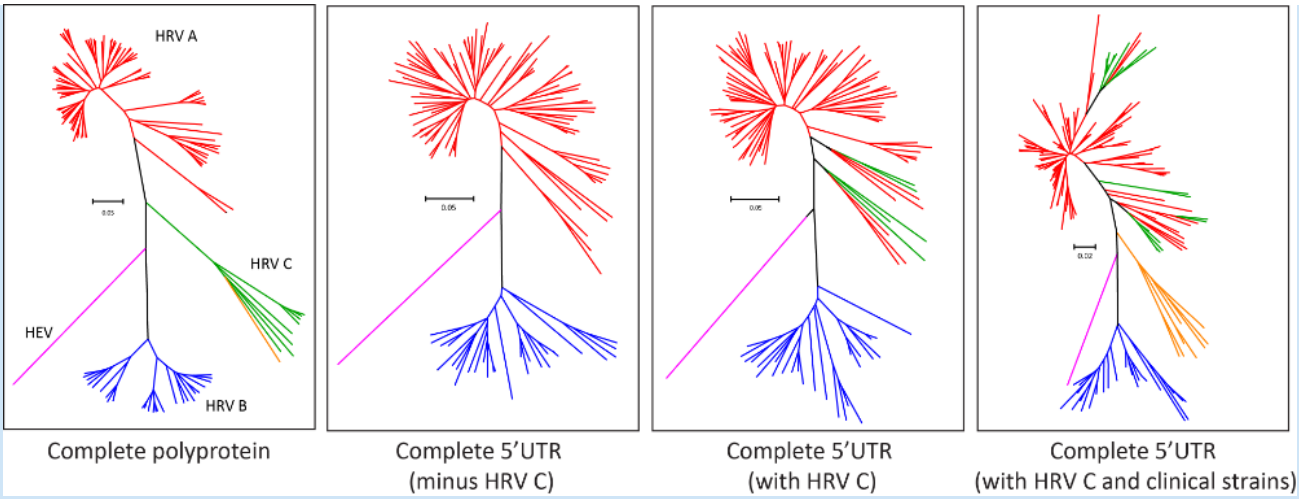
This is a very handy, previously published [Ref 1 and 2], rhinovirus (RV) screening and genotyping assay which I and many others have used on many sample extracts, mostly originating from acutely ill paediatric patients, spanning well over a decade's worth of collection dates.

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.

This assay produces a useful backup subgenomic sequencing target for use primarily should the 'Wisdom VP42' protocol fail to amplify a product for sequencing. It is worth noting that these primers span a target region that can produce confusing results. Recombination within the 5' end of the genome of some members of the genus *Enterovirus* can lead to 5'UTR sequences generated by RV-C genotypes that appear to be RV-As (see the figure below in which green RV-Cs appear in what is expected to be the red RV-A branch of the phylogenetic tree).



Citation: Ian Mackay,Katherine E. Arden Human rhinovirus screening conventional RT-PCR ("Gama assay"). **protocols.io**
dx.doi.org/10.17504/protocols.io.n7gdhjw
Published: 03 Apr 2018

Materials

SensiFAST Probe no ROX one-step kit BIO-76005 by Bioline

Protocol

Oligonucleotide sequences

Step 1.

Name	Sequence (5'-3')
GAMA_OL26_01.1	GCACTTCTGTTTCCCCC
GAMA_OL26_02.1	CGGACACCCAAAGTAG

1. Expected amplicon size 380 base pairs
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 and Ref 2

Reagents

Step 2.

REAGENTS
SensiFAST Probe no ROX one-step kit BIO-76005 by Bioline

Reaction set-up

Step 3.

Reagent	Vol (µl) x1	Final reaction concentration
Nuclease-free water	1.8	N/A
SensiFAST no ROX One-Step Mix(2X)	10	1X
Primers (2µM)[1]	4	400nM
MgCl2 (25mM)	1.6	5mM
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

Amplification

Step 4.

- This protocol has been used successfully on Applied Biosystems PCR System 2400 and GeneAmp® PCR System 2700 thermal cyclers

RT-PCR

45°C	20min	1X
94°C	2min	1X
94°C	20sec	| 40X
55°C	20sec	|
72°C	50sec	|
72°C	10min	1X
15°C		∞

Amplicon detection

Step 5.

- 5µl of amplicon is loaded into the well of a 1.5% agarose gel (TAE or TBE buffer according to your own protocols) and electrophoresed for at least 20min (depending on gel size) at 100-120V.
- Be sure to include at least one known positive sample and a no-template control and load at least one well per gel row with a 100 base-pair ladder (or whatever may be in use at your lab for use with this sized amplicon).

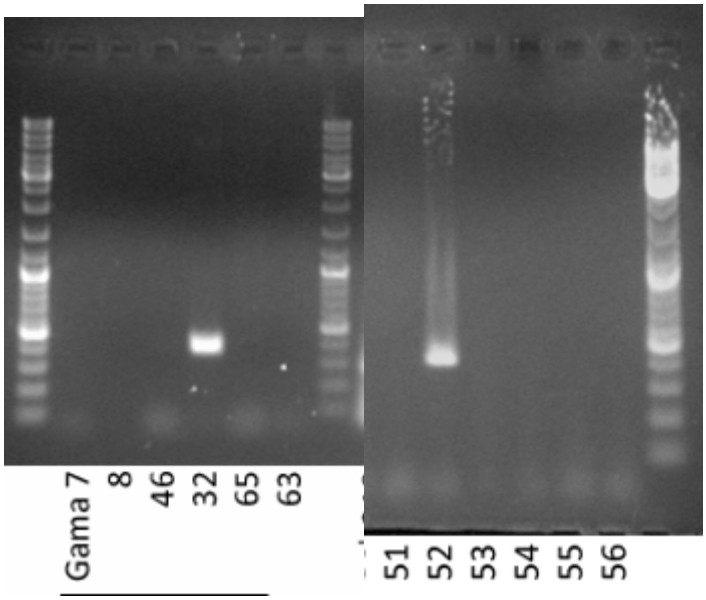


Figure 1. Two examples experiments showing positive results using the Gama assay (1 band per experiment) along with 100-bp ladder).