WHO Collaborating Centre for Reference and Research on Influenza
VIDRL

One-step RT-PCR amplification of Respiratory Syncytial Virus (RSV) in two primer pools for whole-genome sequencing (WGS)

Respiratory syncytial virus (RSV) is a negative-sense, single-stranded, non-segmented RNA virus of approximately 15kb belonging to the Pneumoviridae family. This method utilises SuperScript IV (SSIV) One-Step RT-PCR to amplify the whole RSV genome in two separate reactions (RSV1 and RSV2), three PCR products will be amplified in each reaction. Six sets of overlapping primers are used to produce six amplicons. The successful amplification in each primer pool generates three amplicons of different lengths, which largely facilitate the performance evaluation. The amplicons are then pooled and normalised prior to WGS sequencing.

Primers pools sets for RSV1 and RSV2 multiplex RT-PCR reactions

- Pooled primers for PCR primer set 1

Component	x 1 Volume (µl)	x 10 Volume (µl)
1-1F	0.2	2
1-1R	0.2	2
1-3F_3	0.7	7
1-3R_6	0.7	7
1-5F	0.75	7.5
1-5R	0.75	7.5
Water	0.45	4.5

- Pooled primers for PCR primer set 2

Component	x 1 Volume (µl)	x 10 Volume (µl)
2-2F	0.3	3
2-2R	0.3	3
2-4F	0.75	7.5
2-4R1	0.4	4
2-4R2	0.4	4
2-6F	0.6	6
2-6R	0.5	5
2-6R2	0.5	5

RSV1 and RSV2 multiplex RT-PCR procedure:

1. Label two 1.5 ml tubes:

   MM1 for multiplex PCR mix 1

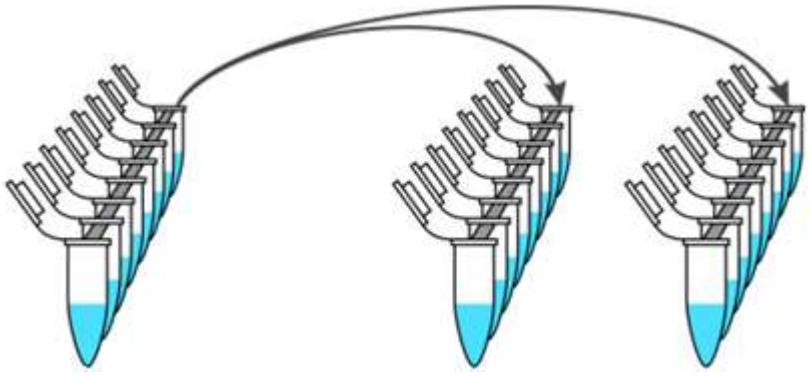
   MM2 for multiplex PCR mix 2

2. Prepare master mix of multiplex PCR mix 1 and 2 for 10 samples using the following table. **Note: the primers were pre-mixed for this workshop, you only need to add pooled primer set 1 to MM1, and primer set 2 to MM2.**

Reagent	Volume (µl) per reaction	Volume (µl) for 10 reactions
Pooled primer set 1 or 2	**3.75**	**37.5**
2x mix	**12.5**	**125**
RT	**0.25**	**2.5**
Total	16.5	165

3. Take out two PCR strip tubes, mark one for RSV1 and one for RSV2. Aliquot 16.5µL of MM1 into each well of the RSV1 strip tubes close the caps. Change the tips and add 16.5 µL of MM2 into each well of the RSV2 strip tubes and close caps.

4. For each sample, positive controls (RSV-A and RSV-B), and negative control, add 8.5 µL of RNA or water to a corresponding RSV1 well, then add another 8.5 µL of RNA or water to the corresponding RSV2 well. Change tips between every well. The total final reaction volume in each well is 25 µL.



RNA RSV1 RSV2

5. Close the caps and mix the contents gently by tapping the tubes for a few times, spin down the tubes briefly to collect all the contents to the bottom of the tubes.

6. Take the strip tubes to a PCR machine and run the following one step RT-PCR program:

RSV WGS PCR conditions		
50°C	10 minutes	x 1
98°C	2 minutes	x 1
98°C	10 seconds	x 40
55°C	30 seconds	
72°C	3 minutes	
72°C	5 minutes	x 1
4°C	∞	