Influenza A and B virus HA/NA/(A-MP) amplification

This protocol is for HA, NA and MP amplification for influenza A, or for HA and NA genes amplification of influenza B. PCR products for both influenza A and B can be amplified in one reaction using FluA or FluB primers and SuperScriptIV One-Step RT-PCR System (ThermoFisher Cat#12594100). The PCR amplicons are then quantified for further library preparation for NGS using chosen library preparation method for NGS.

Primers:

Target	Primer Name	Primer sequence	Pooling Vol (µl)
FluB- HA/NA	B-HANA-UniF	GGGGGGAGCAGAAGCAGAGC	13
	B-HANA-UniR	CCGGGTTATTAGTAGTAACAAGAGC	13
FluA- HA/NA/MP	A-HA-UniF	GGGGGGAGCAAAAGCAGGGGA	5
	A-HA-UniR	CCGGGTTATTAGTAGAAACAAGGGTG	5
	A-NA-UniF	GGGGGGAGCAAAAGCAGGAGT	6
	A-NA-UniR	CCGGGTTATTAGTAGAAACAAGGAGT	6
	A-M-UniF	GGGGGGAGCAAAAGCAGGTAG	2.5
	A-M-UniR	CCGGGTTATTAGTAGAAACAAGGTAG	2.5
Total pool (50 reactions)			53

RT-PCR using SSIV RT-PCR kit

1. **Make Master Mix in a clean room.**

Master Mix	Volume (µl)	
	x 1	**x 10**
Primer Pool	1	10
2 x Master Mix	12.5	125
Reverse Tanscriptase	0.25	2.5
Water	3.25	32.5
Total	**17**	**170**

Label a 1.5 ml tube, make master mix by adding reagents in the above table in the tube. Add Reverse Transcriptase at the last step and vortex briefly to mix and then a quick spin to collect all contents at the bottom of the tube.

2. Add RNA to Master Mix

Take the tube into the extraction room, aliquot **17** µl of master mix into each PCR tube.

Then add **8µl** of RNA to each PCR tubes containing master mix, change tips for every well. The total volume should be **25µl**.

A quick vortex to mix and then spin the tubes briefly to collect all contents at the bottom of the tube.

Put in the PCR machine to start the RT-PCR program:

RT-PCR **FluA/FluB NGS**		
SSIV RT-PCR		
55ºC	10 minutes	
98ºC	2 minutes	
98ºC 45ºC 72ºC	10 seconds 10 seconds 2 minutes	x5
98ºC 57ºC 72ºC	10 seconds 10 seconds 2 minutes	x35
72ºC	5 minutes	
4ºC	∞	