Suggested pre-NGS wet-lab protocol

Flu A Multi-Segment RT-PCR

RT-PCR	
ddH_2O	10 µl
2x RT-PCR Buffer	25 µl
Uni12/Inf1 10µM	0,8 µl
Uni12/Inf3 10µM	1,2 µl
Uni13/Inf1 10µM	2 µl
SSIII/Platinum Taq **HiFi** Enzyme Mix	1 µl
Mix	40 µl
RNA	10 µl
Total	50 µl

42º	60'	
94º	2'	
94º	30"	5x
44º	30"	
68º	3'	
94º	30"	35x
57º	30"	
68º	3'	
68º	5'	
4º	∞	

Flu B Multi-Segment RT-PCR

RT-PCR	
ddH_2O	7 µl
2x RT-PCR Buffer	12,5 µl
FluB universal primer cocktail*	2 µl
SSIII/Platinum Taq **HiFi** Enzyme Mix	0,5 µl
Mix	22 µl
RNA	3 µl
Total	25 µl

45º	60'	
55º	30'	
94º	2'	
94º	20"	5x
40º	30"	
68º	3'30"	
94º	20"	40x
58º	30"	
68º	3'30"	
68º	10'	
4º	∞	

* Universal IBV-GA2 primer cocktail	
B-PBs-UniF (10µM)	100 µl
B-PBs-UniR (10µM)	100 µl
B-PA-UniF (10µM)	50 µl
B-PA-UniR (10µM)	50 µl
B-HANA-UniF (10µM)	100 µl
B-HANA-UniR (10µM)	100 µl
B-NP-UniF (10µM)	60 µl
B-NP-UniR (10µM)	60 µl
B-M-UniF (10µM)	30 µl
B-Mg-UniF (10µM)	30 µl
B-M-UniR (10µM)	60 µl
B-NS-UniF (10µM)	50 µl
B-NS-UniR (10µM)	50 µl
FluB universal primer cocktail	**840 µl**

Adapted* from a RT-PCR assay described by Zhou and colleagues (Zhou et al, 2009, for Influenza A; Zhou et al, 2014, for Influenza B; Zhou and Wentworth DE, 2012).

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