



Sequencing primers and protocol

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The WHO Collaborating Centre for influenza at CDC Atlanta, United States of America, has updated the sequencing primers (table attached) and protocol as follows.

1. RNA extraction performed on 100 μ l viral material (cell or egg) and eluted in 100 μ l volume.
2. Only need to prepare 10-15 μ l PCR reactions using 1 μ l RNA
3. Our cycling conditions using the Promega Access Quick RT-PCR kit is as follows:
 - Cycle 1: 48 °C for 45 min
 - Cycle 2: 94 °C for 2 min
 - Cycle 3: 94 °C for 20 sec
 - Cycle 4: 50 °C for 30 sec
 - Cycle 5: 72 °C for 1 min
 - Cycle 6: Go to Cycle 3 29X
 - Cycle 7: 72 °C for 7 min
 - Cycle 8: 4 °C hold
4. Product size verified on agarose gel
5. Prepare each amplicon to be sequenced with each of M13-forward and M-13 reverse and perform sequence reactions and clean up according to your instruments protocol.

This update replaces the previous version published on 29 April 2009.

Swine Genome Primer Set

		5'	3'	reverse	5'	3'
PB2 forward						
fragment 1	1	TGT AAA ACG ACG GCC AGT CTC GAG CAA AAG CAG GTC AA		575	CAG GAA ACA GCT ATG ACC YAG CTG TGA YTC YGA TGT	
fragment 2	328	tgt aaa acg acg gcc agt gtr aca tgg tgg aay aga a		816	cag gaa aca gct atg acc gct ttg rtc aay atc rtc att	
fragment 3	487	tgt aaa acg acg gcc agt cct ggt cay gca gac ctc ag		1019	cag gaa aca gct atg acc cca aar ctg aag gay gar ctg at	
fragment 4	713	tgt aaa acg acg gcc agt caa gca gtr trt aca ttg aag t		1289	cag gaa aca gct atg acc cct cta act gct ttt ayc atg caa t	
fragment 5	946	tgt aaa acg acg gcc agt ccr acw gaa gaa caa gct gt		1509	cag gaa aca gct atg acc gga gta ttc atc yac acc cat	
fragment 6	1169	tgt aaa acg acg gcc agt aag caa cca gra gat tgr ttc a		1740	cag gaa aca gct atg acc ctg aga cca ytg aat ttt rac a	
fragment 7	1447	tgt aaa acg acg gcc agt cca agy acm gag atg tca atg aga		2186	cag gaa aca gct atg acc ttr ctc art tca ttg atg ct	
fragment 8	1683	TGT AAA ACG ACG GCC AGT CAA CAC TTA TCA ATG GAT AAT		2341	cag gaa aca gct atg acc tag tag aaa caa ggt cgt t	
PB1 forward						
fragment 1	22	TGT AAA ACG ACG GCC AGT AGC AAA AGC AGG CAA ACC AT		534	CAG GAA ACA GCT ATG ACC TCT ATT AGC CTC CCW GAY TCA TT	
fragment 2	233	tgt aaa acg acg gcc agt caa ctc aac ccr att gat ggr cca ct		843	cag gaa aca gct atg acc gtt caa gct ttt crc awa tg	
fragment 3	389	tgt aaa acg acg gcc agt aca agr gtg gac aaa tra c		1041	cag gaa aca gct atg acc ctg aac cay tca ggy tga ttt	
fragment 4	711	tgt aaa acg acg gcc agt tga aca cra tga cca arg a		1278	cag gaa aca gct atg acc ttg aac ctg ccc atc atc aty cca gg	
fragment 5	974	tgt aaa acg acg gcc agt aat caa aay cct mga atg tt		1566	cag gaa aca gct atg acc agc tcc atg ctr aaa ttr gc	
fragment 6	1139	tgt aaa acg acg gcc agt caa ata ccy gca gar atg cta gc		1659	cag gaa aca gct atg acc cca agr tca ttg ttt atc at	
fragment 7	1489	tgt aaa acg acg gcc agt atg agy aaa aag aag tcy ta		1954	cag gaa aca gct atg acc tca aty tcy tta tgg ctg ac	
fragment 8	1532	tgt aaa acg acg gcc agt gcy aat tty agc atg gag ct		2321	cag gaa aca gct atg acc agt aga aac aag gca ttt	
PA forward						
fragment 1	0	tgt aaa acg acg gcc agt agc aaa agc agg tac tga t		493	CAG GAA ACA GCT ATG ACC TAG TCS GCC TTT GTG GCC ATY TC	
fragment 2	235	tgt aaa acg acg gcc agt cca aat gca ctk tta aar cac aga tt		756	cag gaa aca gct atg acc tga gaa agc ttg ccc tca atg	
fragment 3	361	tgt aaa acg acg gcc agt tat gay tac aar gag aa		989	cag gaa aca gct atg acc ggt tct ttc cat cca aag aat gtt	
fragment 4	702	tgt aaa acg acg gcc agt tgc mtt gar aat ttt agr acc ta		1292	cag gaa aca gct atg acc tcr cak gcc ttg ttg aac tca tt	
fragment 5	894	TGT AAA ACG ACG GCC AGT AAA TTR AGC ATT GAR GAY CCG		1662	cag gaa aca gct atg acc tcw agt cty ggg tca gtg ag	
fragment 6	1204	TGT AAA ACG ACG GCC AGT TGT TGG AGA CCT TAA ACA GTA TGA		2037	CAG GAA ACA GCT ATG ACC TCC GAT TGG CCA TGT TTC CGA T	
fragment 7	1444	tgt aaa acg acg gcc agt aat gca tcc tgt gca gca atg ga		2057	cag gaa aca gct atg acc ttg tcc cta aga gcc tga aca a	
fragment 8	1787	tgt aaa acg acg gcc agt atg aar tgg gga atg gag atg ag		2233	cag gaa aca gct atg acc agt aga aac aag gta cct ttt	
HA forward						
fragment 1	1	tgt aaa acg acg gcc agt ata cga cta gca aaa gca ggg g		461	cag gaa aca gct atg acc tca tga ttg ggc cay ga	
fragment 2	351	tgt aaa acg acg gcc agt acr tgt tac ccw ggr gat ttc a		943	cag gaa aca gct atg acc gaa akv gga grc tgg tgt tta	
fragment 3	379	TGT AAA ACG ACG GCC AGT ACR TGT TAC CCA GGR GAT TTC		1204	CAG GAA ACA GCT ATG ACC TCT TTA CCY ACT RCT GTG AA	
fragment 4	736	tgt aaa acg acg gcc agt agr atg rac tat tac tgg ac		1340	cag gaa aca gct atg acc ttc tke att rta wgt cca aa	
fragment 5	1124	tgt aaa acg acg gcc agt tgg atg gta ygg tta yca yca		1541	cag gaa aca gct atg acc tca taa gty cca ttt ytg a	
fragment 6	1204	tgt aaa acg acg gcc agt aag atg aay acr car ttc aca g		1778	cag gaa aca gct atg acc gtg tca gta gaa aca agg gtg ttt	
NP forward						
fragment 1	1	tgt aaa acg acg gcc agt cag ggt aga taa tca ctc ac		553	cag gaa aca gct atg acc aga gca cat yct ggg atc cat	
fragment 2	296	tgt aaa acg acg gcc agt atg gtr ctc tct gct ttt gat ga		757	cag gaa aca gct atg acc ttt gtg cag ctg ttt gaa att tyc ctt t	
fragment 3	513	tgt aaa acg acg gcc agt tgg cat tch aat ttr aat gat		1042	cag gaa aca gct atg acc ctg rct ctt gtg tgc dgg	
fragment 4	619	tgt aaa acg acg gcc agt gct gca gtc aar gga rt		1177	cag gaa aca gct atg acc aag cra ttt gta cyc ctc tag t	
fragment 5	872	TGT AAA ACG ACG GCC AGT TTC TGA GRG GRT CAG TTG CTC		1565	cag gaa aca gct atg acc agt aga aac aag ggt att ttt c	
NA forward						
fragment 1	0	tgt aaa acg acg gcc agt agc aaa agc agg agt		600	cag gaa aca gct atg acc ctg gac crg aaa ttc c	
fragment 2	318	tgt aaa acg acg gcc agt tac aca aaa gac aay agc		740	cag gaa aca gct atg acc ggr cca tvg gtc att atg	
fragment 3	536	tgt aaa acg acg gcc agt ggt cag caa gcg cat gyc atg a		1063	cag gaa aca gct atg acc cat aty tgt atg aaa acc	
fragment 4	726	tgt aaa acg acg gcc agt aat ggr car gcc tcr tac aa		1346	cag gaa aca gct atg acc gct gct ycc rct agt cca gat	
fragment 5	941	tgt aaa acg acg gcc agt tag gat aca tct gca gtg g		1452	cag gaa aca gct atg acc agt aga aac aag gag	
M forward						
fragment 1	0	tgt aaa acg acg gcc agt agc aaa agc agg tag		473	cag gaa aca gct atg acc gca atc tgy tca cak gt	
fragment 2	223	tgt aaa acg acg gcc agt cac cgt gcc cag tga gcg		750	cag gaa aca gct atg acc tca ytt gaa ycg ytg cat	
fragment 3	383	tgt aaa acg acg gcc agt tct gct gdw gca ctt gcc agt tg		1027	cag gaa aca gct atg acc agt agm aac aag gta gt	
NS forward						
fragment 1	24	tgt aaa acg acg gcc agt agc aaa agc agg gtg aca aag aca		482	cag gaa aca gct atg acc tvg gtg aaa gcc ctt a	
fragment 2	250	tgt aaa acg acg gcc agt tga ggc wyt taa aat gac ca		890	cag gaa aca gct atg acc agt aga aac aag ggt gtt ttt tat	
fragment 3	418	tgt aaa acg acg gcc agt aaa gcd aay ttc agt gtg		742	cag gaa aca gct atg acc ttc aat hag cca tct ta	

m13 forward m13 forward tgt aaa acg acg gcc agt
m13 reverse m13 reverse cag gaa aca gct atg acc