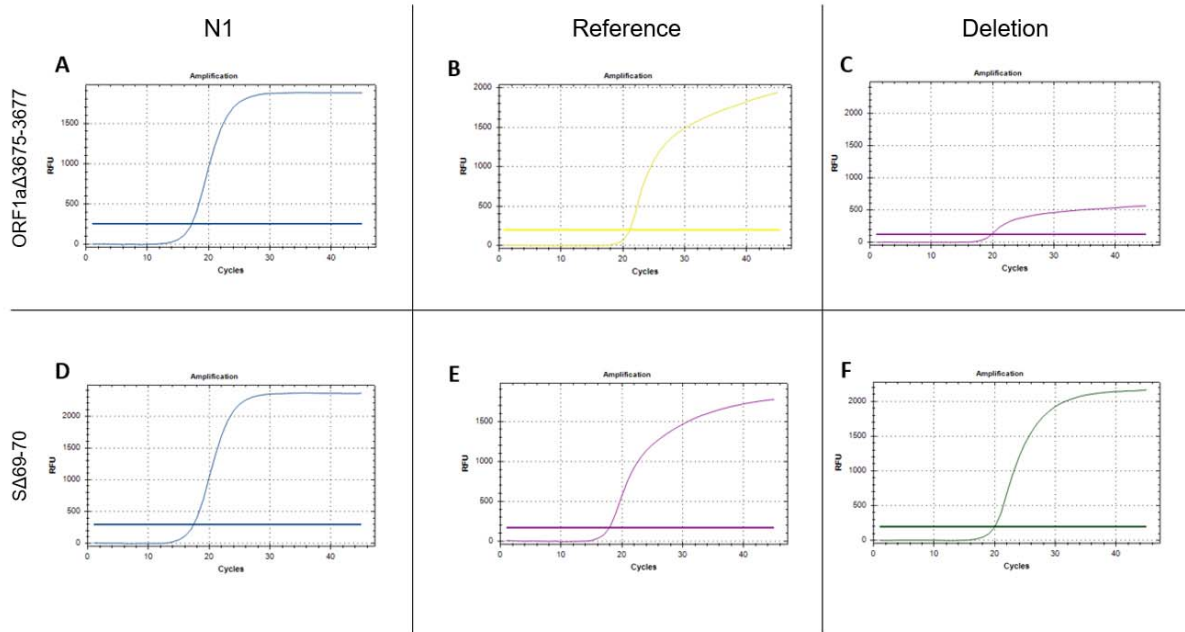


**Supplemental Table 1.** Reaction efficiencies for all primer and probe sets in multiplex.

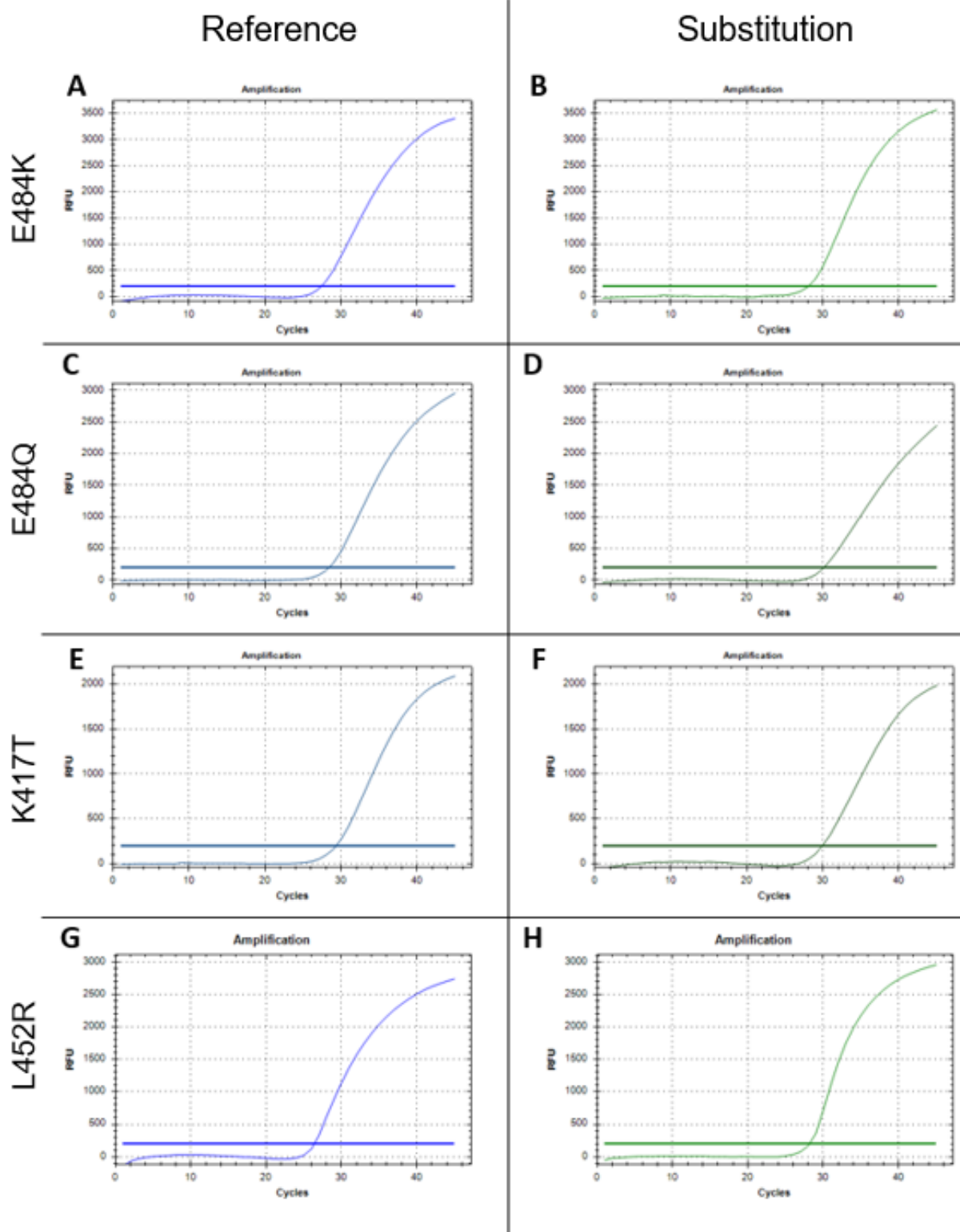
RT-qPCR Assay	Multiplex Reaction Efficiency		
	Internal Control Probe	Reference Probe	Deletion/Substitution Probe
SΔ69-70	95.49%	100.57%	97.12%
ORF1a	101.56%	95.63%	101.44%
Δ3675-3677			
K417T	N/A	101.97%	89.52%
E484K	N/A	99.04%	102.19%
E484Q	N/A	94.69%	101.40%
L452R	N/A	103.99%	112.04%

**Supplemental Table 2.** Thermocycling Conditions for RT-qPCR assays.

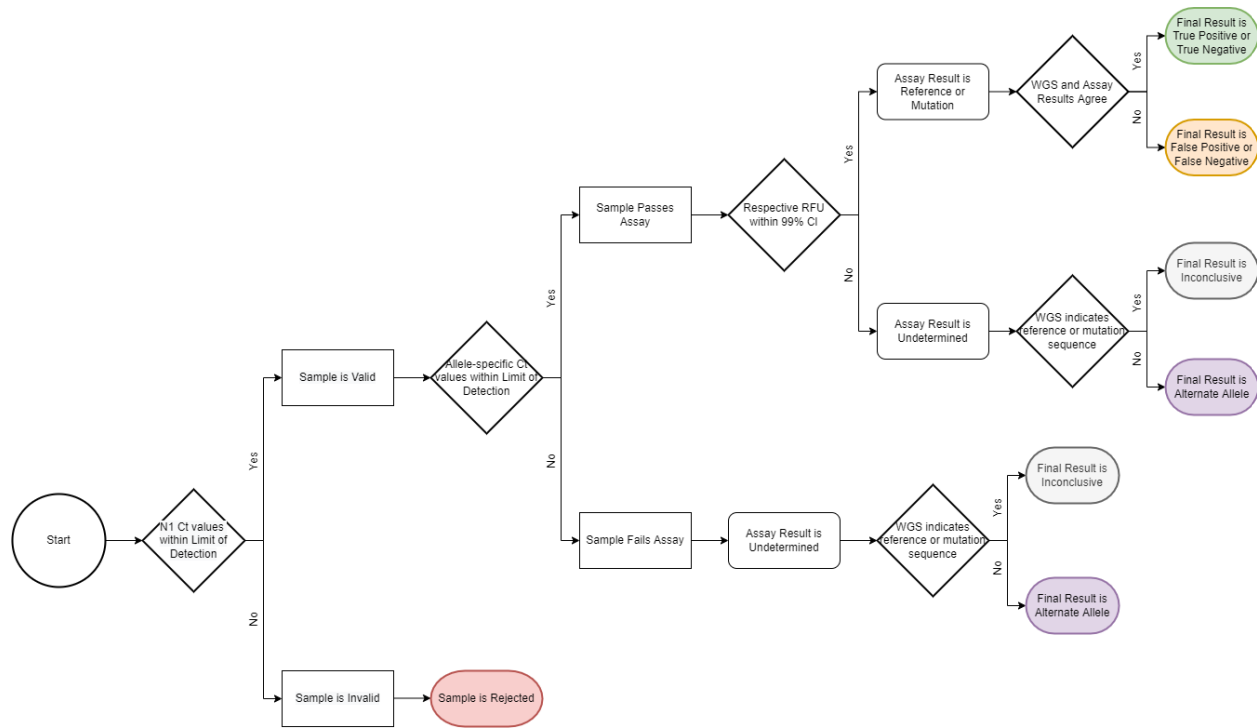
SΔ69-70 and ORF1aΔ3675-3677			
Stage	Temperature (°C)	Duration	Cycles
Warm Up	25.0	2 min	1
Reverse Transcription	50.0	15 min	1
Initial Denaturation	95.0	2 min	1
Touchdown	95.0	3 sec	3
	72.0	30 sec	
	95.0	3 sec	3
	68.0	30 sec	
	95.0	3 sec	3
	64.0	30 sec	
Main Amplification	95.0	3 sec	45
	60.0	30 sec	
TaqPath SNP Assays (K417T, E484K, E484Q, and L452R)			
Stage	Temperature (°C)	Duration	Cycles
Warm Up	25.0	2 min	1
Reverse Transcription	50.0	15 min	1
Initial Denaturation	95.0	2 min	1
Main Amplification	95.0	3 sec	45
	60.0	30 sec	



**Supplemental Figure 1.** Representative curves of RT-qPCR deletion assays.



**Supplemental Figure 2.** Representative curves of TaqPath Spike SNP assays.



**Supplemental Figure 3. Sample Resulting Flowchart.**