

A	Unique Sequence	Abundance	Singles	Ratio	Pass/Fail
	1450A(E484K)	0.115	N/A	1	Pass
	1450A(E484K) 1501T(N501Y)	0.039	0.059	0.653	Fail
	1450A(E484K) 1501T(N501Y) 1709A(A570D)	0.033	0.037	0.890	Fail
	1450A(E484K) 1709A(A570D)	0.038	0.071	0.532	Fail
	1501T(N501Y)	0.626	N/A	1	Pass
	1501T(N501Y) 1709A(A570D)	0.519	0.322	1.610	Pass
	1709A(A570D)	0.621	N/A	1	Pass
	Reference	0.202	N/A	1	Pass

**B**

$$\begin{array}{l} \text{Singles abundance} \quad 0.115 \quad \times \quad 0.626 \quad \times \quad 0.621 \quad = \quad 0.037 \\ \text{Sequence abundance} \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad 0.033 \end{array}$$

**C**

Sequence	Count
1501T(N501Y) 1709A(A570D)	136755    0.519    1.610
1501T(N501Y)	165000
1709A(A570D)	163714
1501T(N501Y) 1709A(A570D)	163714
1501T(N501Y) 165000 - 163714	1286
1709A(A570D)	0

**Figure 4. Schema of Program2's second chimera removal method** A. Calculations for determining if a unique sequence passes the initial check. Sequences pass when they have an Abundance/Singles ratio of 1 or greater. B. Calculation of the singles abundance for one of the unique sequences. C. Passed sequences are processed in order of greatest ratio to least. Counts of the sequence are set to the counts of the least abundant single variant, and that count is removed from all other sequences that share a single variant.