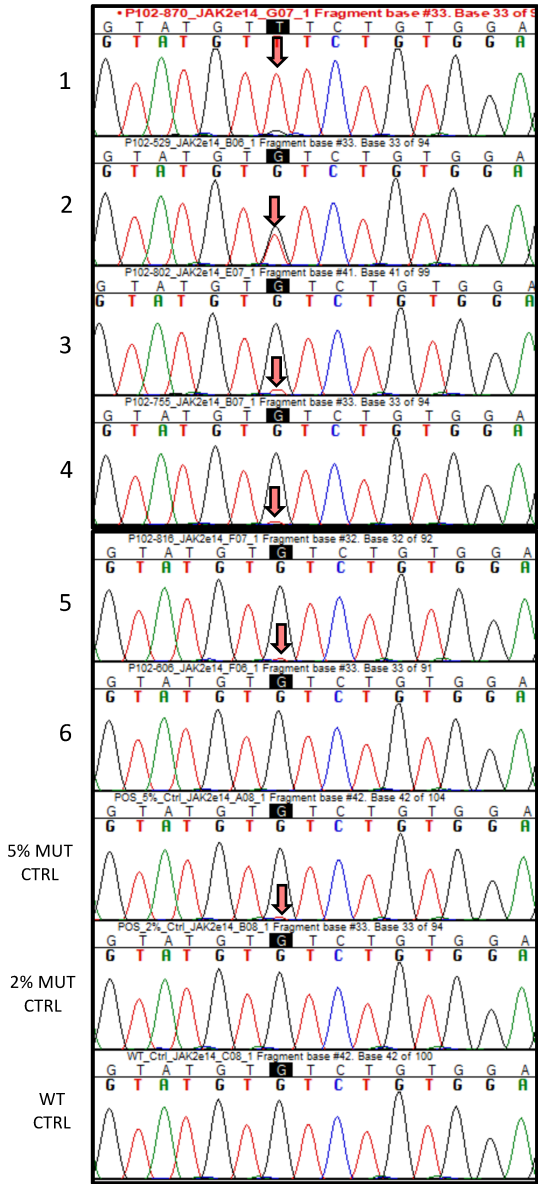


Sample



Sample	Original Sequencing Results with Estimated VAF ^a	Retested Sequencing Results with Pixelated Ruler VAF ^b	ddPCR Results (Fractional Abundance)
1 (P102-870)	c.1849G>T; p.V617F, 95%	c.1849G>T; p.V617F, 91%	93%
2 (P102-529)	c.1849G>T; p.V617F, 45%	c.1849G>T; p.V617F, 44%	50%
3 (P102-802)	c.1849G>T; p.V617F, 5%	c.1849G>T; p.V617F, 10%	10%
4 (P102-755)	c.1849G>T; p.V617F, <5%	c.1849G>T; p.V617F, 5%	7%
5 (P102-816)	c.1849G>T; p.V617F, 5%	c.1849G>T; p.V617F, 4%	6%
6 (P102-606)	NVD	NVD	NVD
7 (P102-478)	c.1849G>T; p.V617F, 10%	-	14%
8 (P102-558)	NVD	-	NVD
9 (P102-596)	c.1849G>T; p.V617F, 5%	-	9%
10 (P102-601)	c.1849G>T; p.V617F, 5%	-	10%
11 (P102-606)	NVD	-	NVD
12 (P102-638)	c.1849G>T; p.V617F, 80%	-	86%
13 (P102-718)	NVD	-	NVD
14 (P102-756)	c.1849G>T; p.V617F, 5%	-	8%
15 (P102-757)	NVD	-	NVD
16 (P102-993)	c.1849G>T; p.V617F, 10%	-	9%
5% MUT CTRL	c.1849G>T; p.V617F, 5%	c.1849G>T; p.V617F, 4%	6%
2% MUT CTRL	N/A	NVD	2%
WT CTRL	NVD	NVD	NVD

Sixteen samples of wild-type and varying mutant variant allele frequency were retested using fresh aliquots of stored DNA using the HRM/Sanger Sequencing protocol and droplet digital PCR for JAK2 Exon 14. Amplification for the HRM/Sanger protocol was performed as originally described. Droplet digital PCR was performed using manufacturer’s recommended conditions.

As seen in the representative electropherograms, the mutant peaks are evident even at low VAF (red arrows). In this study, only the sequence at the mutation area of interest was interrogated relative to baseline and controls. Mutations were called only if they were peak-under-peak centered. The 2% mutant control was not tested in the original cohort.

^a The VAF was determined by visually comparing the peak height of the mutant peak to the estimated total peak height of the wild-type peak and mutant peaks.

^b The VAF was determined by using a pixelated ruler to determine the actual peak height of the mutant peak to the calculated total peak height of the wild-type and mutant peaks.