

SUPPLEMENTAL MATERIAL

Resolving Discrepancies in Idylla *BRAF* Mutational Assay Results Using Targeted Next-Generation Sequencing

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SUPPLEMENTAL TABLES

Supplemental Table S1 Nucleic Acid Variant Concordance

| Sample ID | Result (Idylla) | ΔCq | SPC1 (Cq) | SPC2 (Cq) | SPC3 (Cq) | WT (Cq) | Reference Results | Ref. Meth. | Sample Type |
|-----------|--|-------------|-----------|-----------|-----------|---------|--|---------------|-----------------|
| 1 | BRAF V600E/V600E2/V600D, c.1799T>A/c.1799_1800TG>AA /c.1799_1800TG>AT/ c.1799_1800TG> AC | 1.64 | 37.5 | 38.2 | 39.6 | 38.9 | BRAF c.1799T>A, p.Val600Glu | Send-out NGS | FFPE Lung |
| 2 | BRAF V600E/V600E2/V600D, c.1799T>A/c.1799_1800TG>AA /c.1799_1800TG>AT/ c.1799_1800TG> AC | 0.74 | 34.6 | 34.9 | 34.3 | 35.2 | BRAF c.1799T>A, p.Val600Glu | Send-out NGS | FFPE Lung |
| 3 | BRAF V600E/V600E2/V600D, c.1799T>A/c.1799_1800TG>AA /c.1799_1800TG>AT/ c.1799_1800TG> AC | 3.67 | 32.1 | 32.5 | 32.9 | 31.7 | BRAF c.1799T>A, p.Val600Glu, VAF 8.72 | NGS | FFPE Lung |
| 4 | BRAF V600E/V600E2/V600D, c.1799T>A/c.1799_1800TG>AA /c.1799_1800TG>AT/ c.1799_1800TG> AC | 3.01 | 35.6 | 35.3 | 36.8 | 35.9 | BRAF c.1799T>A, p.Val600Glu | In house BRAF | FFPE Colon |
| 5 | BRAF V600E/V600E2/V600D, c.1799T>A/c.1799_1800TG>AA /c.1799_1800TG>AT/ c.1799_1800TG> AC | 3.10 | 33.4 | 33 | 34.9 | 33.6 | BRAF c.1799T>A, p.Val600Glu | In house BRAF | FFPE Colon |
| 6 | BRAF V600E/V600E2/V600D, c.1799T>A/c.1799_1800TG>AA /c.1799_1800TG>AT/ c.1799_1800TG> AC | 4.65 | 36.6 | 36.7 | 37.8 | 37.3 | BRAF c.1799T>A, p.Val600Glu | Send-out NGS | FFPE Pleura |
| 7* | BRAF V600E/V600E2/V600D, c.1799T>A/c.1799_1800TG>AA /c.1799_1800TG>AT/ c.1799_1800TG> AC | 2.86 | 32.9 | 32.9 | 33.9 | 32.9 | BRAF c.1799T>A, p.Val600Glu, VAF 11.94 | NGS | FFPE Lung |
| 8 | BRAF V600E/V600E2/V600D, c.1799T>A/c.1799_1800TG>AA /c.1799_1800TG>AT/ c.1799_1800TG> AC | 2.23 | 32.6 | 30.9 | 30.9 | 32.5 | BRAF c.1799T>A, p.Val600Glu | In house BRAF | FFPE Lung |
| 9 | BRAF V600E/V600E2/V600D, c.1799T>A/c.1799_1800TG>AA /c.1799_1800TG>AT/ c.1799_1800TG> AC | 0.65 | 34 | 34.4 | 36.6 | 36.2 | BRAF c.1799T>A, p.Val600Glu | NGS | FFPE Lymph node |
| 10 | BRAF V600E/V600E2/V600D, c.1799T>A/c.1799_1800TG>AA /c.1799_1800TG>AT/ c.1799_1800TG> AC | 1.41 | 29.9 | 29.7 | 31.4 | 30.3 | BRAF c.1799T>A, p.Val600Glu | In house BRAF | FFPE Colon |

| | | | | | | | | | |
|----|---|------|------|------|------|------|-----------------------------------|---------------------|--------------------------------------|
| 11 | BRAF V600E/V600E2/V600D, c.1799T>A/c.1799_1800TG>AA/ c.1799_1800TG>AT/ c.1799_1800TG> AC | 1.96 | 31.1 | 31 | 32.8 | 31.8 | BRAF c.1799T>A, p.Val600Glu | In house BRAF | FFPE Colon |
| 12 | BRAF V600E/V600E2/V600D, c.1799T>A/c.1799_1800TG>AA/ c.1799_1800TG>AT/ c.1799_1800TG> AC | 3.35 | 35.5 | 34.9 | 35.9 | 36 | BRAF c.1799T>A, p.Val600Glu | In house BRAF | FFPE Skin |
| 13 | No Mutation detected in BRAF Codon 600 | | 32.9 | 33.6 | 33.9 | 32.1 | No BRAF Mutation detected | Send- out NGS | FFPE Pleural fluid |
| 14 | No Mutation detected in BRAF Codon 600 | | 31.5 | 31.5 | 32.7 | 31.4 | No BRAF Mutation detected | Send- out NGS | FFPE Lymph node |
| 15 | No Mutation detected in BRAF Codon 600 | | 30.1 | 30.2 | 30.9 | 29.8 | No BRAF Mutation detected | Send- out NGS | FFPE Lung |
| 16 | No Mutation detected in BRAF Codon 600 | | 31 | 31.4 | 30.6 | 30.3 | No BRAF Mutation detected | Send- out NGS | FFPE Brain |
| 17 | No Mutation detected in BRAF Codon 600 | | 28.4 | 27.5 | 29.9 | 27 | No BRAF Mutation detected | Send- out NGS | FFPE Metasta tic lung tumor |
| 18 | No Mutation detected in BRAF Codon 600 | | 29.8 | 29.6 | 29.6 | 29.6 | No BRAF Mutation detected | Send- out NGS | FFPE Lung |

Supplemental Table S2 Tissue Variant Concordance

| Sample ID | Result (Idylla) | ΔCq | SPC1 (Cq) | SPC2 (Cq) | SPC3 (Cq) | WT (Cq) | Reference Results | Ref. Meth. | Sample Type |
|--------------|---|-------------|--------------|--------------|--------------|------------|--|---------------------|----------------|
| 1 | BRAF V600E/V600E2/V600D, c.1799T>A/c.1799_1800TG>AA/ c.1799_1800TG>AT/ c.1799_1800TG> AC | 1.64 | 37.5 | 38.2 | 39.6 | 38.9 | BRAF c.1799T>A, p.Val600Glu | Send- out NGS | FFPE Lung |
| 2 | BRAF V600E/V600E2/V600D, c.1799T>A/c.1799_1800TG>AA/ c.1799_1800TG>AT/ c.1799_1800TG> AC | 0.74 | 34.6 | 34.9 | 34.3 | 35.2 | BRAF c.1799T>A, p.Val600Glu | Send- out NGS | FFPE Lung |
| 3 | BRAF V600E/V600E2/V600D, c.1799T>A/c.1799_1800TG>AA/ c.1799_1800TG>AT/ c.1799_1800TG> AC | 3.67 | 32.1 | 32.5 | 32.9 | 31.7 | BRAF c.1799T>A, p.Val600Glu, VAF 8.72 | NGS | FFPE Lung |

| | | | | | | | | | |
|----|---|------|------|------|------|------|---|---------------------|--------------------------|
| 4 | BRAF V600E/V600E2/V600D, c.1799T>A/c.1799_1800TG>AA/ c.1799_1800TG>AT/ c.1799_1800TG> AC | 3.01 | 35.6 | 35.3 | 36.8 | 35.9 | BRAF c.1799T>A, p.Val600Glu | In house BRAF | FFPE Colon |
| 5 | BRAF V600E/V600E2/V600D, c.1799T>A/c.1799_1800TG>AA/ c.1799_1800TG>AT/ c.1799_1800TG> AC | 3.10 | 33.4 | 33 | 34.9 | 33.6 | BRAF c.1799T>A, p.Val600Glu | In house BRAF | FFPE Colon |
| 6 | BRAF V600E/V600E2/V600D, c.1799T>A/c.1799_1800TG>AA/ c.1799_1800TG>AT/ c.1799_1800TG> AC | 4.65 | 36.6 | 36.7 | 37.8 | 37.3 | BRAF c.1799T>A, p.Val600Glu | Send- out NGS | FFPE Pleura |
| 7* | BRAF V600E/V600E2/V600D, c.1799T>A/c.1799_1800TG>AA/ c.1799_1800TG>AT/ c.1799_1800TG> AC | 2.86 | 32.9 | 32.9 | 33.9 | 32.9 | BRAF c.1799T>A, p.Val600Glu, VAF 11.94 | NGS | FFPE Lung |
| 8 | BRAF V600E/V600E2/V600D, c.1799T>A/c.1799_1800TG>AA/ c.1799_1800TG>AT/ c.1799_1800TG> AC | 2.23 | 32.6 | 30.9 | 30.9 | 32.5 | BRAF c.1799T>A, p.Val600Glu | In house BRAF | FFPE Lung |
| 9 | BRAF V600E/V600E2/V600D, c.1799T>A/c.1799_1800TG>AA/ c.1799_1800TG>AT/ c.1799_1800TG> AC | 0.65 | 34 | 34.4 | 36.6 | 36.2 | BRAF c.1799T>A, p.Val600Glu | NGS | FFPE Lymph node |
| 10 | BRAF V600E/V600E2/V600D, c.1799T>A/c.1799_1800TG>AA/ c.1799_1800TG>AT/ c.1799_1800TG> AC | 1.41 | 29.9 | 29.7 | 31.4 | 30.3 | BRAF c.1799T>A, p.Val600Glu | In house BRAF | FFPE Colon |
| 11 | BRAF V600E/V600E2/V600D, c.1799T>A/c.1799_1800TG>AA/ c.1799_1800TG>AT/ c.1799_1800TG> AC | 1.96 | 31.1 | 31 | 32.8 | 31.8 | BRAF c.1799T>A, p.Val600Glu | In house BRAF | FFPE Colon |
| 12 | BRAF V600E/V600E2/V600D, c.1799T>A/c.1799_1800TG>AA/ c.1799_1800TG>AT/ c.1799_1800TG> AC | 3.35 | 35.5 | 34.9 | 35.9 | 36 | BRAF c.1799T>A, p.Val600Glu | In house BRAF | FFPE Skin |
| 13 | No Mutation detected in BRAF Codon 600 | | 32.9 | 33.6 | 33.9 | 32.1 | No BRAF Mutation detected | Send- out NGS | FFPE Pleural fluid |
| 14 | No Mutation detected in BRAF Codon 600 | | 31.5 | 31.5 | 32.7 | 31.4 | No BRAF Mutation detected | Send- out NGS | FFPE Lymph node |
| 15 | No Mutation detected in BRAF Codon 600 | | 30.1 | 30.2 | 30.9 | 29.8 | No BRAF Mutation detected | Send- out NGS | FFPE Lung |
| 16 | No Mutation detected in BRAF Codon 600 | | 31 | 31.4 | 30.6 | 30.3 | No BRAF Mutation detected | Send- out NGS | FFPE Brain |

| | | | | | | | | | |
|----|---|--|------|------|------|------|---------------------------------|---------------------|--------------------------------------|
| 17 | No Mutation detected in BRAF Codon 600 | | 28.4 | 27.5 | 29.9 | 27 | No BRAF Mutation detected | Send- out NGS | FFPE Metastat ic lung tumor |
| 18 | No Mutation detected in BRAF Codon 600 | | 29.8 | 29.6 | 29.6 | 29.6 | No BRAF Mutation detected | Send- out NGS | FFPE Lung |

Supplemental Table S3 Limit of Detection

| | Dilution | Expected VAF (%) | SPC1 (ΔCq) | SPC2 (ΔCq) | SPC3 (ΔCq) | WT (ΔCq) | ΔCq | Idylla Result |
|---|----------|---------------------|---------------|---------------|---------------|-------------|------|---------------|
| HD705 (BRAF c.1799T>A, p.Val600Glu) | N/A | 5 | 33.7 | 33.7 | 35.6 | 34.4 | 4.3 | Detected |
| | 1:2 | 2.5 | 34.4 | 34.1 | 35.7 | 35.1 | 5.0 | Detected |
| | 1:4 | 1.25 | 34.2 | 34.5 | 36.2 | 34.8 | 7.6 | Detected |
| | 1:8 | 0.625 | 34.4 | 34.5 | 34.3 | 34.9 | | Not Detected |
| HD123 (BRAF c.1798_1799G>TdelinsAA , p.Val600Lys) | N/A | 5 | 34.3 | 34.5 | 35.1 | 34.8 | 7.6 | Detected |
| | 1:2 | 2.5 | 33.8 | 34.0 | 35.6 | 34.6 | 10.3 | Detected |
| | 1:4 | 1.25 | 34.1 | 34.6 | 34.2 | 34.5 | 9.1 | Detected |
| | 1:8 | 0.625 | 34.4 | 34.5 | 34.3 | 34.9 | | Not Detected |

Supplemental Table S4.1 Input Limitation for 5% VAF

| HD705 (BRAF c.1799T>A, p.Val600Glu, VAF 5%) | | | | | | | HD123 (BRAF c.1798_1799G>TdelinsAA, p.Val600Lys, VAF 5%) | | | | | | |
|---|--------------|--------------|--------------|------------|-----|------------------|--|--------------|--------------|--------------|------------|-----|------------------|
| Input (ng) | SPC1 (Cq) | SPC2 (Cq) | SPC3 (Cq) | WT (Cq) | ΔCq | Idylla Result | Input (ng) | SPC1 (Cq) | SPC2 (Cq) | SPC3 (Cq) | WT (Cq) | ΔCq | Idylla Result |
| 50 | 33.7 | 33.7 | 35.6 | 34.4 | 4.3 | Detected | 50 | 34.3 | 34.5 | 35.1 | 34.8 | 7.6 | Detected |
| 25 | 35.6 | 36.1 | 37.0 | 35.9 | 5.2 | Detected | 25 | 34.8 | 35.5 | 35.6 | 35.6 | 7.3 | Detected |
| 12.5 | 36.6 | 37.1 | 36.7 | 37.8 | 5.6 | Detected | 12.5 | 35.5 | 36.2 | 36.7 | 36.4 | 6.9 | Detected |

Supplemental Table S4.2 Input Limitation for 1.25% VAF

| HD705 (BRAF c.1799T>A, p.Val600Glu, VAF 1.25%) | | | | | | | HD123 (BRAF c.1798_1799G>TdelinsAA, p.Val600Lys, VAF 1.25%) | | | | | | |
|--|-----------|-----------|-----------|---------|-------------|---------------|---|-----------|-----------|-----------|---------|-------------|---------------|
| Input (ng) | SPC1 (Cq) | SPC2 (Cq) | SPC3 (Cq) | WT (Cq) | Δ Cq | Idylla Result | Input (ng) | SPC1 (Cq) | SPC2 (Cq) | SPC3 (Cq) | WT (Cq) | Δ Cq | Idylla Result |
| 100 | 33.7 | 33.4 | 34.5 | 34.3 | 5.6 | Detected | 100 | 33.2 | 34.5 | 34.6 | 33.8 | 11.1 | Detected |
| 50 | 34.2 | 34.5 | 36.2 | 34.2 | 7.6 | Detected | 50 | 34.1 | 34.6 | 34.2 | 34.5 | 9.1 | Detected |
| 25 | 35.9 | 36.1 | 36.8 | 36.8 | 5.1 | Detected | 25 | 35.6 | 37.3 | 36.2 | 36.6 | | Insufficient |

Supplemental Table S5 Reproducibility

| Specimen No. | Variant | ΔCq | SPC1 (Cq) | SPC2 (Cq) | SPC1 (Cq) | WT (Cq) |
|--------------|---|-------------|-----------|-----------|-----------|---------|
| 3-1 | BRAF V600E/V600E2/V600D, c.1799T>A/c.1799_1800TG>AA/c.1799_1800TG>AT/c.1799_1800TG>AC | 3.7 | 32.1 | 32.5 | 32.9 | 31.7 |
| 3-2 | BRAF V600E/V600E2/V600D, c.1799T>A/c.1799_1800TG>AA/c.1799_1800TG>AT/c.1799_1800TG>AC | 4.1 | 30.9 | 31.6 | 31 | 31.2 |
| 3-3 | BRAF V600E/V600E2/V600D, c.1799T>A/c.1799_1800TG>AA/c.1799_1800TG>AT/c.1799_1800TG>AC | 3.3 | 31 | 30.4 | 32.4 | 31 |
| 7-1 | BRAF V600E/V600E2/V600D, c.1799T>A/c.1799_1800TG>AA/c.1799_1800TG>AT/c.1799_1800TG>AC | 2.9 | 32.9 | 32.9 | 33.9 | 32.9 |
| 7-2 | BRAF V600E/V600E2/V600D, c.1799T>A/c.1799_1800TG>AA/c.1799_1800TG>AT/c.1799_1800TG>AC | 3.2 | 32.9 | 33.3 | 34.8 | 33.2 |
| 7-3 | BRAF V600E/V600E2/V600D, c.1799T>A/c.1799_1800TG>AA/c.1799_1800TG>AT/c.1799_1800TG>AC | 2.7 | 33.6 | 33.2 | 34.9 | 33.1 |
| 5-1 | BRAF V600E/V600E2/V600D, c.1799T>A/c.1799_1800TG>AA/c.1799_1800TG>AT/c.1799_1800TG>AC | 3.1 | 33.4 | 33 | 34.9 | 33.6 |
| 5-2 | BRAF V600E/V600E2/V600D, c.1799T>A/c.1799_1800TG>AA/c.1799_1800TG>AT/c.1799_1800TG>AC | 4.1 | 33 | 32.5 | 34.4 | 33 |
| 5-3 | BRAF V600E/V600E2/V600D, c.1799T>A/c.1799_1800TG>AA/c.1799_1800TG>AT/c.1799_1800TG>AC | 3.6 | 33.6 | 32.7 | 32.9 | 34 |
| 14-1 | No BRAF mutation detected | | 31.5 | 31.5 | 32.7 | 31.4 |
| 14-2 | No BRAF mutation detected | | 30.8 | 32.3 | 30.5 | 30.9 |
| 14-3 | No BRAF mutation detected | | 31.4 | 31.2 | 33 | 31.7 |
| 15-1 | No BRAF mutation detected | | 30.1 | 30.2 | 30.9 | 29.8 |
| 15-2 | No BRAF mutation detected | | 30.6 | 30.5 | 30.3 | 30.2 |
| 15-3 | No BRAF mutation detected | | 30.7 | 30.6 | 31.1 | 30.5 |
| 18-1 | No BRAF mutation detected | | 29.8 | 29.6 | 29.6 | 29.6 |
| 18-2 | No BRAF mutation detected | | 29.9 | 30.7 | 29.6 | 29.8 |
| 18-3 | No BRAF mutation detected | | 30.3 | 29.8 | 29.7 | 29.7 |

SUPPLEMENTAL FIGURES

Idylla amplification profiles of NGS-tested specimens with non-V600 BRAF variants that were ±5 amino acids of the BRAF V600 codon

Figure S1. BRAF Gly596Val
NGS result: BRAF c.1787G>T, p.Gly596Val (VAF: 6%),
HRAS c.34G>A, p.Gly12Ser (VAF: 12%)
Idylla software interpretation: NO MUTATION DETECTED IN BRAF CODON 600

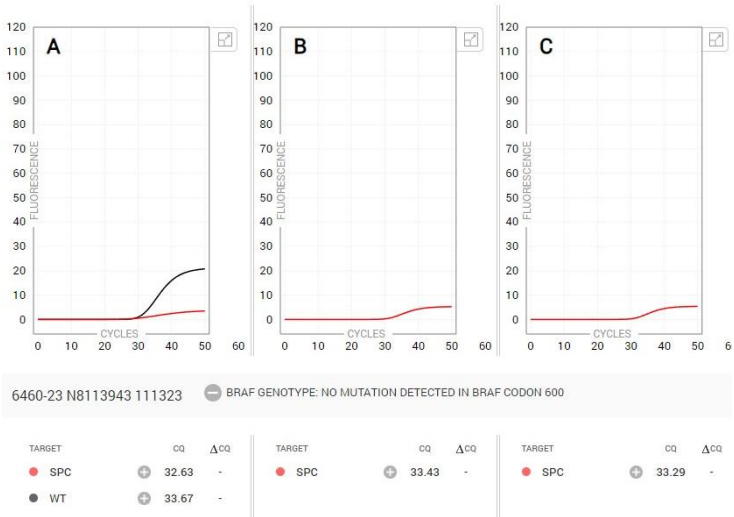


Figure S2. BRAF Leu597Arg
NGS result: BRAF c.1790T>G, p.L597R (VAF: 21%)
Idylla software interpretation: NO MUTATION DETECTED IN BRAF CODON 600

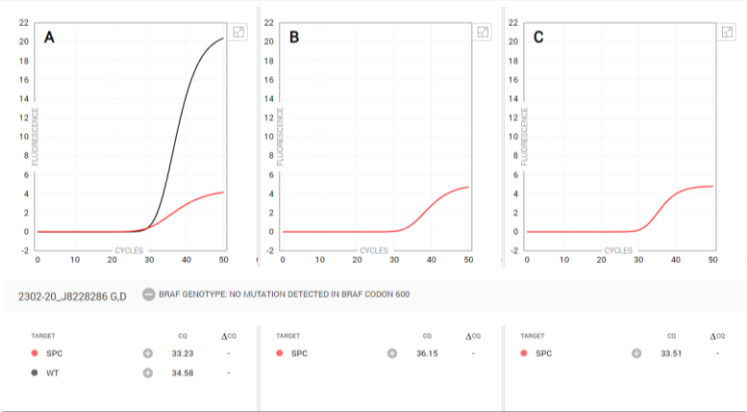


Figure S3. *BRAF* Leu597Val
 NGS result: *BRAF* c.1789C>G, p.L597V (VAF: 42%)
 Idylla software interp.: NO MUTATION DETECTED IN *BRAF* CODON 600

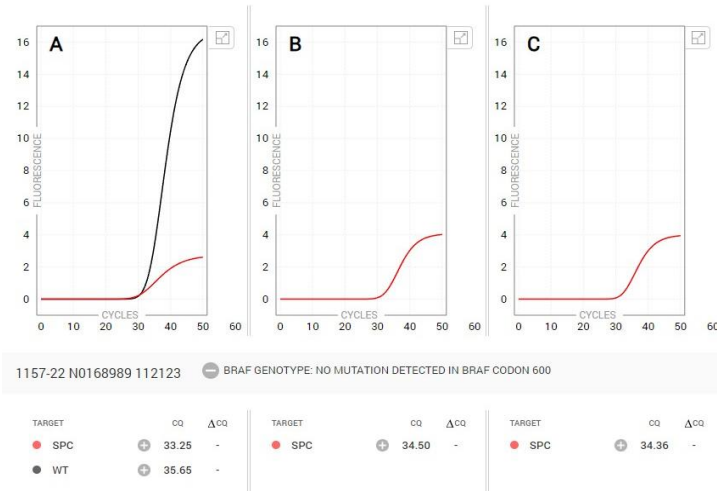


Figure S4. *BRAF* Leu597Gln
 NGS result: *BRAF* c.1790T>A, p.L597Q (VAF: 13%);
KRAS c.38G>A, p.G13D (VAF: 14%),
ROS1 c.5827G>A, E1943K (VAF: 11%)
 Idylla software interpretation: NO MUTATION DETECTED IN *BRAF* CODON 600

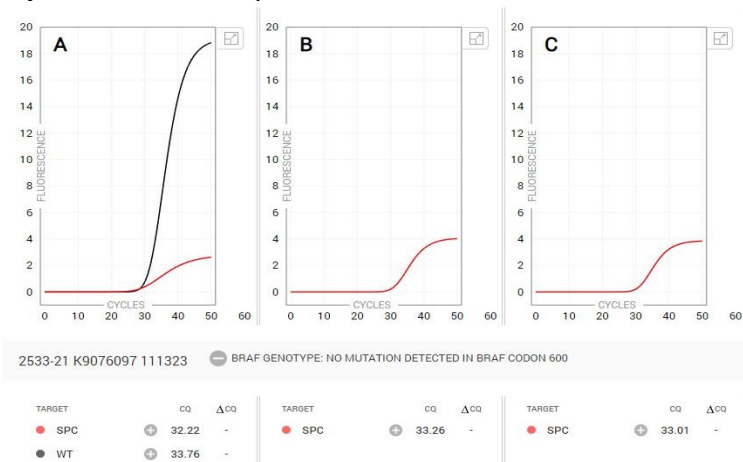


Figure S5. *BRAF* Ala598_Thr599insVal
 NGS result: *BRAF* c.1794_1795insGTT, p.A598_T599insV
 Idylla software interpretation: NO MUTATION DETECTED IN *BRAF* CODON 600

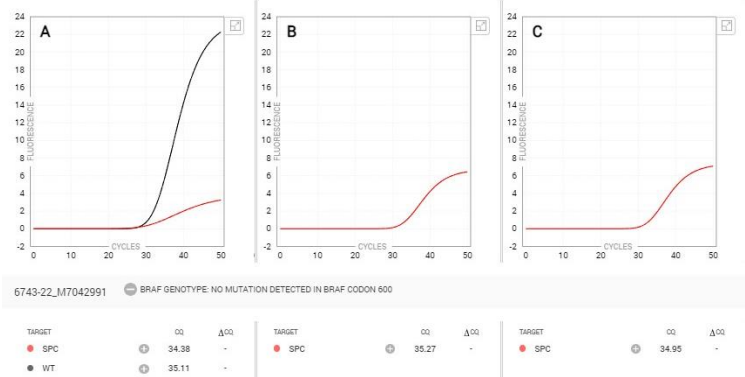


Figure S6. *BRAF* Lys601Glu
 NGS result: *BRAF* c.1801A>G, p.K601E (VAF: 19%)
 Idylla software interpretation: NO MUTATION DETECTED IN *BRAF* CODON 600

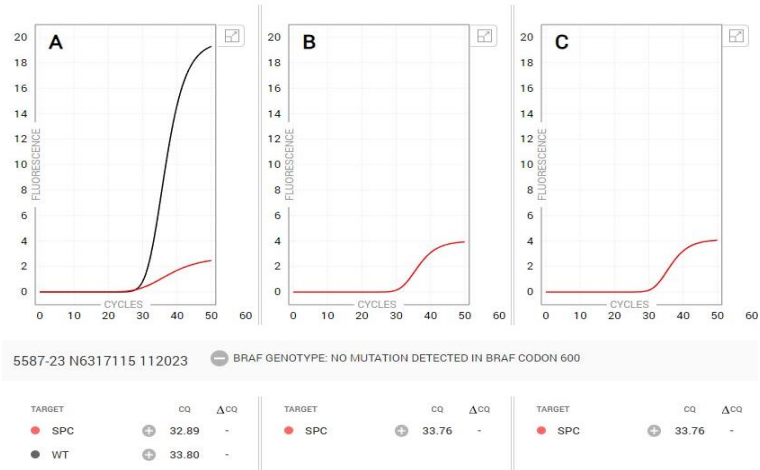


Figure S7. *BRAF* Lys601Thr
 NGS result: *BRAF* c.1802A>C, p.K601T (VAF: 18%)
 Idylla software interpretation: NO MUTATION DETECTED IN *BRAF* CODON 600

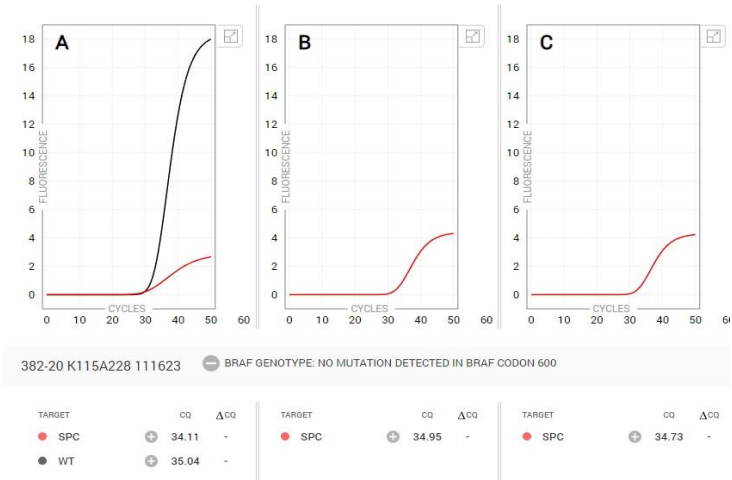


Figure S8. *BRAF* Lys601Asn
 NGS result: *BRAF* c.1803A>C, p.K601N (VAF: 41%)
 Idylla software interpretation: NO MUTATION DETECTED IN *BRAF* CODON 600

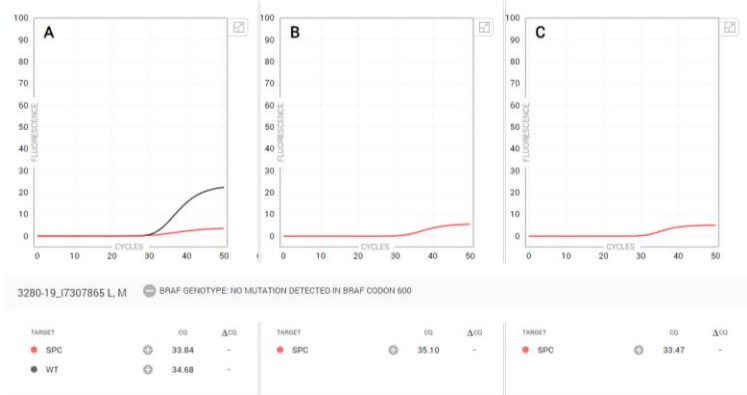


Figure S9. *BRAF* Trp604Cys

NGS result: *BRAF* c.1812G>C, p.W604C (VAF: 47%)

BRAF c.1780G>A, p.D594N (VAF: 47%),

KRAS c.34_35delGGinsTT, p.G12F (VAF: 49%)

Idylla result: NO MUTATION DETECTED IN *BRAF* CODON 600

