

Sample with BRAF mutation	Gene with additional mutations															
	None	R173	E29V	KDR	TP53	PDGFRA	AKT1	FRXN7	ERBB4	KIT	PIK3CA	CDKN2A	APC	HRAS	KRAS	NRAS
<b>Activating BRAF mutations</b>																
MEL2 (p.Val600Glu)	X															
MEL4 (p.Val600Glu)										p.A680T <sup>er</sup>						
MEL6 (p.Val600Glu)											p.M611D <sup>31E</sup>					
MEL11 (p.Val600Glu complex)													p.T17L <sup>129M</sup> <sup>er</sup>			
MEL5 (p.Val600Arg)													p.A68V <sup>57G</sup> <sup>ly</sup>	p.A621T <sup>65S</sup>	p.G1627G <sup>lu</sup>	
MEL12 (p.Val600Lys)																p.S61L <sup>27P</sup> <sup>is</sup>
MEL13 (p.Val600_Lys601delinsGlu)																X
MEL15-MEL16 (p.Val600Glu at 3.6%)																X
MEL21 (p.Val600Arg at 4.9%)															p.G16E <sup>15Y</sup>	
<b>Intermediate activity BRAF mutations</b>																
MEL7 (p.Asn581Ser)																p.C604D <sup>20A</sup> <sup>rg</sup>
MEL9 (p.Leu597Gln)																p.T17P <sup>22L</sup> <sup>eu</sup>
MEL20-MEL19 (p.Gly466Arg)																p.G16L <sup>137G</sup> <sup>in</sup>
<b>Inactivating BRAF mutations</b>																
MEL8 (p.Asp594Asn)																p.G16I <sup>24S</sup>
MEL14 (p.Asp594Asn)																p.G16E <sup>11H</sup> <sup>is</sup>
MEL10 (p.Gly596Val)																p.P468S <sup>5S</sup> <sup>er</sup>
<b>BRAF wild-type</b>																
MEL1																p.L612S <sup>7T</sup> <sup>ro</sup>
MEL3																p.G16Y <sup>69E</sup> <sup>du</sup>
MEL18-MEL17																p.S61L <sup>11P</sup> <sup>he</sup>

ClinVar/SIFT/PolyPhen: Pathogenic or likely pathogenic  
ClinVar/SIFT/PolyPhen: conflicting interpretation  
ClinVar/SIFT/PolyPhen: benign

**Figure S1. Identified mutations in FFPE samples.** All the identified variants are reported. Each column represents a mutated gene and each row represents a sample. Samples are categorized in relation to the ascribed effect of the mutation on the BRAF protein activity. The additional mutation boxes are highlighted on the basis of the predicted effect.

**Table S1.** Quality parameters and coverage of FFPE samples performed by NGS.

Sample MEL	Library (pmol/L)	Bases	>=Q20	Reads	Reads Mean length (bp)	Mapped Reads	On Target	Mean Depth	% of amplicon with at least 500x	% of amplicon with at least 100x	Uniformity	Amplicons with lower than 500 reads	Amplicons with lower than 100 reads
1	266	106728410	101938290	935415	114	934005	85.46%	3713	98,6	100,0	98.52%	3	0
2	188	132124958	126292682	1153026	115	1150097	88.83%	4750	99,5	100,0	94.91%	1	0
3	51	110421513	105780845	983297	112	958773	96.53%	4344	100,0	100,0	100.00%	0	0
4	150	107194613	102524590	967973	111	965754	87.47%	3881	99,5	100,0	98.03%	1	0
5	186	112826451	107891647	1007614	112	1005357	84.76%	3965	99,0	100,0	98.52%	2	0
6	311	118383119	112991229	999407	118	998243	88.16%	4156	99,5	100,0	98.17%	1	0
7	187	114968508	109009271	1022823	112	1020411	97.01%	4543	95,7	100,0	89.00%	9	0
8	90	65428547	61979152	608819	107	607085	93.48%	2553	94,7	100,0	92.75%	11	0
9	75	55847911	52996111	572171	98	570357	82.08%	2006	94,7	100,0	96.29%	11	0
10	662	114570811	108203065	979578	117	978676	95.54%	4426	99,0	100,0	98.22%	2	0
11	128	75338077	71497953	687284	110	686401	96.68%	2992	98,1	100,0	96.77%	4	0
12	386	92883182	88223655	812907	114	812294	97.38%	3693	99,5	100,0	98.08%	1	0
13	1169	105638869	100157652	904544	117	903833	97.04%	4101	99,0	99,5	97.95%	2	1
14	412	128754379	122066099	1134610	113	1133565	96.75%	5086	99,5	100,0	98.46%	1	0
15	99	50348751	47598296	467207	108	466411	92.21%	1919	86,5	99,0	87.68%	28	2
16	492	73629701	69815499	643732	114	643152	93.58%	2795	97,6	99,5	96.79%	5	1
17	376	72330552	68350119	629290	115	628600	96.12%	2837	98,6	100,0	96.46%	3	0
18	132	54799548	51938524	505571	108	505091	97.83%	2227	97,1	100,0	96.68%	6	0
19	117	53572368	50636789	485481	110	484838	94.30%	2098	97,1	100,0	97.59%	6	0
20	34	38650584	36522475	363853	106	362584	96.41%	1545	78,3	97,6	87.86%	45	5
21	228	49452569	46956005	456092	108	455708	97.20%	2002	98,1	100,0	98.27%	4	0

**Table S2.** Analysis of samples with classical BRAF or NRAS variants: comparison of results among different methods.

SAMPLE	NGS (mutant allele fraction)	Conventional methods		
		Sequenom	HRMA+Sanger	castPCR for BRAF p.Val600Glu (mutant allele fraction)
MEL-1	BRAF wt	BRAF wt	BRAF wt	BRAF wt
	NRAS p.Gln61Leu (40.1%)	NRAS p.Gln61Leu	not performed	not performed
	KRAS wt	KRAS wt	not performed	not performed
	PIK3CA wt	PIK3CA wt	not performed	not performed
MEL-2	BRAF p.Val600Glu (47.7%)	BRAF p.Val600Glu	not performed	not performed
	NRAS wt	NRAS wt	not performed	not performed
	KRAS wt	KRAS wt	not performed	not performed
	PIK3CA wt	PIK3CA wt	not performed	not performed
MEL-3	BRAF wt	BRAF wt	BRAF wt	BRAF wt
	NRAS p.Gln61Arg (8.4%)	NRAS p.Gln61Arg	not performed	not performed
	KRAS wt	KRAS wt	not performed	not performed
	PIK3CA wt	PIK3CA wt	not performed	not performed
MEL-4	BRAF p.Val600Glu (31.3%)	BRAF p.Val600Glu	not performed	not performed
	NRAS wt	NRAS wt	not performed	not performed
	KRAS wt	KRAS wt	not performed	not performed
	PIK3CA wt	PIK3CA wt	not performed	not performed
MEL-5	BRAF p.Val600Arg (23.8%)	BRAF p.Val600Arg	not performed	not performed

	NRAS wt	NRAS wt	not performed	not performed
	KRAS wt	KRAS wt	not performed	not performed
	PIK3CA wt	PIK3CA wt	not performed	not performed
<b>MEL-6</b>	BRAF p.Val600Glu (46.1%)	BRAF p.Val600Glu	not performed	not performed
	NRAS wt	NRAS wt	not performed	not performed
	KRAS wt	KRAS wt	not performed	not performed
	PIK3CA p.Met1043Ile (44.2%); p.Ile391Met (63.9)	PIK3CA p.Met1043Ile; wt*	not performed	not performed
<b>MEL-11</b>	BRAF p.Val600Glu complex (38.1%)	BRAF p.Val600Glu complex/p.Val600Asp	not performed	not performed
	NRAS wt	NRAS wt	not performed	not performed
	KRAS wt	KRAS wt	not performed	not performed
	PIK3CA wt	PIK3CA wt	not performed	not performed
<b>MEL-12</b>	BRAF p.Val600Lys (60.9%)	BRAF p.Val600Lys	not performed	not performed
	NRAS wt	NRAS wt	not performed	not performed
	KRAS wt	KRAS wt	not performed	not performed
	PIK3CA wt	PIK3CA wt	not performed	not performed
<b>MEL-13</b>	BRAF p.Val600_Lys601delinsGlu (44.0%)	BRAF p.Val600_Lys601delinsGlu	not performed	not performed
	NRAS wt	NRAS wt	not performed	not performed
	KRAS wt	KRAS wt	not performed	not performed
	PIK3CA wt	PIK3CA wt	not performed	not performed

\*mutation not covered by the panel

**Table S3.** Analysis of samples with BRAF mutated allele at low frequency: comparison of results among different methods.

SAMPLE	NGS (mutant allele fraction)	Sequenom	HRMA+Sanger	Conventional methods
				castPCR for BRAF p.Val600Glu (mutant allele fraction)
<b>MEL-15</b>	BRAF p.Val600Glu (3.62%)	BRAF wt	BRAF wt	BRAF p.Val600Glu (1.49%)
	NRAS wt	NRAS wt	not performed	not performed
	KRAS wt	KRAS wt	not performed	not performed
	PIK3CA wt	PIK3CA wt	not performed	not performed
<b>MEL-21</b>	BRAF p.Val600Arg (4.9%)	BRAF wt	BRAF <b>ambiguous</b>	BRAF p.Val600Arg (8.99%)
	NRAS p.Gln61Lys (19.5%)	NRAS wt	NRAS p.Gln61Lys	not performed
	KRAS wt	KRAS wt	not performed	not performed
	PIK3CA wt	PIK3CA wt	not performed	not performed

**Table S4.** Analysis of samples with a rarer BRAF mutation: comparison of results among different methods.

SAMPLE	NGS (mutant allele fraction)	Sequenom	HRMA+Sanger	Conventional methods
				castPCR for BRAF p.Val600Glu (mutant allele fraction)
<b>MEL-7</b>	BRAF p.Asn581Ser (44.2%)	BRAF wt *	BRAF p.Asn581Ser	not performed
	NRAS wt	NRAS wt	not performed	not performed
	KRAS wt	KRAS wt	not performed	not performed
	PIK3CA p.Cys420Arg (13.9%)	PIK3CA p.Cys420Arg	not performed	not performed

<b>MEL-8</b>	BRAF p.Asp594Asn (52.1%)	BRAF wt *	BRAF p.Asp594Asn	not performed
	NRAS p.Gly12Asp (63.4%)	NRAS p.Gly12Asp	NRAS p.Gly12Asp	not performed
	KRAS wt	KRAS wt	not performed	not performed
	PIK3CA wt	PIK3CA wt	not performed	not performed
<b>MEL-9</b>	BRAF p.Leu597Gln (25.3%)	BRAF wt *	BRAF p.Leu597Gln	not performed
	NRAS wt	NRAS wt	not performed	not performed
	KRAS wt	KRAS wt	not performed	not performed
	PIK3CA wt	PIK3CA wt	not performed	not performed
<b>MEL-10</b>	BRAF p.Gly596Val (52.6%)	BRAF wt *	BRAF p.Gly596Val	not performed
	NRAS wt	NRAS wt	not performed	not performed
	KRAS wt	KRAS wt	not performed	not performed
	PIK3CA wt	PIK3CA wt	not performed	not performed
<b>MEL-14</b>	BRAF p.Asp594Asn (36.8%)	BRAF wt *	BRAF p.Asp594Asn	not performed
	NRAS wt	NRAS wt	not performed	not performed
	KRAS p.Gln61His (61.2%)	KRAS p.Gln61His	KRAS p.Gln61His	not performed
	PIK3CA wt	PIK3CA wt	not performed	not performed

\*mutation not covered by the panel

**Table S5.** Analysis of wild type samples both for *BRAF* and *NRAS* genes by conventional methods: comparison of results with NGS analysis.

SAMPLE	NGS (mutant allele fraction)	Sequenom	Conventional methods	
			HRMA+Sanger	castPCR for <i>BRAF</i> p.Val600Glu (mutant allele fraction)
<b>MEL-16</b>	BRAF wt	BRAF wt	BRAF wt	BRAF wt
	NRAS wt	NRAS wt	not performed	not performed
	KRAS wt	KRAS wt	not performed	not performed
	PIK3CA wt	PIK3CA wt	not performed	not performed
<b>MEL-17</b>	BRAF wt	BRAF wt	BRAF wt	BRAF wt
	NRAS wt	NRAS wt	not performed	not performed
	KRAS wt	KRAS wt	not performed	not performed
	PIK3CA wt	PIK3CA wt	not performed	not performed
<b>MEL-18</b>	BRAF wt	BRAF wt	BRAF wt	BRAF wt
	NRAS wt	NRAS wt	not performed	not performed
	KRAS wt	KRAS wt	not performed	not performed
	PIK3CA wt	PIK3CA wt	not performed	not performed
<b>MEL-19</b>	BRAF p.Gly466Arg in exon11 (36.0%)	BRAF wt*	BRAF wt	BRAF wt
	NRAS wt	NRAS wt	not performed	not performed
	KRAS wt	KRAS wt	not performed	not performed
	PIK3CA wt	PIK3CA wt	not performed	not performed
<b>MEL-20</b>	BRAF p.Gly466Arg in exon11 (31.5%)	BRAF wt*	BRAF wt	BRAF wt
	NRAS wt	NRAS wt	not performed	not performed
	KRAS wt	KRAS wt	not performed	not performed
	PIK3CA wt	PIK3CA wt	not performed	not performed

\*mutation not covered by the panel

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