

# Detection of *BRAF V600E* mutation by droplet digital PCR in FFPE tissues

## Supplementary Information

All FFPE samples (n = 87) were histologically confirmed melanomas selected by clinical pathologists. Tested samples were containing tumor cells in interval between 30-90%. For the extraction of total DNA from the sections (5 µm thick, 50 mg ± 10%) Black PREP FFPE DNA kit (Life Science-Analytik Jena, Germany) kit was used. DNA was quantified on a Qubit Fluorometer 2.0 (Invitrogen California).

For the comparison different methods with different sensitivity were used. In general, the limit of detection (LOD) by Sanger sequencing is approximately 20 to 25% mutant alleles. However, detection of lower percentage of the mutant allele may be achieved, based on the context of the target sequences. When compared to Sanger Sequencing, allele-specific PCR sensitivity in tumor tissues is significantly higher (with a detection limit of 0.65% mutant alleles) [14]. Both in case of Sanger sequencing as well as allele-specific PCR, 20 ng of genomic DNA in total volume of 25 µl was put into the reaction. Detection limit of The Cobas® 4800 BRAF V600 Mutation Test (Roche Molecular Diagnostics, Pleasanton, CA) is ≥5% mutation level. PCR using this kit was performed in a 50-µL volume containing 50 to 100 ng genomic DNA according to the manufacturer's protocol, which is 2.5-5 times more than amount of DNA used in previous methods.

Detection limit of ddPCR was established at 0.001%. The sensitivity of ddPCR was tested and optimal concentration was selected in serial dilutions of the RKO cell line as described in the manuscript. DNA of final amount 132 ng were added in total volume of 20 µL to ddPCR reaction mixes, as recommended by Bio-rad provider.

**Supplementary Table 1.** Mutation analysis was performed by the characterized RKO line in varying dilutions. The mutation was detected with 0.001% sensitivity using ddPCR

		ddPCR	
		<i>BRAF V600E</i>	Wild type
Sanger	<i>BRAF V600E</i>	19	4
	WT	12	52
		ddPCR	
		<i>BRAF V600E</i>	Wild type
AS-PCR	<i>BRAF V600E</i>	20	3
	WT	11	53
		Sanger	
		<i>BRAF V600E</i>	Wild type
AS-PCR	<i>BRAF V600E</i>	21	2
	WT	2	62
		Cobas	
		<i>BRAF V600E</i>	Wild type
Sanger	<i>BRAF V600E</i>	6	1
	WT	2	11
		Cobas	
		<i>BRAF V600E</i>	Wild type
AS-PCR	<i>BRAF V600E</i>	6	1
	WT	2	11
		Cobas	
		<i>BRAF V600E</i>	Wild type
ddPCR	<i>BRAF V600E</i>	8	1
	WT	0	11