Oncology Research and Treatment

Research Article

Oncol Res Treat
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Supplemental Material

Supplemental Table 1. Primers and PCR conditions

Assay	Primers	PCR composition	PCR product length	PCR conditions / Device
EGFR T790M: allele-specific PCR	Wild-type-specific: TCCACCGTGCAGCTCATTAC Mutation-specific: TCCACCGTGCAGCTCATTAT Common: CTTCCCTGATTACCTTTGCGA	1 µl template, 1 u of hot-start polymerase, 1x PCR buffer (pH 8.3), 2.0 mM $MgCl_2$, 200 µM dNTPs, 0.2 µM of each primer, 0.2× SYBR Green I in a total volume of 20 µl	133 bp	Activation of Taq polymerase (95°C for 10 min) followed by 50 cycles (95°C for 10 sec, 55°C for 20 sec, 72°C for 30 sec) Device: CFX96 Thermal Cycler (Bio-Rad)
EGFR T790M: digital droplet PCR	Forward: TCTGCCTCACCTCCACCGT Reverse: GGACATAGTCCAGGAGGCA Wild-type-specific probe: ATCA**C**GCAGCTCATGCCCTTC Mutation-specific probe: ATCA**T**GCAGCTCATGCCCTTC	1 µl template, 10 µl PCR Supermix (Bio-Rad), 0.5 µM of each primer and 0.5 µM of each probe in a total volume of 20 µl	69 bp	Activation of Taq polymerase (95°C for 10 min), followed by 45 cycles (95°C for 15 sec, 60°C for 60 sec) Devices: CFX96 Thermal Cycler (Bio-Rad); QX100 Droplet Digital PCR system (Bio-Rad)
EGFR T790M: PCR amplification prior to NGS	Forward: ACCATGCGAAGCCACACTG Reverse: TTGAGCAGGTACTGGGAGC	1 µl template, 1 u of hot-start polymerase, 1× PCR buffer (pH 8.3), 2.0 mM $MgCl_2$, 200 µM dNTPs, 0.3 µM of each primer in a total volume of 20 µl	205 bp	Activation of Taq polymerase (95°C for 10 min), followed by 50 cycles (95°C for 10 sec, 67°C for 20 sec, 72°C for 30 sec) Device: CFX96 Thermal Cycler (Bio-Rad)
EGFR T790M: determination of allelic context by PCR and PAGE (T790M allele-specific design)	Wild-type-specific: CGAAGGGCATGAGCTACG Mutation-specific: CGAAGGGCATGAGCTACA Common: AGAAAGTTAAAATTCCCGTCG	1 µl cDNA template, 1 unit of hot-start polymerase, 1× PCR buffer (pH 8.3), 2.0 mM $MgCl_2$, 200 μM dNTPs, 0.3 μM of each primer in a total volume of 20 µl	180 bp (shorter in case of ex19 deletion)	Activation of Taq polymerase (95°C for 10 min), followed by 50 cycles (95°C for 10 sec, 60°C for 20 sec, and 72°C for 30 sec) Device: CFX96 Thermal Cycler (Bio-Rad)
EGFR T790M: determination of	Mutation-specific (ex19 c.2240_2257del18): CCGTCGCTATCAAGGAATCG	1 µl template, 1 u of hot-start polymerase,	151 bp	Activation of Taq polymerase (95°C for 10 min), followed by 50 cycles

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Assay	Primers	PCR composition	PCR product length	PCR conditions / Device
allelic context by PCR and sequencing (exon 19 deletion-specific design)	Reverse: CAGCCGAAGGGCATGAGC Mutation-specific (ex19 c.2235_2249del15): ATTCCCGTCGCTATCAAAACAT Reverse: AGTCCAGGAGGCAGCCGAA	1× PCR buffer (pH 8.3), 2.0 mM $MgCl_2$, 200 μM dNTPs, 0.3 μM of each primer in a total volume of 20 µl	169 bp	(95°C for 10 sec, 60°C for 20 sec, and 72°C for 30 sec) Devices: CFX96 Thermal Cycler (Bio-Rad); CEQ 8000 (Beckman-Coulter)

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Supplemental Table 2. Performance characteristics of the three assays for detection of EGFR T790M mutation

	AS-PCR	ddPCR	NGS
DNA from lymphocytes of healthy donors	n = 43 (mean delta Ct = 12.4) LOB*: delta Ct = 9.8 LOD*: delta Ct = 7.9 (≈ 0.2% of mutant alleles)	n = 89 Mean number of mutation-positive droplets: 1.39 Mean fraction of mutated DNA copies: 0.05% LOB** (at 95% confidence level): 4 mutation-positive droplets LOD*** (at 95% confidence level): 9 mutant DNA copies (0.24% of mutant alleles)	-
DNA from non-LC FFPE tissues	n = 37 (mean delta Ct = 8.5) LOB*: delta Ct = 4.1 LOD*: delta Ct = 0.54 (≈ 20% of mutant alleles)	n = 67 Mean number of mutation-positive droplets: 5.90 Mean fraction of mutated DNA copies: 0.23% LOB** (at 95% confidence level): 11 mutation-positive droplets LOD** (at 95% confidence level): 18 mutant DNA copies (0.44% of mutant alleles)	n = 2 Mean number of mutant reads: 0.25%
Analytical sensitivity determined with serial dilutions of commercial T790M DNA	1:250 (0.2% of mutant alleles)	1:1000 (0.05% of mutant alleles)	1:50 (1% of mutant alleles)

*LOB (limit of blank) and LOD (limit of detection) for AS-PCR were calculated by the subtraction of 1.645 and 3 standard deviations, correspondingly, from the mean value [Armbruster DA, Pry T. Limit of blank, limit of detection and limit of quantitation. Clin Biochem Rev 2008;29 Suppl 1:S49-52].
**LOB and LOD for ddPCR were calculated as described in [Milbury CA, Zhong Q, Lin J et al. Determining lower limits of detection of digital PCR assays for cancer-related gene mutations. Biomol Detect Quantif 2014;1:8-22].

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Supplemental Table 3. Raw ddPCR data for determination of limit of blank (LOB) from blood-derived and archival wild-type DNA samples

Sample type	Sample ID	WT events (droplets)	MUT events (droplets)	Total events (WT+MUT +negative)	DNA concentration (WT+MUT), copies/µl	DNA concentration (MUT), copies/µl	Amount of analyzed DNA molecules	% of MUT DNA copies
Blood	2539	276	0	9902	31.10	0.00	307.95	0.00
Blood	2590	508	4	11841	48.57	0.37	575.13	0.76
Blood	2604	784	0	7568	120.00	0.00	908.16	0.00
Blood	2496	849	0	12114	79.80	0.00	966.70	0.00
Blood	2576	872	0	7327	139.00	0.00	1018.45	0.00
Blood	2645	961	0	12267	89.60	0.00	1099.12	0.00
Blood	2667	1014	1	12396	93.89	0.09	1163.84	0.09
Blood	2550	1154	0	10545	127.00	0.00	1339.22	0.00
Blood	2579	1244	2	10281	142.21	0.21	1462.10	0.15
Blood	2584	1316	3	10472	148.32	0.32	1553.15	0.21
Blood	2596	1359	0	9857	163.00	0.00	1606.69	0.00
Blood	2669	1468	2	10337	168.21	0.21	1738.82	0.13
Blood	2559	1673	1	11474	173.10	0.10	1986.10	0.06
Blood	2566	1680	0	9668	210.00	0.00	2030.28	0.00
Blood	2514	1690	0	8576	241.00	0.00	2066.82	0.00
Blood	2630	1724	0	10345	200.00	0.00	2069.00	0.00
Blood	2606	1803	0	10050	217.00	0.00	2180.85	0.00
Blood	2589	1816	3	10124	217.33	0.33	2200.21	0.15
Blood	2648	2050	1	11341	219.10	0.10	2484.78	0.04
Blood	2608	2154	0	13090	198.00	0.00	2591.82	0.00
Blood	2605	2114	0	9237	286.00	0.00	2641.78	0.00
Blood	2541	2051	1	7420	356.15	0.15	2642.62	0.04
Blood	2662	2140	1	10026	264.11	0.11	2647.97	0.04
Blood	2498	2179	1	10751	249.10	0.10	2678.10	0.04
Blood	2639	2257	3	11691	236.28	0.28	2762.37	0.12
Blood	2561	2269	0	10624	264.00	0.00	2804.74	0.00
Blood	2545	2326	0	12063	235.00	0.00	2834.81	0.00
Blood	2652	2318	1	11204	255.10	0.10	2858.12	0.04
Blood	2681	2300	1	9334	311.12	0.12	2903.98	0.04
Blood	2500	2288	3	8815	330.37	0.37	2912.25	0.11
Blood	2656	2305	8	8538	347.03	1.03	2962.94	0.30
Blood	2513	2339	0	8014	379.00	0.00	3037.31	0.00
Blood	2601	2485	3	9747	323.34	0.34	3151.58	0.10
Blood	2614	2525	1	10322	308.11	0.11	3180.27	0.03
Blood	2588	2565	3	11487	278.29	0.29	3196.68	0.10
Blood	2506	2443	0	7984	401.00	0.00	3201.58	0.00
Blood	2617	2625	0	12840	251.00	0.00	3222.84	0.00

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Blood	2510	2552	1	10271	314.11	0.11	3226.19	0.03
Blood	2621	2552	2	10101	320.22	0.22	3234.52	0.07
Blood	2675	2541	1	9238	353.12	0.12	3262.11	0.03
Blood	2568	2501	0	8120	405.00	0.00	3288.60	0.00
Blood	2631	2621	2	10819	305.20	0.20	3301.99	0.07
Blood	2628	2702	0	12498	268.00	0.00	3349.46	0.00
Blood	2523	2738	1	13027	259.08	0.08	3375.09	0.03
Blood	2595	2716	0	10560	327.00	0.00	3453.12	0.00
Blood	2598	2787	4	11129	317.40	0.40	3532.29	0.12
Blood	2660	2860	3	12104	296.27	0.27	3586.08	0.09
Blood	2529	2764	1	9548	376.12	0.12	3591.15	0.03
Blood	2657	2867	1	10319	358.11	0.11	3695.30	0.03
Blood	2633	2962	0	11415	330.00	0.00	3766.95	0.00
Blood	2528	2816	1	8330	453.13	0.13	3774.59	0.03
Blood	2671	2889	2	9677	390.23	0.23	3776.23	0.06
Blood	2571	2919	3	9982	380.33	0.33	3796.45	0.09
Blood	2519	2935	2	10214	372.22	0.22	3801.80	0.06
Blood	2557	2938	0	10071	379.00	0.00	3816.91	0.00
Blood	2654	3011	1	11757	325.09	0.09	3822.12	0.03
Blood	2665	2925	5	9718	394.57	0.57	3834.39	0.14
Blood	2503	2866	2	8272	467.27	0.27	3865.22	0.06
Blood	2564	3238	0	11422	366.00	0.00	4180.45	0.00
Blood	2627	3168	1	9966	420.11	0.11	4186.82	0.03
Blood	2661	3332	0	11932	360.00	0.00	4295.52	0.00
Blood	2640	3437	1	12178	364.09	0.09	4433.89	0.02
Blood	2533	3466	1	9692	486.11	0.11	4711.41	0.02
Blood	2565	3389	4	8740	539.50	0.50	4715.26	0.09
Blood	2674	3596	1	11178	427.10	0.10	4774.10	0.02
Blood	2646	3560	0	10335	464.00	0.00	4795.44	0.00
Blood	2673	3589	1	10722	448.10	0.10	4804.55	0.02
Blood	2655	3623	1	10383	472.11	0.11	4901.88	0.02
Blood	2659	3744	2	11953	413.18	0.18	4938.79	0.04
Blood	2505	3626	0	8791	584.00	0.00	5133.94	0.00
Blood	2563	3668	1	8960	579.12	0.12	5188.94	0.02
Blood	2647	3839	4	9730	551.45	0.45	5365.63	0.08
Blood	2616	4001	0	9473	603.00	0.00	5712.22	0.00
Blood	2663	4155	4	11142	513.40	0.40	5720.25	0.08
Blood	2586	4175	1	10067	589.11	0.11	5930.56	0.02
Blood	2620	4321	2	11645	510.19	0.19	5941.15	0.04

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Blood	2582	4220	0	10167	589.00	0.00	5988.36	0.00
Blood	2668	4441	1	11468	538.10	0.10	6170.88	0.02
Blood	2679	4167	1	8682	719.13	0.13	6243.46	0.02
Blood	2547	4325	0	9849	635.00	0.00	6254.12	0.00
Blood	2540	4279	2	9197	688.24	0.24	6329.73	0.03
Blood	2569	4430	2	10230	624.22	0.22	6385.72	0.03
Blood	2625	4491	3	10753	594.31	0.31	6390.58	0.05
Blood	2676	4349	1	8651	768.13	0.13	6645.07	0.02
Blood	2650	4672	5	10283	666.53	0.53	6853.97	0.08
Blood	2680	4743	1	9972	709.11	0.11	7071.24	0.02
Blood	2553	4744	0	9053	816.00	0.00	7387.25	0.00
Blood	2511	4590	3	7250	1100.46	0.46	7978.30	0.04
Blood	2517	5931	6	10769	879.61	0.61	9472.54	0.07
	Mean	**2805.56**	**1.39**	**10241.29**	**376.53**	**0.15**	**3764.51**	**0.05**
Sample type	Sample ID	WT events (droplets)	MUT events (droplets)	Total events (WT+MUT +negative)	DNA concentration (WT+MUT), copies/µl	DNA concentration (MUT), copies/µl	Amount of analyzed DNA molecules	% of MUT DNA copies
FFPE	BC2638	3728	1	8535	631	0.13	5385.59	0.02
FFPE	BC2639	1822	0	9916	223	0.00	2211.27	0.00
FFPE	BC2645	277	0	9055	34.1	0.00	308.78	0.00
FFPE	BC2646	2653	1	8670	401	0.13	3476.67	0.03
FFPE	BC2649	938	0	8515	128	0.00	1089.92	0.00
FFPE	BC2650	2529	1	8653	380	0.13	3288.14	0.03
FFPE	BC2652	1599	0	9095	212	0.00	1928.14	0.00
FFPE	BC2664	679	2	11058	69.6	0.20	769.64	0.29
FFPE	BC2668	2538	1	10818	294	0.10	3180.49	0.03
FFPE	BC2669	735	1	10874	76.9	0.10	836.21	0.13
FFPE	BC2678	683	0	9302	83.8	0.00	779.51	0.00
FFPE	BC2680	5339	2	9414	920	0.23	8660.88	0.03
FFPE	BC2681	1702	0	10027	204	0.00	2045.51	0.00
FFPE	BC2682	1576	1	8345	230	0.13	1919.35	0.06
FFPE	BC2683	783	0	7298	125	0.00	912.25	0.00
FFPE	CC154	3644	11	6672	868	1.81	5791.30	0.21
FFPE	CC42	5140	18	6950	1480	2.85	10286.00	0.19
FFPE	CC46	820	9	6478	149	1.53	965.22	1.03
FFPE	CC83	382	5	6396	67.7	0.86	433.01	1.27
FFPE	CC93	2616	12	5686	677	2.32	3849.42	0.34
FFPE	OvCa33	2330	2	8654	304	0.25	2630.82	0.08
FFPE	OvCa34	3127	0	10013	411	0.00	4115.34	0.00

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FFPE	OvCa35	3533	0	8587	582	0.00	4997.63	0.00
FFPE	OvCa36	837	2	9472	102	0.29	966.14	0.28
FFPE	OvCa37	1020	0	8883	134	0.00	1190.32	0.00
FFPE	OvCa38	1162	0	9204	148	0.00	1362.19	0.00
FFPE	OvCa39	6007	6	8547	1330	0.60	11367.51	0.04
FFPE	OvCa40	3628	2	8968	570	0.34	5111.76	0.06
FFPE	OvCa41	5937	26	9462	1099	3.02	10398.74	0.27
FFPE	OvCa43	5285	17	9388	910	1.99	8543.08	0.22
FFPE	OvCa44	2805	6	9541	383	0.68	3654.20	0.18
FFPE	OvCa45	6913	20	9964	1300	2.21	12953.20	0.17
FFPE	OvCa47	7166	34	10048	1370	3.72	13765.76	0.27
FFPE	OvCa48	4146	18	11330	501	1.75	5676.33	0.35
FFPE	OvCa50	4423	7	9842	656	0.78	6456.35	0.12
FFPE	OvCa51	5789	21	8340	1300	2.77	10842.00	0.21
FFPE	OvCa52	1725	8	9976	209	0.88	2084.98	0.42
FFPE	OvCa56	4011	13	7003	935	2.04	6547.81	0.22
FFPE	OvCa57	3703	5	9385	551	0.59	5171.14	0.11
FFPE	OvCa58	532	1	10684	56.1	0.10	599.37	0.18
FFPE	OvCa59	4323	4	10094	614	0.44	6197.72	0.07
FFPE	OvCa62	1405	1	7739	220	0.14	1702.58	0.06
FFPE	OvCa67	3521	7	9047	542	0.85	4903.47	0.16
FFPE	OvCa74	537	0	9752	62.2	0.00	606.57	0.00
FFPE	OvCa75	2047	2	9130	279	0.24	2547.27	0.09
FFPE	OvCa81	1157	7	6149	370	1.25	2275.13	0.34
FFPE	OvCa82	6038	42	7564	1760	6.12	13312.64	0.35
FFPE	PC10	249	2	7929	35.1	0.28	278.31	0.79
FFPE	PC11	1985	6	7437	341	0.89	2536.02	0.26
FFPE	PC17	2241	7	9707	288	0.79	2795.62	0.28
FFPE	PC18	267	2	10552	28.2	0.21	297.57	0.74
FFPE	PC19	5450	10	8270	1180	1.33	9758.60	0.11
FFPE	PC20	699	2	7203	112	0.31	806.74	0.27
FFPE	PC9	6314	22	9228	1270	2.62	11719.56	0.21
FFPE	THYR20	281	1	9090	34.5	0.12	313.61	0.35
FFPE	THYR21	381	0	10922	39	0.00	425.96	0.00
FFPE	THYR22	208	1	11250	20.5	0.10	230.63	0.49
FFPE	THYR23	2890	4	9974	376	0.44	3750.22	0.12
FFPE	THYR24	1568	0	9945	189	0.00	1879.61	0.00
FFPE	THYR25	58	2	10979	5.82	0.20	63.90	3.44
FFPE	THYR26	349	1	9530	41	0.12	390.73	0.28

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FFPE	THYR28	6533	6	10284	1110	0.64	11415.24	0.06
FFPE	THYR29	5117	6	9121	905	0.72	8254.51	0.08
FFPE	THYR30	1248	0	11133	131	0.00	1458.42	0.00
FFPE	THYR32	3107	3	9185	454	0.36	4169.99	0.08
FFPE	THYR34	1538	2	8445	221	0.26	1866.35	0.12
FFPE	THYR35	2249	2	6466	470	0.34	3039.02	0.07
	Mean	**2627.19**	**5.90**	**9032.43**	**465.72**	**0.77**	**4082.80**	**0.23**

Supplemental Table 4. Raw ddPCR data for DNA isolated with organic extraction protocol and commercial kits

Sample type	Sample ID	Extraction	WT events (droplets)	MUT events (droplets)	Total events (WT+MUT +negative)	DNA concentration (WT+MUT), copies/µl	DNA concentration (MUT), copies/µl	Amount of analyzed DNA molecules	% of MUT DNA copies
Blood	11946	Organic	500	0	7356	77.40	0.00	569.35	0.00
Blood		Kit	479	0	7271	74.90	0.00	544.60	0.00
Blood	11947	Organic	1735	1	8190	262.13	0.13	2146.88	0.05
Blood		Kit	1358	1	9099	178.12	0.12	1620.72	0.07
Blood	11948	Organic	920	1	9447	113.12	0.12	1068.61	0.10
Blood		Kit	603	0	7533	91.70	0.00	690.78	0.00
Blood	11949	Organic	537	0	6111	101.00	0.00	617.21	0.00
Blood		Kit	731	0	8094	104.00	0.00	841.78	0.00
Blood	11950	Organic	889	0	8539	121.00	0.00	1033.22	0.00
Blood		Kit	656	0	7007	108.00	0.00	756.76	0.00
Blood	11951	Organic	1072	0	11361	109.00	0.00	1238.35	0.00
Blood		Kit	1059	4	10625	115.41	0.41	1226.27	0.36
Blood	11930	Organic	1765	0	9045	239.00	0.00	2161.76	0.00
Blood		Kit	1709	3	10055	205.33	0.33	2064.57	0.16
Blood	11931	Organic	553	1	5759	111.19	0.19	640.35	0.17
Blood		Kit	644	3	8140	91.01	0.41	740.78	0.45
Blood	11932	Organic	1090	0	7573	171.00	0.00	1294.98	0.00
Blood		Kit	905	0	9780	107.00	0.00	1046.46	0.00
Blood	11937	Organic	1349	0	5762	293.00	0.00	1688.27	0.00
Blood		Kit	1385	0	6075	284.00	0.00	1725.30	0.00
FFPE	8489	Organic	871	4	10649	94.21	0.41	1003.27	0.44
FFPE		Kit	1008	7	9740	120.79	0.79	1176.49	0.65
FFPE	8446	Organic	1271	3	9957	150.33	0.33	1496.85	0.22
FFPE		Kit	1083	4	8807	144.50	0.50	1272.60	0.35
FFPE	7697	Organic	148	1	8463	19.53	0.13	165.28	0.67

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FFPE		Kit	115	1	8994	14.22	0.12	127.91	0.86
FFPE	7637	Organic	1769	3	9223	234.36	0.36	2161.48	0.15
FFPE		Kit	1455	5	6673	270.82	0.82	1807.21	0.30
FFPE	7588	Organic	244	1	8127	33.64	0.14	273.35	0.40
FFPE		Kit	231	1	7860	32.94	0.14	258.91	0.43
FFPE	7592	Organic	392	0	8814	50.00	0.00	440.70	0.00
FFPE		Kit	378	0	9349	45.40	0.00	424.44	0.00
FFPE	8891	Organic	2640	7	8771	394.88	0.88	3463.47	0.22
FFPE		Kit	2642	4	8450	412.52	0.52	3485.79	0.13
FFPE	6908	Organic	226	0	8475	29.70	0.00	251.71	0.00
FFPE		Kit	206	0	8458	27.10	0.00	229.21	0.00
FFPE	7572	Organic	1997	5	7177	358.77	0.77	2574.86	0.21
FFPE		Kit	3269	9	6766	726.46	1.46	4915.23	0.20

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Supplemental Table 5. Raw ddPCR data for DNA samples extracted with and without UDG treatment

Sample number	Sample type	WT events (droplets)	MUT events (droplets)	Total events (WT+MUT +negative)	DNA concentration (WT+MUT), copies/µl	DNA concentration (MUT), copies/µl	Amount of analysed DNA molecules	% of MUT DNA copies
1	UDG-	2914	5	11571	318.97	0.47	3690.85	0.15
	UDG+	3494	6	10017	472.66	0.66	4734.63	0.14
2	UDG-	10417	12	11038	3171.15	1.15	35001.57	0.04
	UDG+	10446	6	10741	4855.61	0.61	52151.67	0.01
3	UDG-	8912	12	10905	1866.20	1.20	20350.95	0.06
	UDG+	8702	15	10913	1756.51	1.51	19168.79	0.09
4	UDG-	7162	11	10810	1191.13	1.13	12875.47	0.09
	UDG+	5111	3	10411	736.80	0.30	7670.43	0.04
5	UDG-	5339	17	10647	776.21	1.71	8264.25	0.22
	UDG+	5350	12	10015	843.32	1.32	8445.43	0.16
6	UDG-	4294	3	9910	623.26	0.26	6176.21	0.04
	UDG+	3794	3	10001	524.28	0.28	5243.29	0.05
7	UDG-	5426	15	9381	956.70	1.70	8974.76	0.18
	UDG+	5156	10	10157	779.53	1.03	7917.65	0.13
8	UDG-	11007	10	11679	3255.90	0.90	38025.63	0.03
	UDG+	12199	6	12558	3905.53	0.53	49045.68	0.01

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Supplemental Table 6. Raw ddPCR data for paired DNA samples extracted from fresh-frozen (FF) and FFPE tissues

Pair number	Sample type	WT events (droplets)	MUT events (droplets)	Total events (WT+MUT +negative)	DNA concentration (WT+MUT), copies/µl	DNA concentration (MUT), copies/µl	Amount of analyzed DNA molecules	% of MUT DNA copies
1	FFPE	2536	5	9477	346.91	0.58	3287.59	0.17
	FF	1546	2	9411	197.56	0.23	1859.30	0.11
2	FFPE	2422	4	9251	330.53	0.53	3057.61	0.16
	FF	513	0	9022	64.23	0.03	579.49	0.05
3	FFPE	4929	7	9560	796.13	0.80	7611.29	0.10
	FF	8951	5	9105	4487.32	0.65	40858.54	0.01
4	FFPE	5069	6	10179	758.33	0.66	7719.05	0.09
	FF	1790	3	7325	304.08	0.41	2227.49	0.14
5	FFPE	727	1	9816	84.57	0.07	830.21	0.09
	FF	3001	2	10822	354.54	0.21	3836.99	0.06
6	FFPE	3337	3	9797	455.35	0.35	4461.21	0.08
	FF	5797	3	9479	1040.68	0.35	9864.60	0.03
7	FFPE	4522	5	10981	603.14	0.48	6623.10	0.08
	FF	9934	6	9961	6595.66	0.66	65699.34	0.01
8	FFPE	6068	7	7877	1627.84	1.17	12821.94	0.07
	FF	4623	1	8240	907.18	0.18	7475.14	0.02
9	FFPE	7650	9	10023	1597.69	1.03	16013.69	0.06
	FF	8174	3	11358	1400.32	0.32	15904.84	0.02
10	FFPE	4021	3	9698	588.68	0.34	5708.78	0.06
	FF	4627	1	8891	807.08	0.08	7175.50	0.01
11	FFPE	7062	7	10127	1317.46	0.80	13341.52	0.06
	FF	7666	4	8699	2350.53	0.53	20448.05	0.02
12	FFPE	7751	5	10062	1620.51	0.51	16306.11	0.03
	FF	4355	2	10015	639.17	0.17	6401.12	0.03
13	FFPE	10132	10	10459	3807.72	1.06	39826.24	0.03
	FF	6174	2	9588	1136.92	0.26	10900.82	0.02
14	FFPE	6953	2	7455	2960.30	0.30	22069.00	0.01
	FF	12228	3	12304	5626.93	0.27	69235.65	0.00

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Supplemental Table 7. Raw ddPCR data for pretreatment LC samples, which were subsequently exposed to TKI therapy

Sample ID	WT events (droplets)	MUT events (droplets)	Total events (WT+MUT +negative)	DNA concentration (WT+MUT), copies/µl	DNA concentration (MUT), copies/µl	Amount of analyzed DNA molecules	% of MUT DNA copies	Sample status
281	466	14	8934	60	1.72	536.04	2.87	ND
282	116	1	12420	10.3	0.09	127.93	0.86	ND
492	1886	1	14169	157	0.08	2224.53	0.05	WT
597	1080	1	8424	151	0.13	1272.02	0.09	ND
721	7232	26	8666	1980	3.30	17158.68	0.17	WT
778	93	1	10352	9.92	0.11	102.69	1.07	ND
786	120	3	9197	14.8	0.36	136.12	2.43	ND
792	584	4	9375	71	0.48	665.63	0.68	ND
803	71	2	9846	10.6	0.10	104.37	0.96	ND
805	5590	3	9719	941	0.32	9145.58	0.03	WT
908	144	0	12195	13.1	0.00	159.75	0.00	ND
917	3522	3	9308	522	0.53	4858.78	0.10	WT
1037	2543	10	10690	300	1.03	3207.00	0.34	WT
1052	499	19	6971	84.6	0.57	589.75	0.67	ND
1055	392	7	11699	38	0.66	444.56	1.73	ND
1139	109	3	12814	9.4	0.26	120.45	2.73	ND
1153	1398	1	11513	142	0.10	1634.85	0.07	WT
1195	79	4	10124	13.8	0.31	139.71	2.21	ND
1250	1151	19	8596	160	2.43	1375.36	1.52	ND
1259	368	2	9853	42	0.22	413.83	0.53	ND
1273	1482	2	9242	192	0.24	1774.46	0.12	WT
1287	142	2	11516	13.6	0.19	156.62	1.40	ND
1352	899	8	11373	91	0.77	1034.94	0.85	ND
1403	258	1	14714	19.4	0.08	285.45	0.39	ND
1471	64	4	12832	5.8	0.34	74.43	5.91	ND
1563	374	10	12231	35	0.90	428.09	2.57	ND
1595	1136	2	11301	116	0.19	1310.92	0.17	ND
1772	8742	0	9306	3080	0.00	28662.48	0.00	WT
2280	51	1	11507	4.88	0.10	56.15	2.05	ND
2899	64	3	10570	6.67	0.31	70.50	4.68	ND
2925	106	0	10512	11.1	0.00	116.68	0.00	ND
3057	5865	3	10472	902	0.32	9445.74	0.03	WT
3636	819	5	11804	79.5	0.47	938.42	0.59	ND
3726	335	52	6729	64.5	8.53	434.02	13.22	ND
4127	8477	10	8731	3890	1.26	33963.59	0.03	WT
4429	10221	7	10679	3460	0.72	36949.34	0.02	WT
4791	6236	16	11532	855	1.53	9859.86	0.18	WT

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Sample ID	WT events (droplets)	MUT events (droplets)	Total events (WT+MUT +negative)	DNA concentration (WT+MUT), copies/µl	DNA concentration (MUT), copies/µl	Amount of analyzed DNA molecules	% of MUT DNA copies	Sample status
4879	705	34	7499	113	5.00	847.39	4.42	ND
4880	226	3	9619	26.1	0.34	251.06	1.31	ND
4881	11	9	12839	1.17	0.77	15.02	65.90	ND
5936	1182	0	12458	110	0.00	1370.38	0.00	ND
5973	3582	6	10973	434.6	0.60	4768.87	0.14	WT
6142	3671	2	8753	597	0.13	5225.54	0.02	WT
6146	3095	5	9084	458.6	0.61	4165.92	0.13	WT
6443	549	9	12388	54.8	0.80	678.86	1.46	ND
6453	4574	4	10418	635	0.42	6615.43	0.07	WT
6577	775	14	10618	84.7	1.45	899.34	1.71	ND
6846	2529	3	9640	334	0.34	3219.76	0.10	WT
6869	1266	3	10662	139	0.31	1482.02	0.22	ND
7087	4246	3	11287	519	0.29	5857.95	0.06	WT
7158	31	2	8951	3.81	0.25	34.10	6.46	ND
7171	3319	6	8052	584	0.79	4702.37	0.13	WT
7287	1814	249	9232	270	26.50	2492.64	46.33	MUT
7290	3275	8	13287	311	0.66	4132.26	0.21	WT
7396	345	4	12103	32	0.36	387.30	1.13	ND
7507	1114	24	11959	109.2	0.80	1305.92	0.73	ND
7661	9036	37	9194	4474.43	4.43	41137.91	0.10	WT
7791	55	0	8389	7.23	0.00	60.65	0.00	ND
7834	3033	13	8836	463.6	1.62	4096.37	0.35	WT
7877	7086	6	11905	994	0.89	11833.57	0.09	WT
8236	2049	5	10452	240	0.53	2508.48	0.22	WT
8470	2595	5	9279	360	0.59	3340.44	0.16	WT
8802	5360	18	9717	881	1.77	8560.68	0.20	WT
8956	687	2	10954	71	0.24	777.73	0.34	ND
9166	587	2	13316	49.5	0.17	659.14	0.33	ND
9329	1593	4	11992	157	0.37	1882.74	0.23	WT
9551	4264	18	9142	692	2.17	6326.26	0.31	WT
Mean	**2169.67**	**11.16**	**10491.25**	**459.16**	**1.22**	**4471.87**	**2.75**	

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Supplemental Table 8. Clinical characteristics and T790M genotyping results for 68 metastatic lung cancer cases treated by tyrosine kinase inhibitors

Patient ID	TKI	Treatment line	Gender	Age	Stage	EGFR mutation	Response (RECIST)	PFS/TTP (months)	OS (months)	AS-PCR	ddPCR (% of mutant DNA copies)	NGS (% of mutant reads)
281	Gefitinib	2	F	70	IV	L858R	SD	5	20	WT	ND	WT
282	Gefitinib	3	F	88	IIIb	L858R	SD	28	46	WT	ND	WT
492	Gefitinib	1	F	55	IV	ex19del	SD	23	41	WT	WT	WT
597	Gefitinib	1	M	48	IV	Ex19del	CR	10	14	WT	ND	WT
721	Gefitinib	1	F	76	IV	L858R	SD	0.7	0.9	WT	WT	WT
736	Gefitinib	1	F	55	IV	Ex19del	SD	8	26	WT	ND	WT
778	Gefitinib	1	F	61	IV	Ex19del	SD	5	6	WT	ND	WT
786	Gefitinib	1	F	70	IV	Ex19del	SD	7	14	WT	ND	WT
792	Gefitinib	1	F	69	IV	L858R	SD	2	6	WT	ND	ND
803	Gefitinib	1	M	80	IV	Ex19del	PR	10	12	WT	ND	ND
805	Gefitinib	1	F	67	IV	Ex19del	PR	3	8	WT	WT	WT
908	Gefitinib	1	F	81	IV	L858R	SD	25	25	WT	ND	ND
917	Gefitinib	1	F	55	IV	Ex19del	PR	73	80	WT	WT	WT
1037	Gefitinib	1	F	54	IV	L858R	PR	8	14	WT	WT	WT
1052	Gefitinib	1	F	68	IV	Ex19del	SD	4	4	WT	ND	WT
1055	Gefitinib	1	F	79	IIIA	L858R	SD	7	13	WT	ND	WT
1139	Gefitinib	1	M	70	IV	Ex19del	PR	9	13	WT	ND	WT
1153	Gefitinib	1	F	62	IV	L858R	PR	18	20	WT	WT	WT
1195	Gefitinib	1	F	75	IV	L858R	SD	14	19	WT	ND	WT
1250	Erlotinib	2	F	35	IIIb	ex19del	SD	17	32	WT	ND	WT
1259	Erlotinib	1	F	60	IV	Ex19del	SD	75	92	WT	ND	WT

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Patient ID	TKI	Treatment line	Gender	Age	Stage	EGFR mutation	Response (RECIST)	PFS/TTP (months)	OS (months)	AS-PCR	ddPCR (% of mutant DNA copies)	NGS (% of mutant reads)
1273	Gefitinib	1	F	69	IV	L858R	PR	8	12	WT	WT	WT
1287	Gefitinib	1	M	72	IV	Ex19del	PR	6	6	WT	ND	WT
1352	Erlotinib	2	F	62	IV	ex19del	PR	9	22	WT	ND	WT
1403	Erlotinib	2	F	73	IV	ex19del	CR	25	26	WT	ND	WT
1471	Erlotinib	2	M	38	IV	ex19del	PR	10	17	WT	ND	WT
1563	Erlotinib	2	F	71	IV	ex19del	PR	21	33	WT	ND	WT
1595	Gefitinib	2	F	62	IV	ex19del	PR	11	37	WT	ND	WT
1772	Erlotinib	2	F	62	IIIb	L858R	PR	8	24	WT	WT	WT
2280	Afatinib	1	F	68	IV	ex19del	SD	14	29	WT	ND	ND
2899	Erlotinib	2	F	63	IV	ex19del	SD	10	21	WT	ND	WT
2925	Erlotinib	2	F	77	IV	L858R	PR	25	25	WT	ND	WT
3057	Erlotinib	2	F	83	IV	L858R	SD	7	13	WT	WT	WT
3636	Erlotinib	2	F	71	IV	L858R	SD	4	14	WT	ND	WT
3726	Erlotinib	2	M	67	IV	ex19del	CR	7	14	WT	ND	**MUT (2.88%)**
4127	Erlotinib	2	F	60	IV	L858R	PR	5	ND	WT	WT	WT
4429	Gefitinib	1	F	57	IV	Ex19del	SD	21	31	WT	WT	ND
4791	Gefitinib	1	F	60	IV	Ex19del	PR	8	21	WT	WT	WT
4879	Gefitinib	1	F	62	IV	Ex19del	PR	30	30	WT	ND	WT
4880	Gefitinib	1	F	61	IV	L858R	SD	9	13	WT	ND	WT
4881	Gefitinib	1	M	76	IV	L858R	PR	21	21	WT	ND	WT
5936	Gefitinib	1	F	62	IV	L858R	SD	13	18	WT	ND	WT
5973	Gefitinib	1	F	78	IV	Ex19del	CR	12	15	WT	WT	ND

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Patient ID	TKI	Treatment line	Gender	Age	Stage	EGFR mutation	Response (RECIST)	PFS/TTP (months)	OS (months)	AS-PCR	ddPCR (% of mutant DNA copies)	NGS (% of mutant reads)
6142	Gefitinib	1	F	75	IIIA	Ex19del	SD	14	14	WT	WT	WT
6146	Gefitinib	1	F	66	IV	L858R	SD	7	7	WT	WT	WT
6443	Gefitinib	1	F	61	IV	L858R	SD	21	23	WT	ND	WT
6453	Gefitinib	1	F	56	IV	Ex19del	SD	19	19	WT	WT	WT
6577	Gefitinib	1	F	67	IV	L858R	SD	16	18	WT	ND	WT
6846	Gefitinib	1	F	67	IV	L858R	SD	22	22	WT	WT	WT
6869	Gefitinib	1	M	72	IV	Ex19del	SD	17	18	WT	ND	ND
7087	Gefitinib	1	M	73	IV	L858R	SD	21	21	WT	WT	WT
7158	Gefitinib	1	F	64	IV	Ex19del	PR	21	21	WT	ND	WT
7171	Gefitinib	1	F	63	IV	Ex19del	SD	21	21	WT	WT	WT
7287	Gefitinib	1	F	56	IV	Ex19del	PR	16	21	**MUT**	**MUT (46.33%)**	**MUT (47.22%)**
7290	Gefitinib	1	F	65	IV	Ex19del	SD	13	13	WT	WT	ND
7396	Gefitinib	1	F	57	IV	Ex19del	PR	12	14	WT	ND	ND
7507	Gefitinib	1	F	58	IIIB	L858R	SD	4	4	WT	WT	WT
7661	Gefitinib	1	F	53	IV	Ex19del	SD	8	15	WT	WT	WT
7791	Gefitinib	1	F	69	IIIB	Ex19del	SD	18	18	WT	ND	ND
7834	Gefitinib	1	M	75	IV	L858R	SD	16	16	WT	WT	WT
7877	Gefitinib	1	F	59	IV	Ex19del	PR	9	15	WT	WT	WT
8236	Gefitinib	1	F	65	IV	L858R	PR	12	12	WT	WT	ND
8470	Gefitinib	1	F	58	IV	L858R	SD	9	10	WT	WT	WT
8802	Gefitinib	1	F	62	IV	L858R	SD	36	44	WT	WT	WT
8956	Gefitinib	1	F	63	IV	L858R	SD	13	13	WT	ND	WT

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Patient ID	TKI	Treatment line	Gender	Age	Stage	EGFR mutation	Response (RECIST)	PFS/TTP (months)	OS (months)	AS-PCR	ddPCR (% of mutant DNA copies)	NGS (% of mutant reads)
9166	Gefitinib	1	F	71	IV	L858R	SD	7	7	WT	ND	WT
9329	Gefitinib	1	F	63	IV	Ex19del	PR	7	7	WT	WT	WT
9551	Gefitinib	1	F	59	IV	L858R	PR	8	8	WT	WT	WT

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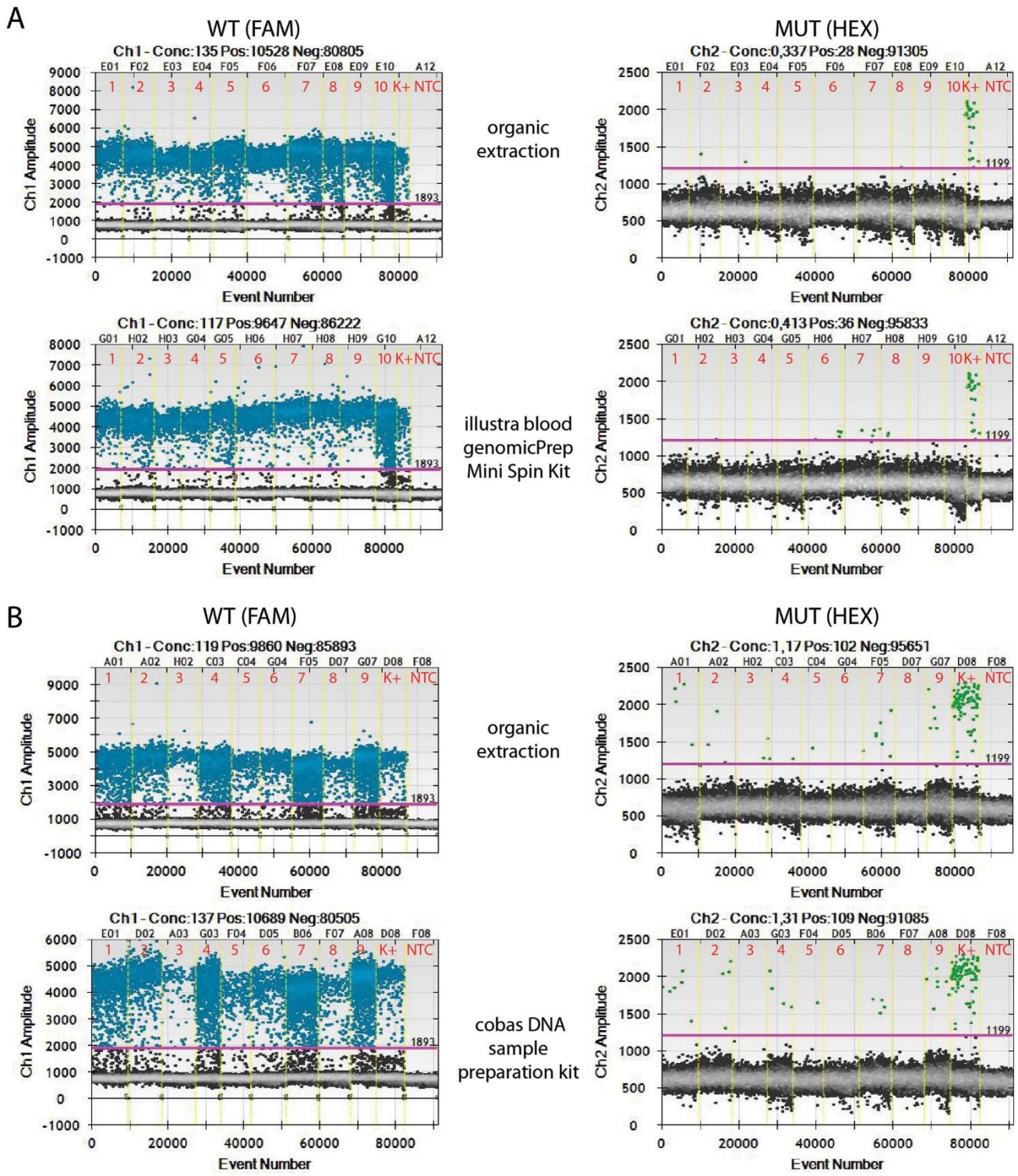
Supplemental figures



Supplemental Figure S1. Graphical representation of results of ddPCR and NGS T790M detection assays obtained for blood DNA samples from healthy donors (HD, n = 89) as well as FFPE-derived DNA specimens (non-lung (non-LC): n = 67; lung cancer (LC): n = 30 for ddPCR and n = 57 for NGS).

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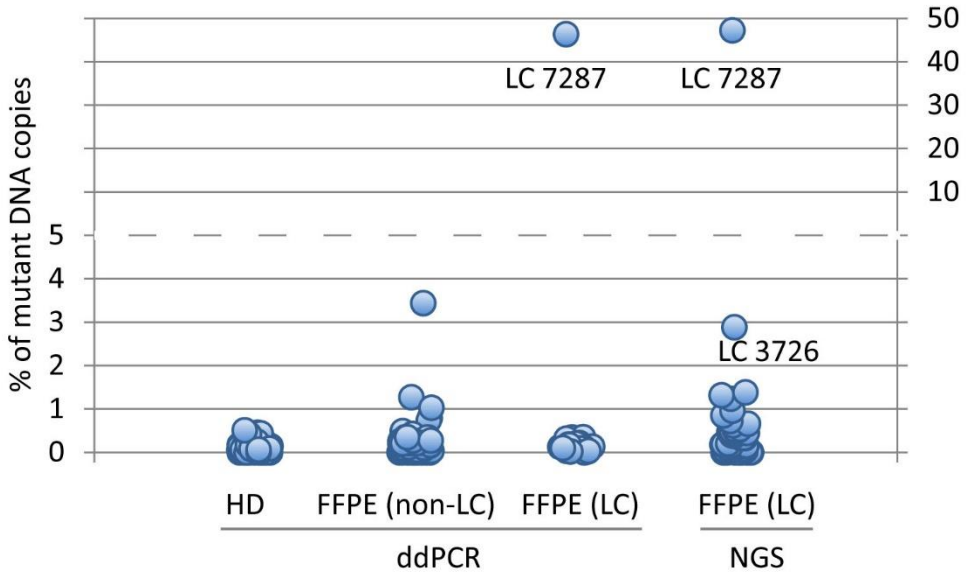

Supplemental Figure S2. DNA extraction method has no major influence on the rate of false-positive T790M signals in ddPCR assay, irrespectively whether the analysis involves blood-derived DNA (**A**) or FFPE specimens (**B**); wild-type-specific droplets have blue color (FAM channel), while mutation-positive signals are green (HEX channel); numbers in red designate tested samples, K+ corresponds to the mutation-positive control, NTC means no template control.

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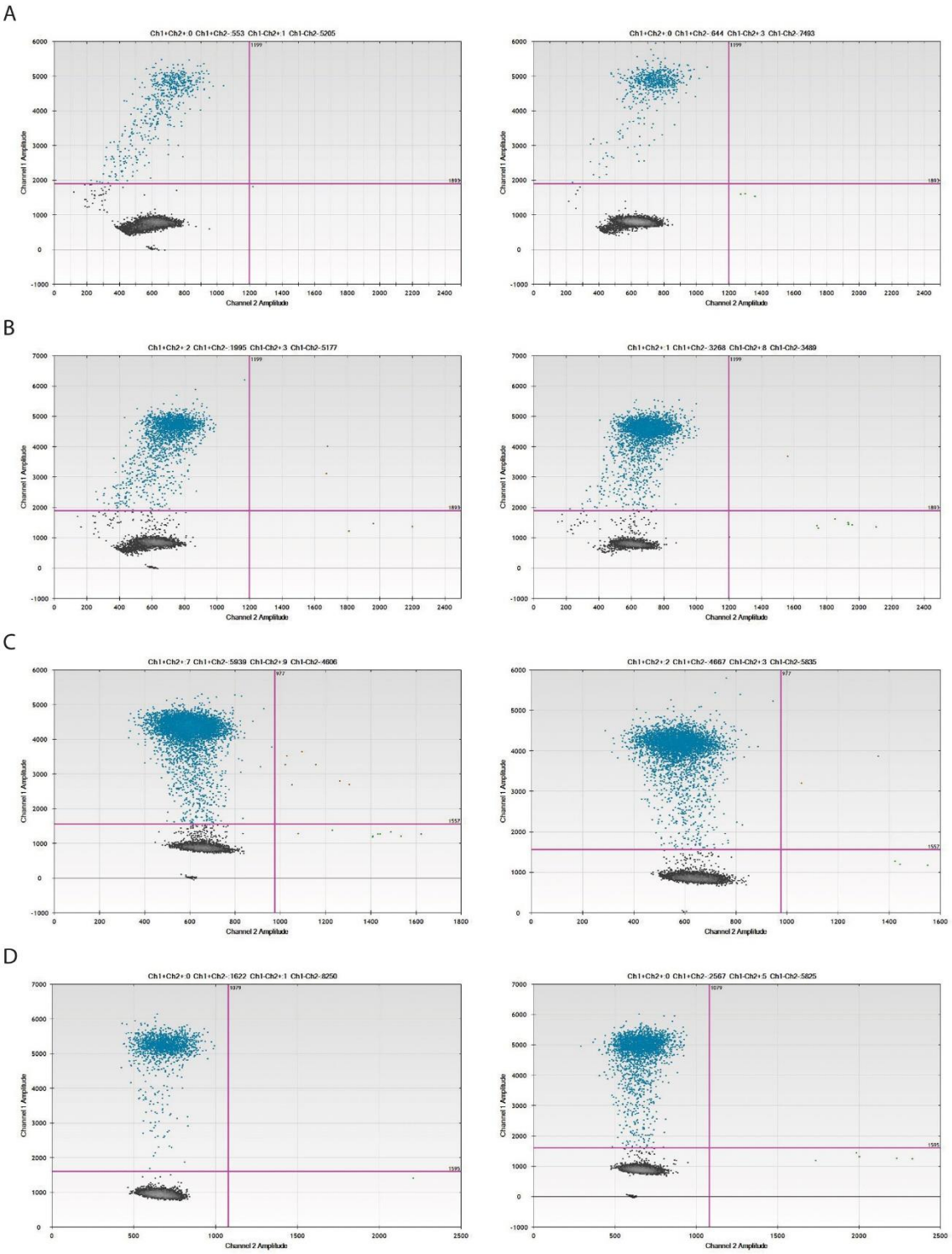

Supplemental Figure S3. Examples of ddPCR 2D-plots. **A**. DNA extracted from blood by organic extraction protocol (left) and the same sample extracted with GE Healthcare illustra blood genomicPrep Mini Spin Kit (right). **B**. DNA extracted from FFPE by organic extraction protocol (left) and the same sample extracted with Roche cobas® DNA Sample Preparation Kit (right). **C**. DNA sample extracted without (left) and with (right) UDG pretreatment. **D**. Paired samples from a lung cancer patient: DNA extracted from fresh-frozen (left) and FFPE (right) tissues.