

Standardization of the liquid biopsy for pediatric diffuse midline glioma using ddPCR

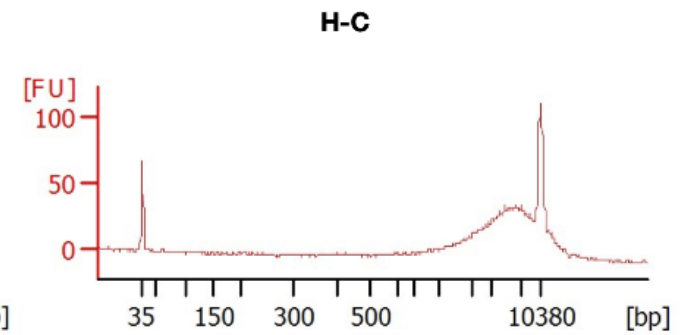
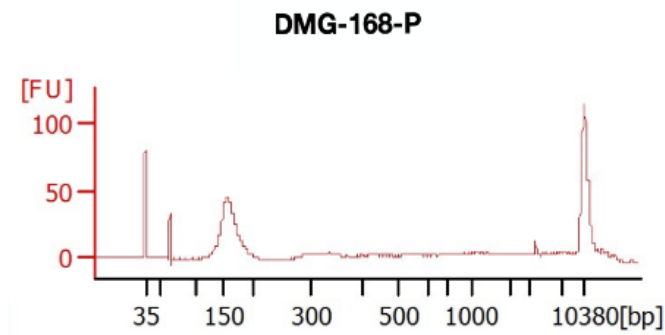
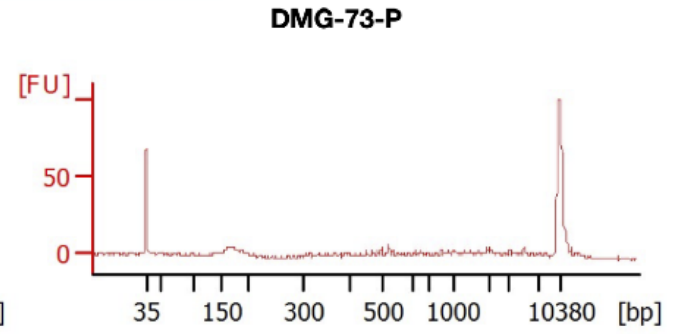
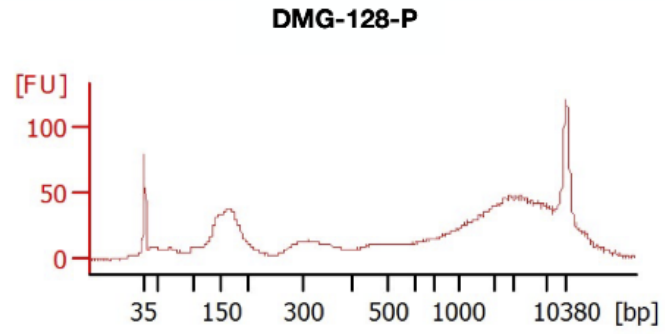
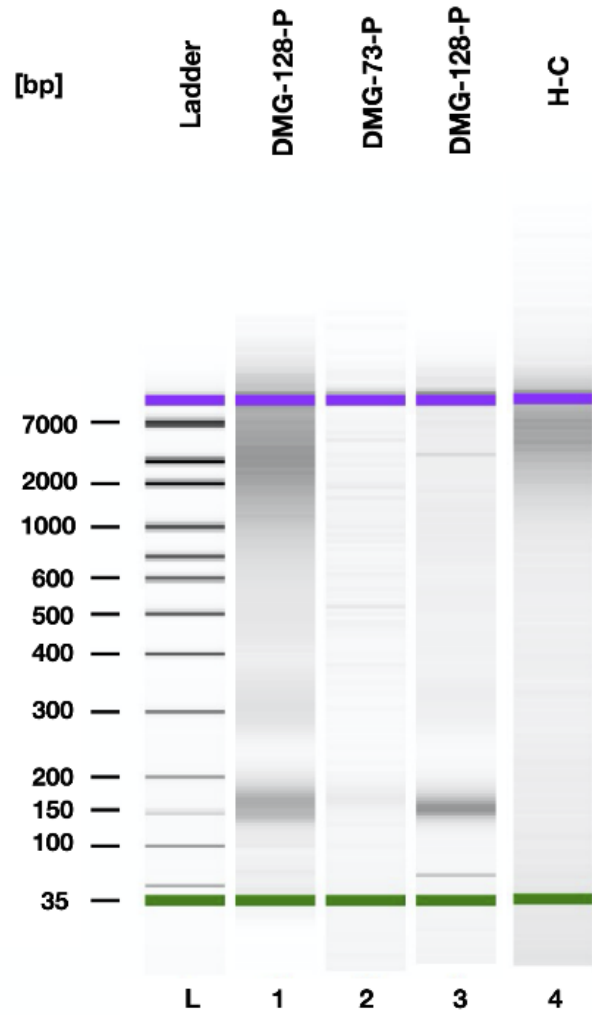
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SUPPLEMENTARY MATERIAL

Supplemental Figures: 6

Supplemental Tables: 2

SUPPLEMENTARY FIGURE 1

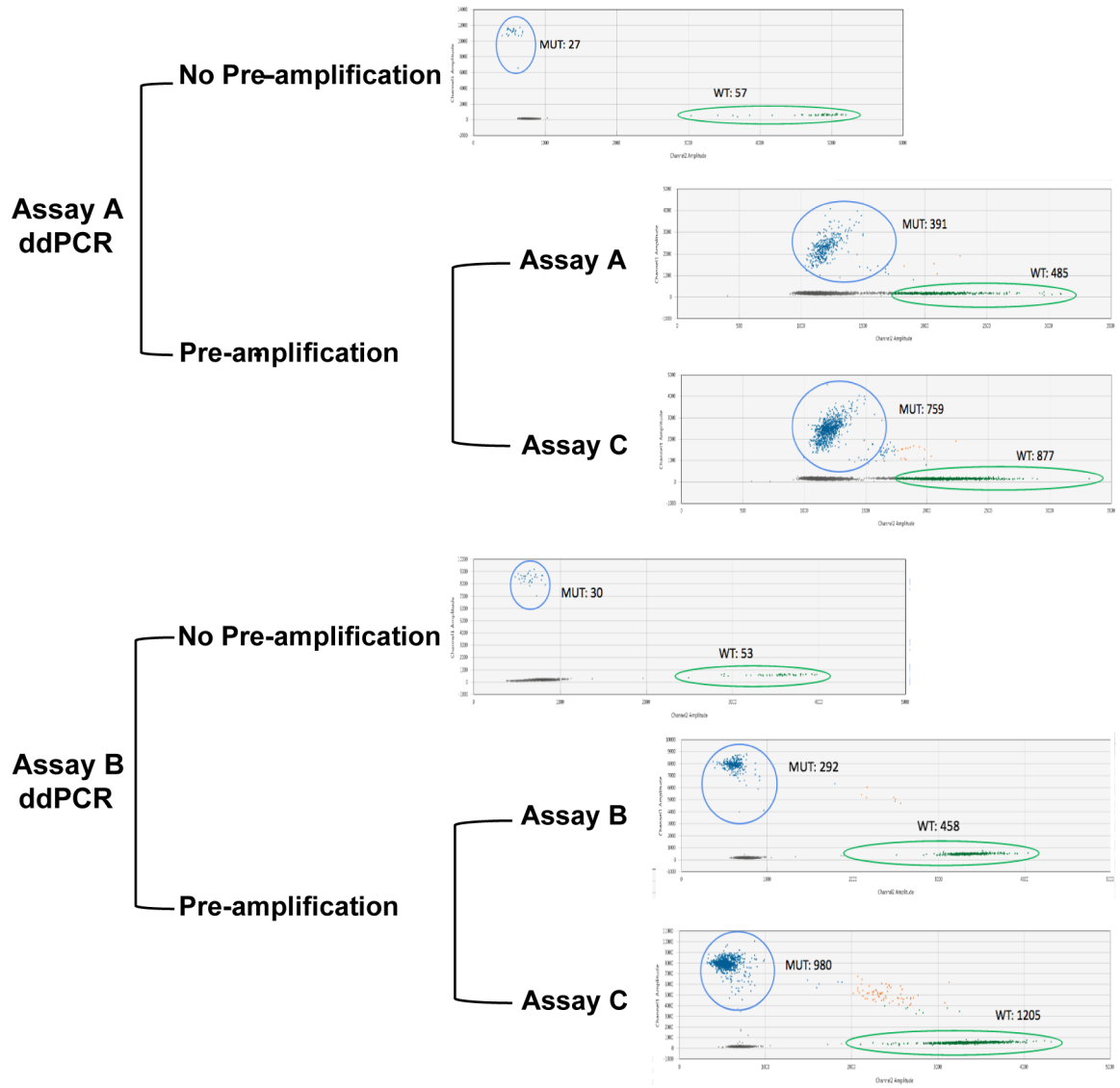


SUPPLEMENTARY FIGURE 2



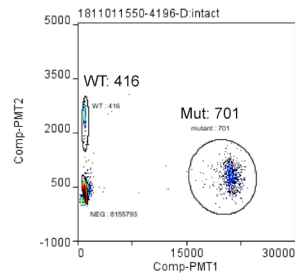
SUPPLEMENTARY FIGURE 3

A



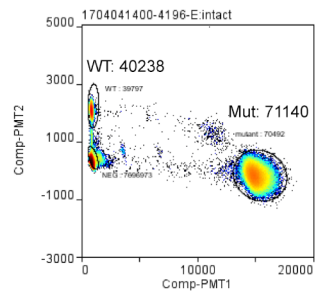
B

**No pre-amplification
Assay A**

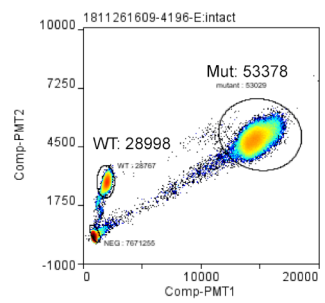


**Pre-amplification
Assay A**

**ddPCR
Assay A**



**ddPCR
Assay B**



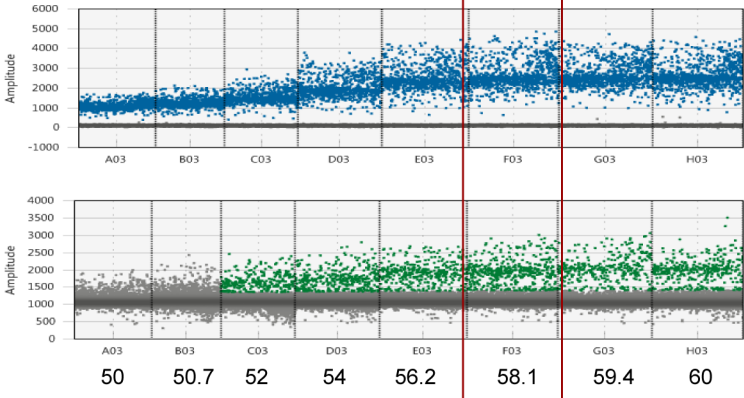
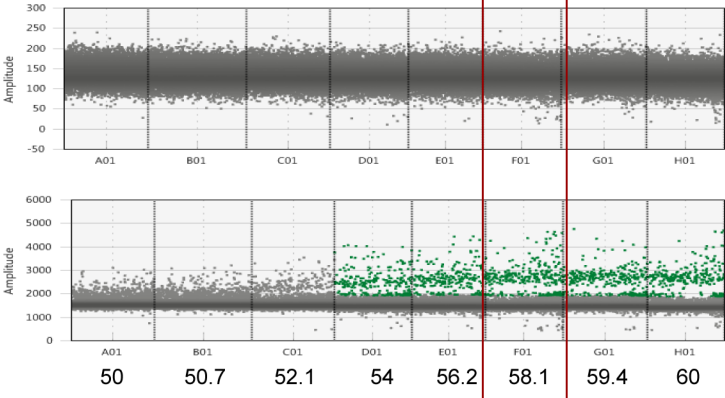
SUPPLEMENTARY FIGURE 4

A

KNS 42 (H3F3A WT)

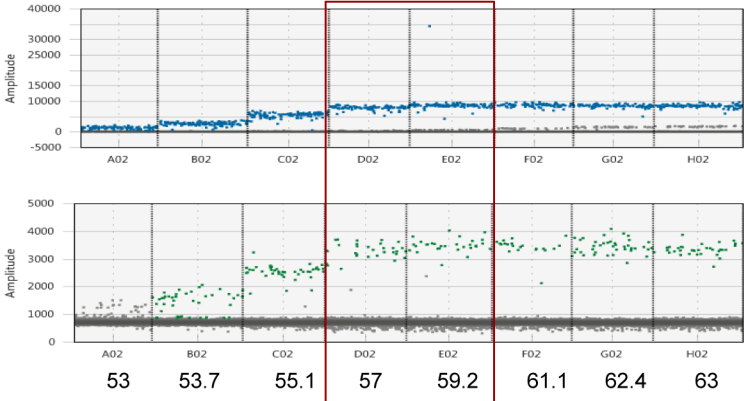
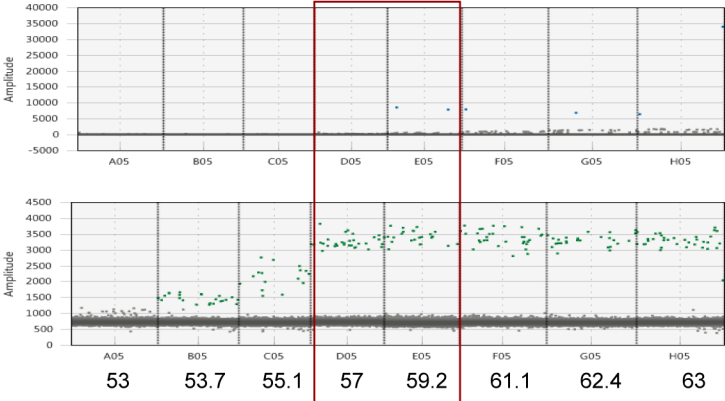
SF7761 (H3K27M)

Assay A

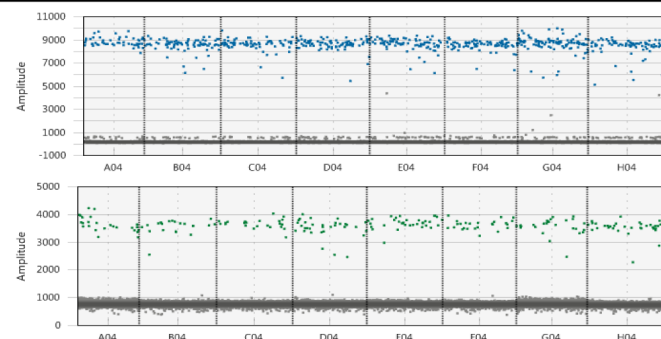
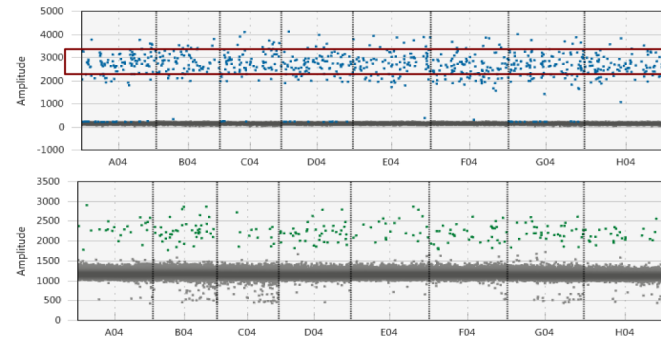
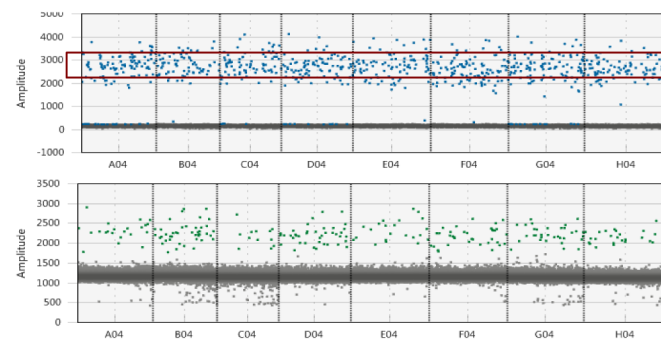
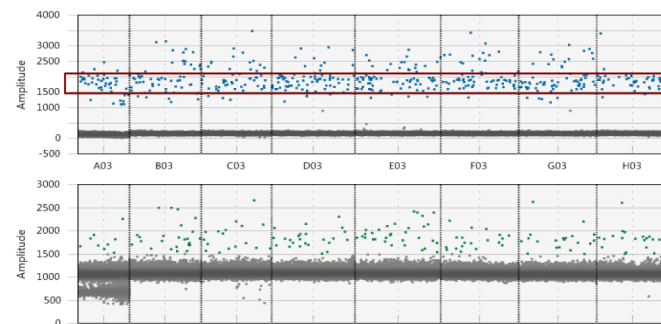
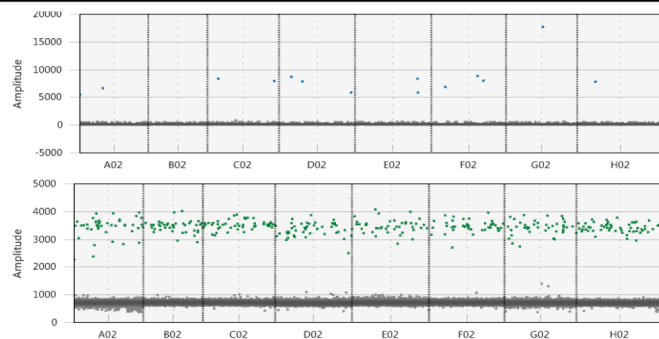
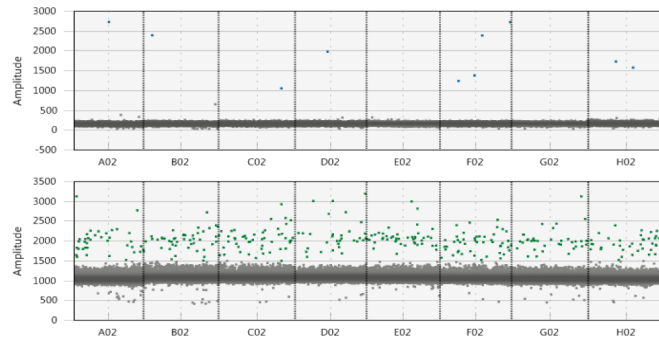
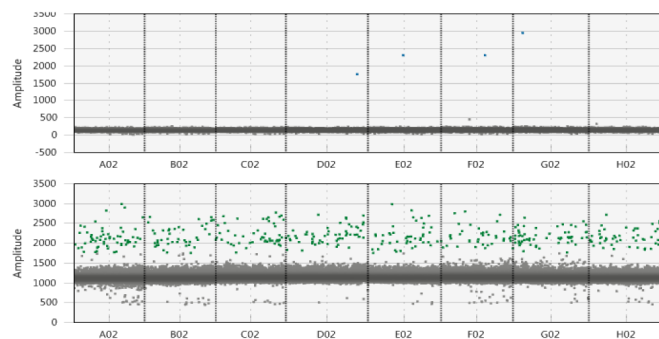
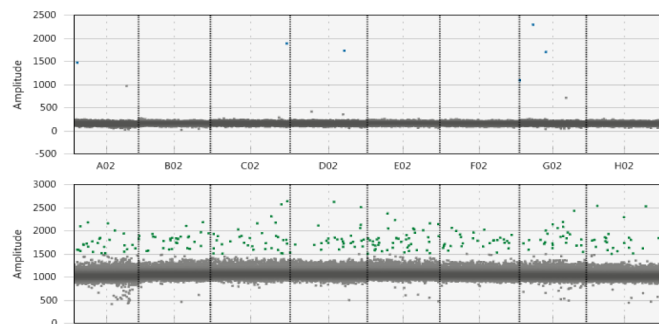


Temperature (°C):

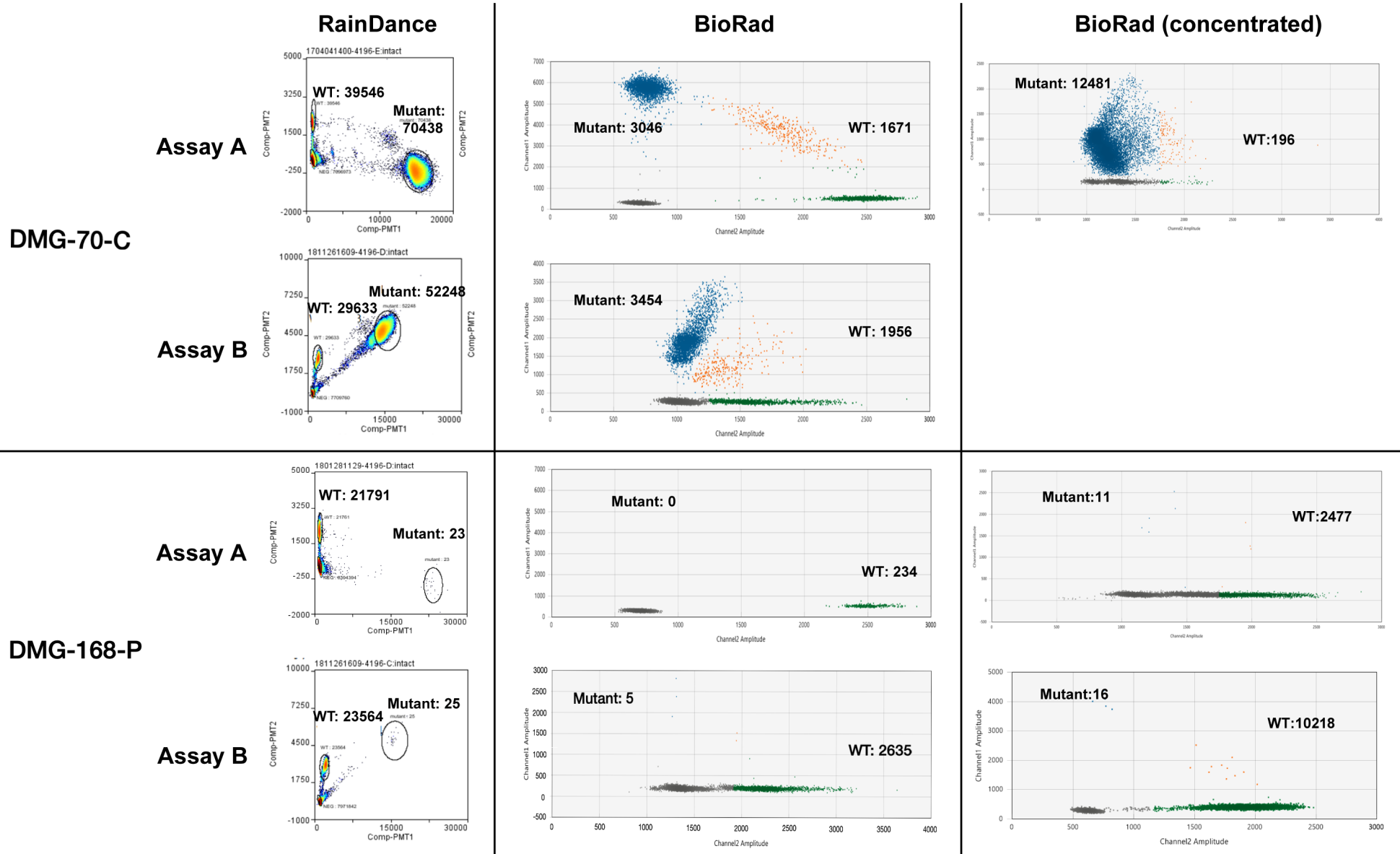
Assay B



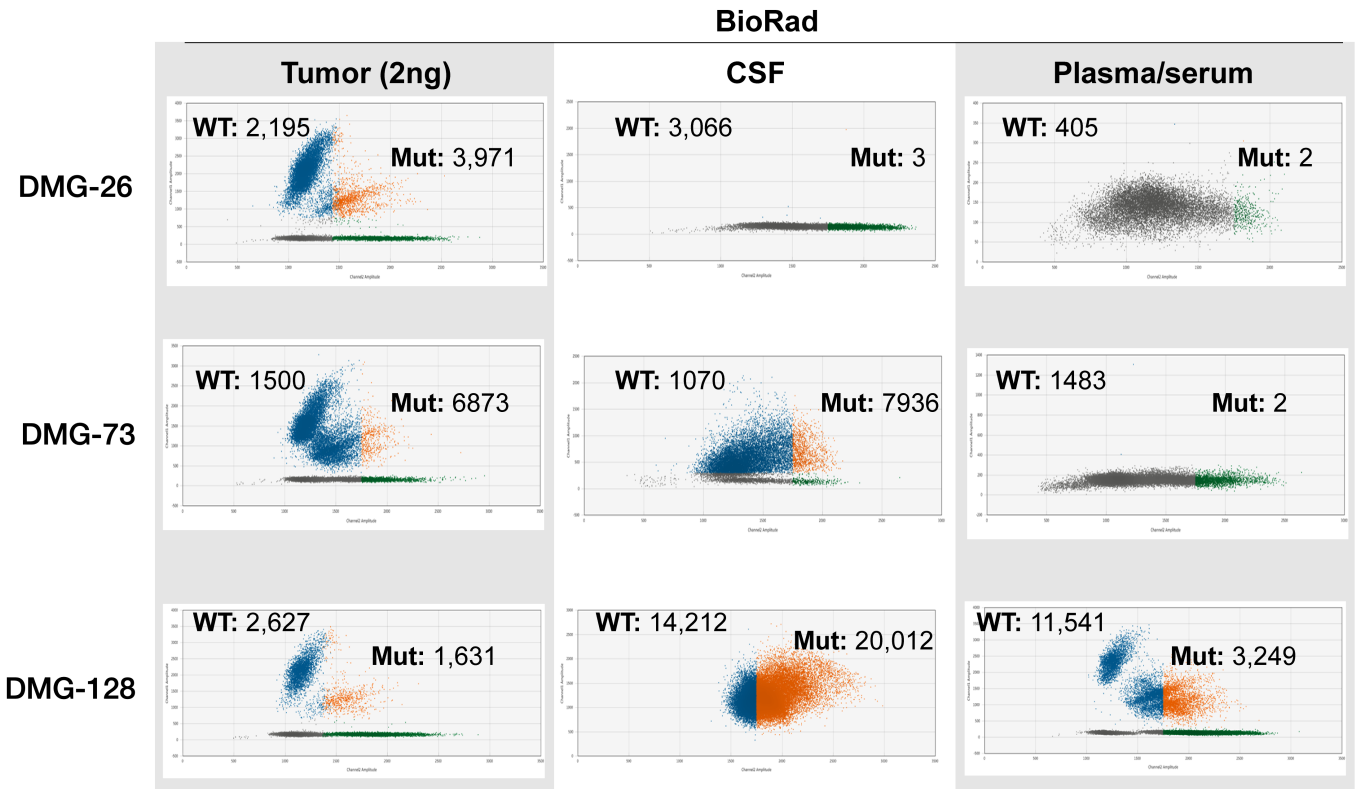
Temperature (°C):

B**KNS 42 (H3F3A WT)****SF7761 (H3K27M)****Assay A**
(40 cycles)+5
cycles**Assay A**
(45 cycles)+5
cycles**Assay A**
(50 cycles)**Assay B**
(40 cycles)

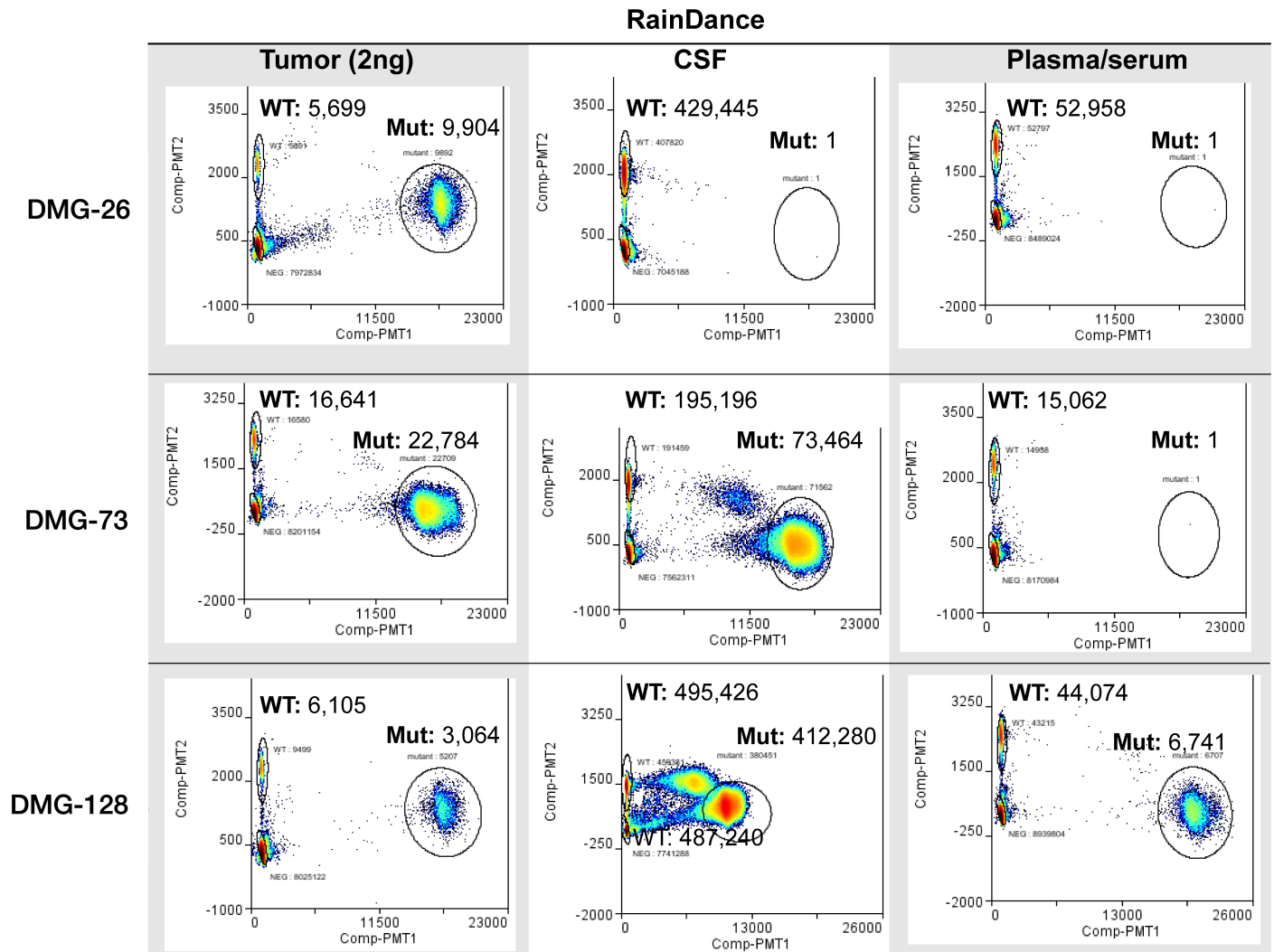
SUPPLEMENTARY FIGURE 5



A



B



Supplementary Table 1. Biological Specimens used. All samples de-identified and labeled as follows in subsequent figures.

Sample	Type	Time point of Collection	Diagnosis	H3K27 status
DMG-26	Tumor	Postmortem	DMG	H3.3K27M Mutant
	CSF	Postmortem		
	Serum	During treatment		
DMG-73	Tumor	Postmortem	DMG	H3.3K27M Mutant
	CSF	Postmortem		
	Plasma	During treatment		
DMG-128	Tumor	Postmortem	DMG	H3.3K27M Mutant
	CSF	Postmortem		
	Plasma	Postmortem		
DMG-51-T	Tumor	Postmortem	DMG	H3.3K27M Mutant
DMG-1-C	CSF	During treatment	DMG	H3.3K27M Mutant
DMG-70-C	CSF	During treatment	DMG	H3.3K27M Mutant
DMG-168-P	Plasma	During treatment	DMG	H3.3K27M Mutant
SF 7761	Cell line	n/a	DMG	H3.3K27M Mutant
KNS 42	Cell line	n/a	Supratentorial GBM	Wild-Type
H-C	CSF	During treatment	Hydrocephalus	Wild-Type

Abbreviations: CSF = cerebrospinal fluid, DMG = diffuse midline glioma, GBM = glioblastoma multiforme

Supplementary Tables 2A-D. Cumulative ddPCR data generated through ddPCR analysis on BioRad Q200x and RainDance platforms. Abbreviations: avg = average, C =

Cerebrospinal fluid, M = positive mutant droplet count, MAF = mutant-allelic frequency, P = plasma, P/A = pre-amplification, S = serum, stdev = standard deviation, T = tumor, V/C = vacuum concentration, WT = positive wildtype droplet count

A: Data generated for Figure 1A-C. ddPCR analysis of CSF ctDNA with and without pre-amplification

Figure	Specimen	Assay	Conditions	BioRad				RainDance					
				M	WT	MAF (%)	Avg MAF (%± stdev)	M	WT	MAF (%)	Avg MAF (%± stdev)		
1A	Blank	A	No P/A	0	0	0	0±0						
				0	0	0							
				0	0	0							
	H-C			No P/A	0	3	0	0±0					
					0	18	0						
					0	15	0						
	DMG-1-C				No P/A	29	55	34.52	37.86±3.14				
						29	42	40.85					
						36	55	39.56					
			27			42	39.13						
			30			52	36.59						
			27			57	32.14						
			40	59		40.40							
	29		44	39.73									
	Blank		P/A with assay A	0		0	0	0±0					
				0	0	0							
				0	2	0							
	H-C			P/A with assay A	0	167	0	0±0					
					0	188	0						
					0	183	0						
					0	176	0						
DMG-1-C	P/A with assay A	373			464	44.56	44.85±1.69						
		358			434	45.20							
		391	385		44.63								
		488	595		45.06								
		336	425		44.15								
		363	503	41.92									
		393	476	45.22									
451		487	48.08										
Blank		P/A with assay C	0	0	0	0±0							
	0		0	0									
	0		2	0									
H-C	P/A with assay C		0	246	0	0±0							
			0	264	0								
			0	223	0								
			0	228	0								
DMG-1-C			P/A with assay C	852	1029	45.30	46.66±0.77						
				897	976	47.89							
		781		892	46.68								
		893		1015	46.80								
		670		746	47.32								
	874	1018		46.19									
	857	979		46.68									
759	877	46.39											
1B	Blank	B		No P/A	0	0	0	0±0					
				0	0	0							
				0	0	0							

				0	0	0					
	H-C			0	13	0	0±0				
				0	19	0					
				0	27	0					
				0	20	0					
				0	0	0					
	DMG-1-C			24	53	31.17	35.82±5.69				
				19	49	27.94					
				30	53	36.14					
				27	40	40.30					
				39	51	43.33					
				35	50	41.18					
				32	55	36.78					
				36	85	29.75					
	Blank			0	1	0	0±0				
				0	0	0					
				0	0	0					
				0	0	0					
	H-C			2	146	1.35	0.70±0.68				
				0	150	0					
				1	135	0.74					
	DMG-1-C		P/A with assay B	346	512	40.33	39.08±0.95				
				292	458	38.93					
				397	633	38.54					
				359	543	39.80					
				366	568	39.19					
				312	522	37.41					
				316	475	39.95					
				343	549	38.45					
	Blank			0	0	0	0±0				
				0	0	0					
				0	0	0					
				0	0	0					
	H-C			0	111	0	0.25±0.49				
				0	95	0					
				0	104	0					
				1	101	0					
	DMG-1-C		P/A with assay C	818	1021	44.48	44.29±2.03				
				799	1069	42.77					
				884	1080	45.01					
				980	1025	48.88					
				825	1115	42.53					
				778	1013	43.44					
				906	1152	44.02					
				942	1238	43.21					
1C	DMG-70-C	A	No P/A					701	416	62.76	61.73±1.73
								577	389	59.73	
								528	314	62.71	
		A	P/A with assay A					70438	39546	64.04	64.44±0.56
								69026	37542	64.83	
		B	P/A with assay A					52589	29866	63.78	
						52573	29095	64.37	64.17±0.34		
						65434	36228	64.36			

B: Data generated for Figure 2A and B. Optimization of ctDNA detection across technical platforms and institutions

Figure	Specimen	Assay	Conditions	BioRad				RainDance			
				M	WT	MAF (%)	Avg MAF (%± stdev)	M	WT	MAF (%)	Avg MAF (%± stdev)
2A	DMG-70-C	A	P/A, 12uL					70438	39546	64.04	64.44±0.56

								69026	37542	64.83	
		B	P/A, 12uL					52589 52573 65434	29866 29095 36228	63.78 64.37 64.36	64.17±0.34
		A	P/A, 1uL, no V/C	3046 3101	1671 1701	64.57 64.58	64.58±1.64E ⁻⁵				
		B	P/A, 1uL, no V/C	3454 2844	1956 1630	63.84 63.57	63.70±0.19				
2B	DMG-168- P	A	P/A, 12uL					23 24 20	21791 23166 21477	0.11 0.10 0.09	0.10±0.01
			P/A, 1uL, no V/C	1 13 5	1220 2931 2635	0.08 0.44 0.19	0.24±0.18				
			P/A, 1uL, V/C	10 11	2477 1362	0.40 0.80	0.60±0.28				
		B	P/A, 12uL					29 18 25	30637 22040 23598	0.095 0.082 0.106	0.09±0.01
			P/A, 1uL, no V/C	0 0	254 268	0 0	0±0				
			P/A, 1uL, V/C	16 13	10218 10448	0.16 0.12	0.14±0.03				

C: Data generated for Figure 3. H3.3K27M mutant DNA is detectable in patient-matched tumor tissue, CSF and plasma or serum specimens. All specimens were pre-amplified and analyzed using Assay A primers and/or probes.

Specimen	T/C/S/P	BioRad (V/C)				RainDance			
		M	WT	MAF (%)	Avg MAF (%± stdev)	M	WT	MAF (%)	Avg MAF (%± stdev)
DMG-26	T	3255 3843 3971	1618 1999 2195	66.80 65.78 64.40	65.66±1.20	9709 9904	5668 5699	63.14 63.47	63.31±2.37
	C	2 3	1215 3066	0.16 0.10	0.13±0.05	1 0	418466 429445	0.0002 0	0.0001±0
	S	0 0	405 14	0 0	0±0	0 1 0	51230 52958 50727	0 0.002 0	0.0006±0
DMG-73	T	6231 6873	937 1500	86.93 82.09	84.51±3.42	19724 22784	14351 16641	57.88 57.80	57.84±0.07
	C	4844 7936	344 1070	93.37 88.12	90.74±3.71	72042 73464	190741 195196	27.42 27.35	27.38±0.05
	P	0 2 0	518 1483 225	0 0.13 0	0.04±0.08	0 1 1	14683 14971 15062	0 0.007 0.007	0.004±0.004
DMG-128	T	1340 1437 1631	2242 2317 2627	37.41 38.28 38.30	38.00±0.51	3064 3046	6105 5554	33.42 35.42	34.42±1.42
	C	20012 18686	13094 14212	60.45 56.80	58.62±2.58	412280 396924	495426 479054	45.42 45.31	45.37±0.08
	P	2868 3159 3249	10213 9900 11541	21.92 24.19 21.97	22.69±1.30	6317 6741 6718	41542 43350 44074	13.20 13.46 13.23	13.29±0.14

D: Data generated for Figure 4A and B. ddPCR results are consistent across institutions, irrespective of specimen preparation location. All specimens were pre-amplified with Assay C primers.

Figure	Specimen	Assay	BioRad				RainDance			
			M	WT	MAF (%)	Avg MAF (%± stdev)	M	WT	MAF (%)	Avg MAF (%± stdev)
4A	SF7761	A	465	142	76.61	77.45±1.92	942	298	75.97	75.00±1.38
			542	134	80.18		852	299	74.02	
				562	180	75.74				
			820	241	77.29					
		B	134	21	86.45	83.90±3.60	933	299	75.73	75.73±0
			144	33	81.36					
4B	DMG-1-C	A	335	415	44.67	43.62±0.98	1142	1708	40.07	41.17±1.55
			377	496	43.18		986	1347	42.26	
				381	482	44.15				
			409	554	42.47					
		B	176	204	46.32	45.02±1.83	1013	1389	42.17	41.05±0.97
			171	220	43.73		1046	1540	40.45	
							961	1410	40.53	