cancers

MDPI

Supplementary Material

Design and Validation of a Gene-Targeted, Next-Generation Sequencing Panel for Routine Diagnosis in Gliomas

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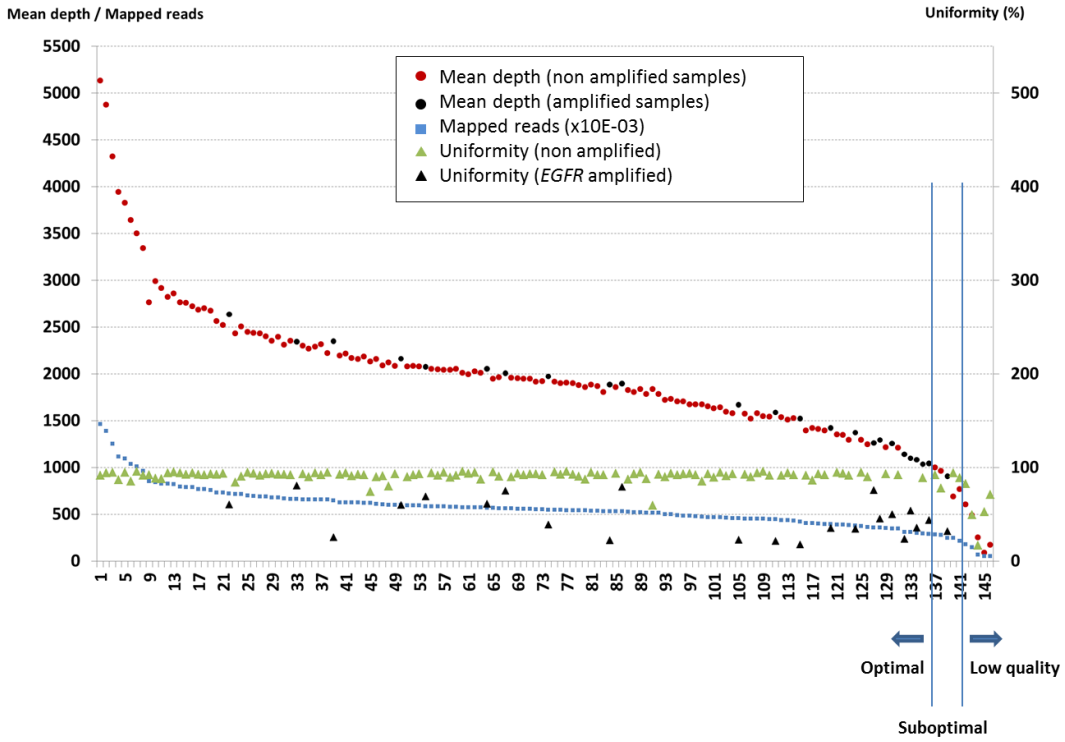

Figure S1. Graphical representation, for each sample, of the mean depth, mapped reads, and uniformity obtained in the NGS assays. Squares indicate the number of mapped reads, dots indicate the mean depth, and triangles indicate percentage of uniformity (note that in order to represent two of the variables under the same scale, the real mapped read values are 1000 times more than what is specified in the y-axis). The different colors are used to distinguish samples with *EGFR* amplification (black).

Figure S2. Visualization using Integrative Genomic Viewer of the AcroMetrix engineered DNA and a *PTEN* mutant sample. The green bars represent the length of the whole amplicons (insert and primers), while the blue bars above them represent the insert amplicons without the primers. The position of the described AcroMetrix mutations in the region are indicated by the blue squares and numbered 1 to 5. As shown, the variants 1 and 2 are located in the primer sequence of the right amplicon, and interfere with its amplification and the detection of variants 3, 4, and 5. Similarly, variant 3 is located in the primer sequence of the left amplicon, and impedes the amplification and detection of variants 1 and 2 that are located in the amplicon.

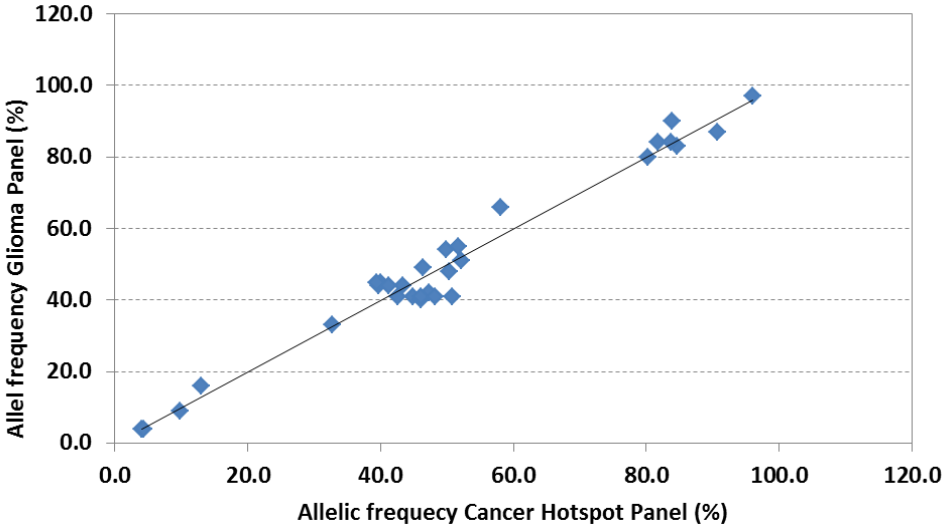

Figure S3. Comparative analyses of the allelic frequencies obtained with the cancer hotspot and glioma NGS panels. Allelic frequencies of 33 mutations from 22 glioma samples that were assessed for mutation detection with the CHP and glioma panels showed a high correlation between both panels ($R = 0.99$), even for those variants with an AF close to the limit of detection (4%).

Table S1. Validation of the NGS 1p/19q codeletion detection by fluorescence in situ hybridization (FISH).

NGS LOH Patterns	FISH Results				
	1p/19q Codeletion	No Codeletion	1p36 del	19q del	Total
1p/19q LOH	21	0	0	0	21
No LOH	1	18	1	0	20
LOH 1q	0	0	3	0	3
LOH 19q	0	0	0	5	5
Whole chr 1 LOH	0	2	0	0	2
Whole chr 19 LOH	0	6	1	0	7
Other patterns	0	2	0	0	2
Noninformative	0	3	1	1	5
Total	22	31	6	6	

Table S2. Summary of samples and patients included in the study.

		Samples			Patients		
Subtype	**WHO Grade**	**Total**	**Validation**	**Prospective**	**Total**	**Validation**	**Prospective**
Pilocytic	I	8	1	7	8	1	7
Oligodendroglioma IDH mutant, 1p/19q codeleted	II	15	11	4	14	10	4
	III	10	6	4	9	6	3
Astrocytoma, IDH mutant	II	14	6	8	11	4	7
	III–IV	17	7	10	14	7	7
Astrocytoma, IDH wildtype	II	8	0	8	5	0	5
	III–IV	52	16	36	50	14	36
Glioblastoma-H3.3 K27M mutant	IV	5	3	2	5	3	2
Other diagnosis		10	1	9	9	1	8
Glioneuronal tumor		3	1	2	2	1	1
Subependymal giant cell astrocytoma		1	0	1	1	0	1
Tumor vs non tumor		5	0	5	5	0	5
Undifferentiated tumor		1	0	1	1	0	1
Non classified*		4	1	3	4	1	3
Total		143	52	91	129	47	82

*These cases could not be classified according to the 2016 WHO, due to inconclusive NGS results.

Table S3. SNPs used in the 1p/19q LOH analysis.

Chromosome arm	Start*	SNP
Chr 1p	16112795	rs7663
	19683301	rs169957
	23210600	rs309481
	26213991	rs159525
	29245406	rs157208
	36168038	rs6425953
	40306898	rs7315
	45976472	rs7903
	53307957	rs504816
	55316322	rs7374
	60594980	rs87061
	65952428	rs11811946
	71477315	rs5680
	76990862	rs191142
	88776278	rs54396
	91604522	rs106075
	95394352	rs1132
	101338324	rs8888
	109289487	rs6604120
	115110683	rs8128
Chr 1q	156030820	rs2275073
	162301446	rs347303
	170198294	rs4575136
	181286871	rs898114
	189241655	rs1342566
	195608036	rs12744553
	204839154	rs2802849
	210951585	rs1770214
	219383192	rs6692892
	228876199	rs16848862
	156030820	rs2275073
	162301446	rs347303
	170198294	rs4575136
	181286871	rs898114
	189241655	rs1342566

Chromosome arm	Start*	SNP
Chr 19p	751553	rs13345388
	2720281	rs7256720
	5063649	rs36115836
	6818628	rs164020
	8990761	rs57167556
	10265248	rs2114724
	12505873	rs7246440
	15120104	rs10419689
	16842052	rs8107776
	18108956	rs4808732
Chr 19q	30106659	rs7283
	31883906	rs2542297
	34011248	rs33841
	35615179	rs12852
	38229378	rs1291
	39926509	rs17628
	40931717	rs166539
	44090195	rs3817
	47112648	rs10113
	48833800	rs8355
	51359497	rs11573
	53073605	rs193040
	53611187	rs3814
	56030428	rs10217
	59093239	rs10448

*Start position according to GRCh37 (hg19).