Supplementary Material

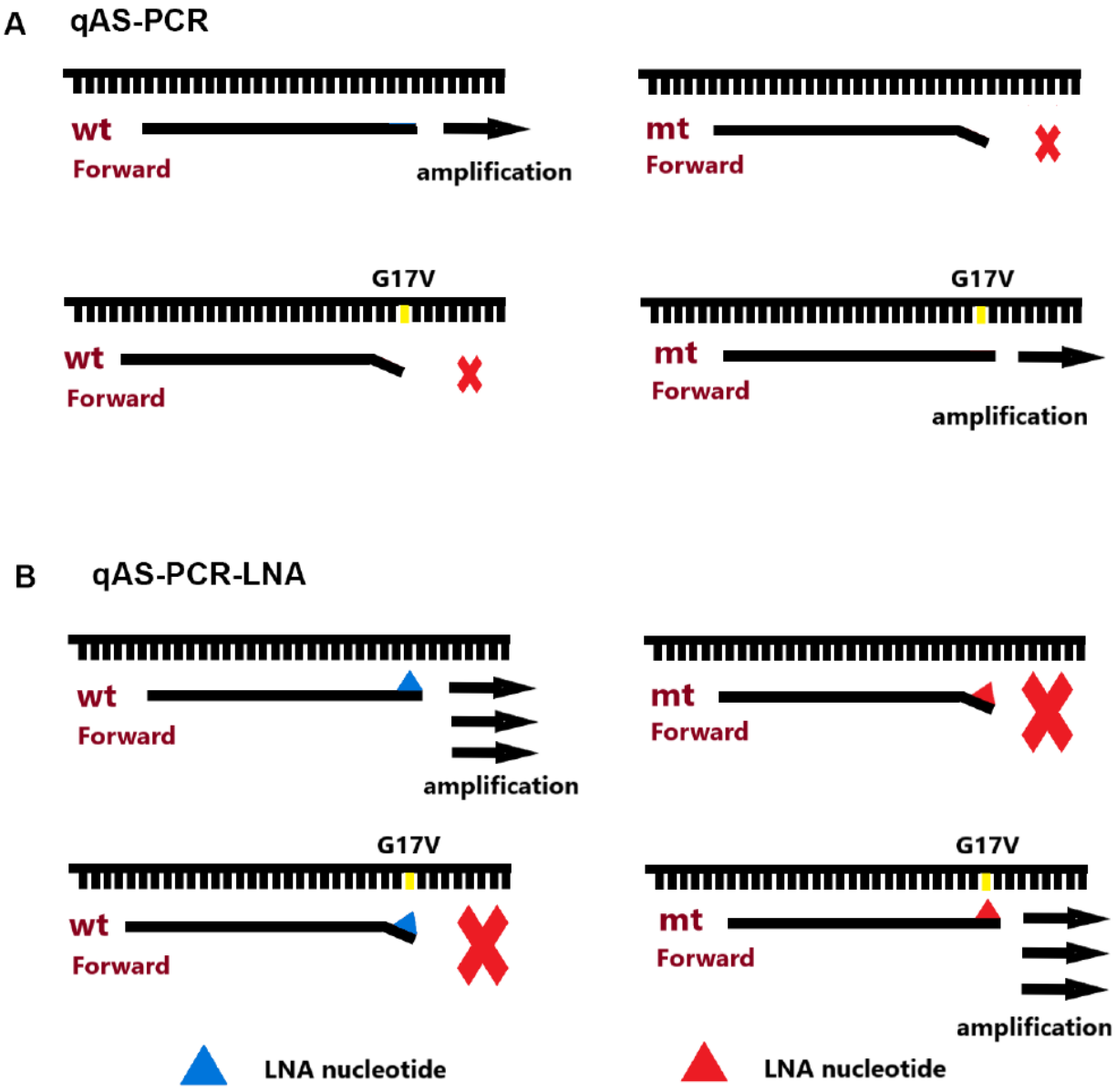

Figure 1: Schematic representation of allele-specific PCR with conventional primers (**A**) and LNA-modified (**B**).

A. Amplification with two types of allele-specific primers –wt (wild type) and mt (mutant type). In the absence of nucleotide replacement wt primer is working much better than mt primer. Conversely, in the case of mutated DNA.

B. LNA-modification of the nucleotide at the 3' end of allele-specific primer allows to increase the stability of bond between nucleotides therefore enhancing the specific and reducing non-specific amplification.

Table 1: Percentage of Cells with RHOA Gly17Val Mutation at Initial Diagnosis in Different Tissues of 32 Patients Positive for RHOA Gly17Val. Bone Marrow Involvement Estimated According to Histological Data. Samples with T-cell Monoclones are Highlited. LN –Lymph Nodes, BM -Bone Marrow, PB -Peripheral Blood."nd" –no Data

# case	LN	Blood	BM	Skin	BM involvement
1	1	0.06	nd	nd	+
2	10	0.8	0.45	nd	-
3	7	0.4	0.06	nd	+
4	9	nd	0.4	nd	+
5	8.2	nd	0.03	nd	+
6	0.7	0.06	0.05	nd	-
7	2.1	nd	nd	nd	nd
8	4.12	1.6	0.07	nd	+
9	nd	0.04	0.5	0.3	-
10	25	10.1	5.7	nd	+
11	2	0.07	0.03	nd	-
12	10.2	0.8	0.4	0.22	+
13	1.27	0.34	0.24	nd	+
14	nd	nd	0.8	nd	+
15	29	19	12	nd	+
16	20	nd	0.05	2	-
17	3.85	nd	nd	nd	nd
18	7.18	0.05	0.05	nd	-
19	2.7	nd	0.05	1.9	-
20	8.8	0.42	0.63	nd	+
21	14.35	1.2	0.6	4.4	-
22	4.7	nd	0.2	4.7	+
23	6.5	0.42	0.55	nd	+
24	2.2	nd	nd	nd	+

25	0.5	0.1	neg (≤0.02)	nd	+
26	4.2	nd	neg (≤0.02)	nd	+
27	4.12	1.6	nd	nd	+
28	1.92	0.4	0.13	0.5	-
29	nd	0.12	0.34	nd	+
30	1.5	0.2	0.05	nd	-
31	2.5	0.15	nd	nd	-
32	47	12.5	2	19	+
Number of positive samples / total	29/29	22/22	24/26	8/8	19/30
Average (%)	8.33	2.3	0.98	4.12	-
Min-max (%)	0.5-47	0.04-19	0.02-12	0.22-19	-

Table 3: Results of MRD Monitoring of RHOA Gly17Val Mutation during the Course of Therapy in Blood. *-The Presence of CD4 + CD10 + Lymphocytes in the Blood Shown by flow Cytometry

Case #	Before trearment	6 months	1 year	1,5 year	2 year	2,5 year	3 year	3,5 year	4 year	4,5 year	5 year
1	0,8	0,5	0,1	0,16	0,32	1,1	-	-	-	-	-
2	0,4	≤0,02	0,04	≤0,02	-	-	-	-	-	-	-
3	0,06	≤0,02	≤0,02	≤0,02	≤0,02	≤0,02	-	-	-	-	-
4	0,04	0,09	0,5	0,05	0,05	-	0,1	-	0,3	-	-
5	0,05	0,06	≤0,02	0,04	0,04	0,05	0,1	-	-	-	-
6	0,42	≤0,02	-	-	-	-	-	-	-	-	-
7	1,2	0,78	0,16	-	0,21	0,9	1	-	-	-	-
8	1,6	0,07	0,1	-	0,08	0,1	0,05	-	0,2*	-	0,2
9	0,2	≤0,02	-	-	-	-	-	-	-	-	-
10	1,4	0,08	0,15*	-	-	-	-	-	-	-	-
11	0,4	0,08	0,04	-	-	-	-	-	-	-	-
12	0,07	0,05	-	-	-	-	-	-	-	-	-
13	10,1	0,8	0,27	-	-	-	-	-	-	-	-
14	19	0,2	0,34	0,04	-	-	-	-	-	-	-
15	0,3	0,16	≤0,02	-	-	-	-	-	-	-	-
16	0,42	0,4	0,2	-	-	-	-	-	-	-	-

Table 4: Results of MRD Monitoring of RHOA Gly17Val Mutation during the Course of Therapy in Bone Marrow

Case #	Before trearment	6 months	1 year	1,5 year	2 year	2,5 year	3 year
1	0,45	0,55	0,06	0,07	0,23	1,2	-
2	0,06	≤0,02	≤0,02	≤0,02	≤0,02	≤0,02	-
3	0,05	≤0,02	≤0,02	≤0,02	-	-	-
4	0,03	0,06	≤0,02	-	≤0,02	-	0,03
5	0,05	≤0,02	≤0,02	0,05	0,11	-	-
6	0,63	≤0,02	-	-	-	-	-
7	0,6	0,15	0,08	0,09	0,2	-	-
8	0,2	0,1	≤0,02	≤0,02	≤0,02	0,1	0,17
9	0,05	≤0,02	-	-	-	-	-
10	0,36	0,04	0,3	-	-	-	-
11	0,13	0,16	0,2	-	-	-	-
12	0,03	0,019	-	-	-	-	-
13	5,7	0,1	0,08	-	-	-	-
14	12	0,11	0,03	-	-	-	-
15	0,2	0,175	0,04	≤0,02	-	-	-
16	0,55	0,2	0,1	-	-	-	-