

Additional File 7:

rUN Blocked Primer	Template Sequence				
		A	C	G	T
	A	11.4	2.5	12.2	0
	C	6.4	10.4	0	9.0
	G	13.8	0	4.6	3.0
	T	0	11.1	11.9	2.9

rCN Blocked Primer	Template Sequence				
		A	C	G	T
	A	5.6	1.8	10.2	0
	C	8.8	9.6	0	8.6
	G	9.8	0	3.2	0.3
	T	0	2.1	0.2	0

rAN Blocked Primer	Template Sequence				
		A	C	G	T
	A	3.1	1.0	6.1	0
	C	9.3	10.2	0	8.3
	G	13.2	0	2.5	5.9
	T	0	5.0	7.1	4.0

rGN Blocked Primer	Template Sequence				
		A	C	G	T
	A	6.2	3.0	11.4	0
	C	9.5	7.3	0	4.7
	G	13.1	0	6.0	3.2
	T	0	4.5	11.5	0.3

Figure S5. Mismatch discrimination using rhPCR with the mismatch positioned at the “+1” position relative to the RNA base.

Sixteen synthetic oligonucleotide targets were employed where the base complementary to the single RNA residue in the blocked-cleavable primers was fixed (A, C, G, or T) and the base paired opposite position “+1” immediately 5′-to the RNA base in the primer was varied (A, C, G, or T). Likewise a set of 16 “rDDDDx” blocked-cleavable primers was employed where the RNA base was fixed (rA, rC, rG, or rU) and the base at the “+1” position was varied (A, C, G, or T). The target sequence and primers were otherwise the same as in Figure 2. Assay conditions and calculations of ΔC_q values were the same as in Figure 7 in the manuscript. All reactions were run in triplicate.