Additional File 6:

NrU Blocked Primer

Template Sequence

	A	C	G	T
A	16.1	8.7	12.6	0
C	7.6	3.9	0	12.0
G	13.8	0	12.4	5.9
T	0	5.2	2.4	6.2

NrC Blocked Primer

Template Sequence

	A	C	G	T
A	13.0	8.2	10.5	0
C	5.0	3.3	0	3.5
G	8.3	0	7.0	0.8
T	0	5.4	2.1	4.6

NrA Blocked Primer

Template Sequence

	A	C	G	T
A	14.2	8.6	11.8	0
C	6.9	12.6	0	6.8
G	12.8	0	12.6	8.9
T	0	5.1	1.4	8.6

NrG Blocked Primer

Template Sequence

	A	C	G	T
A	12.4	4.8	10.4	0
C	4.5	11.1	0	2.5
G	10.3	0	10.1	3.8
T	0	3.5	2.2	5.3

Figure S4. 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, except that the control non-discriminatory primer was one base shorter on the 3′-end. Assay conditions and calculations of ΔCq values were the same as in Figure 7 in the manuscript. All reactions were run in triplicate.