Synthesis of SAMRS-containing oligonucleotides

SAMRS-containing oligonucleotides were synthesized on an ABI 394 DNA synthesizer using the following SAMRS phosphoramidites (A* = 2-aminopurine-CE phosphoramidite, T* = 2-Thio-dT-CE-phosphoramidite, G* = dI-CE phosphoramidite, and C* = N^4-Et-dC-CE phosphoramidite, Glen Research), ultramild CE phosphoramidites (for standard nucleotides), and ultramild CPG supports (from Glen Research). The coupling time for SAMRS phosphoramidites is 3 min; the rest of synthesis conditions are following standard procedures. After completion of the synthesis, the CPG support was treated with NH_4OH (1.0 mL) for 12-20 hour at room temperature, and the support was removed by filtration. The filtrate was lyophilized and SAMRS-containing oligonucleotides were purified by 20% denatured PAGE (7 M urea) or Ion-exchange HPLC.

Table S1: Primers used in **Supplementary Figure S1**

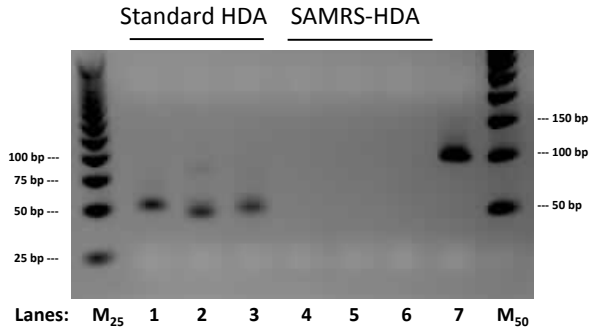
Name	Sequence
FLT3-F-Std:	5`-CGGGAAAGTGGTGAAGATATGTGAC-3`
FLT3-R-Std:	5`-CCCTGACAACATAGTTGGAATCACT-3`
FLT3-F-SAMRS8:	5`-CGGGAAAGTGGTGAAG**ATATGTGA**C-3`
FLT3-R-SAMRS8:	5`-CCCTGACAACATAGTT**GGAATCAC**T-3`
KIT-F-Std:	5`-ACAAAGATTTGTGATTTTGGTCTAGCCAG-3`
KIT-R-Std:	5`-GGACTGTCAAGCAGAGAATGGGTACTCAC-3`
KIT-F-SAMRS8:	5`-ACAAAGATTTGTGATTTTGG**TCTAGCCA**G-3`
KIT-R-SAMRS8:	5`-GGACTGTCAAGCAGAGAATG**GGTACTCA**C-3`
TSHR-F-Std:	5`-CCGCAGTACAACCCAGGGGACAAAG-3`
TSHR-R-Std:	5`-ATGAGAGGCTTGTTCAGAATTGCTG-3`
TSHR-F-SAMRS8:	5`-CCGCAGTACAACCCAG**GGGACAAA**G-3`
TSHR-R-SAMRS8:	5`-ATGAGAGGCTTGTTCA**GAATTGCT**G-3`

Nucleotides in bold and underlined are SAMRS components.

Table S2: Primers used in Figure 3, S5, and S6.

Name	Sequence[a]
Gag_92_F:	5`-AAACACCATGCTAAACACAGTGGGG**GGAC**A-3`
Gag-92_R:	5`-ATCTATCCCATTCTGCAGCTTCCTCA**TTGA**T-3`
1_Gag_119_F:	5`-GAACCCTTTAGAGACTATGTA**GACC**G-3`
1_Gag_119_R:	5`-CAATCTGGGTTCGCATTTT**GGAC**C-3`
2_Pol_110_F:	5`-CGACCCCTCGTCTCAATAAA**GATA**G-3`
2_Pol_110_R:	5`-GGTTTCCATCTTCCTGGCA**AATT**C-3`
3_Env_94_F:	5`-ACTTATCTGGGACGATCTG**CGGA**G-3`
3_Env_94_R:	5`-CAGAAGTTCCACAGTCCTC**GTTA**C-3`
4_Env_101_F:	5`-TAGGCAGGGATATTCACCATTGTC**GTTT**C-3`
4_Env_101_R:	5`-TGTCTCTCTCTCCACCTTCTTCTT**CTGT**T-3`
5_Gag_103_F:	5`-TATAGTATGGGCAAGCAGGGAGC**TAGA**A-3`
5_Gag_103_R:	5`-GGCTGGTTGTAGCTGTCCCAG**TATT**T-3`
6_Pol_109_F:	5`-GCCAAGTAACAAATTCAGCCACCG**TAAT**G-3`
6_Pol_109_R:	5`-TTTCTGGCTATGTGCCCTTCTT**TACC**A-3`

[a] Nucleotides in bold and underlined are SAMRS components. All SAMRS-containing primers were derived from their standard primers.

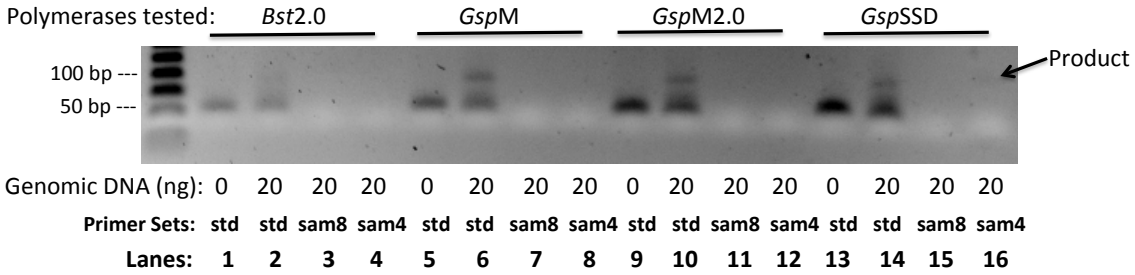


Supplementary Figure S1. HDA assays using standard primers or primers containing eight SAMRS components to amplify three cancer genes, showing inefficient priming with eight SAMRS components.
Lanes 1, 2, and 3: With standard primers, artifacts (~ 50 bp) were generated with standard HDA assays targeting three cancer genes (FLT3, KIT, or TSHR, respectively).
Lanes 4, 5, and 6: With primers containing eight SAMRS components, neither amplicons nor artifacts were generated in HDA assays targeting three cancer genes (FLT3, KIT, or TSHR) (see Table S1 for key).
Lane 7: Desired amplicon was generated with control primers and target from BioHelix Kit.
M_{25} and M_{50}: 25 bp and 50 bp DNA ladders.

Screening of various DNA polymerases for their ability to amplify DNA using primers containing four SAMRS components

A mixture of male human genomic DNA (20 ng, Promega) and primers (either standard primers or SAMRS-containing primers, final 200 nM, Table S1) was incubated at 65 °C for 3 minutes and then slowly cooled to 25 °C (at a rate of 0.1 °C / s). The HDA amplification mixture consists of 1x annealing buffer II (BioHelix), $MgSO_4$ (final 4 mM), NaCl (final 40 mM), 1x IsoAmp dNTP (BioHelix), 1x IsoAmp enzyme mix III (BioHelix), and various DNA polymerase (*Bst*2.0 (NEB), *Gsp*M (OptiGene), *Gsp*M2.0 (OptiGene), or *Gsp*SSD (OptiGene)). Genomic DNA and primers were mixed with amplification mixture at room temperature, then, amplification reactions were performed at 65 °C for 90 minutes. Upon the completion of HDA assays, each assay was analyzed by 3% agarose gel to resolve amplicons. The results are shown in **Supplementary Figure S2.**

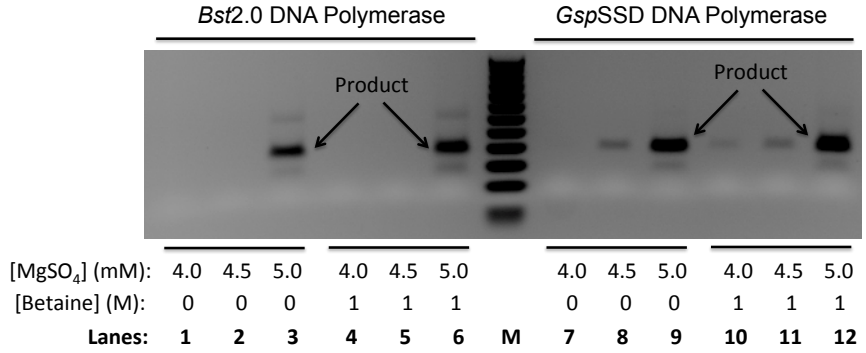
Supplementary Figure S2. Screening of various added DNA polymerases for their ability to amplify v-kit Hardy-Zuckerman (KIT) gene in human genomic DNA using standard and SAMRS-containing primers (Table 1 and Table S1). Standard primers gave primarily primer dimer and small amount of product with all polymerases tested. SAMRS-containing primers gave only a trace amount of desired product with *Gsp*SSD (lane 16), and no primer artifacts were generated with SAMRS-containing primers for all tested polymerases.



Lanes 1, 2, 5, 6, 9, 10, 13, and 14: standard primer sets (std, KIT-F-Std + KIT-R-Std) were used in these assays.
Lanes 3, 7, 11, and 15: primers containing eight SAMRS components (sam8, KIT-F-SAMRS8 + KIT-R-SAMRS8) were used.
Lanes 4, 8, 12, and 16: primers containing four SAMRS components (sam4, KIT-F-SAMRS4 + KIT-R-SAMRS4) were used.

Optimization of SAMRS-HDA assays using *Bst*2.0 and *Gsp*SSD DNA polymerases

SAMRS-HDA assays with IsoAmp enzyme mix III plus either *Bst*2.0 or *Gsp*SSD DNA polymerase were tested under various $MgSO_4$ concentrations (4.0 mM, 4.5 mM, or 5.0 mM) in the presence or absence of betaine (1 M). Briefly, the KIT cancer gene in human genomic DNA (20 ng of total DNA, ~ 3000 copies, Promega) and SAMRS-containing primers (KIT-F-SAMRS4 and KIT-R-SAMRS4, final 200 nM) were mixed with 1x annealing buffer II (BioHelix), NaCl (final 40 mM), 1x IsoAmp dNTP (BioHelix), 1x IsoAmp enzyme mix III (BioHelix), $MgSO_4$ (final 4.0 mM, 4.5 mM, or 5.0 mM), and betaine (0 or 1 M). *Bst*2.0 (2 µL, NEB) or *Gsp*SSD (2 µL, OptiGene) DNA polymerase was added to each assay at room temperature to give a final volume of 25 µL. Each assay was incubated at 65 °C for 90 minutes, followed by 3% agarose gel to resolve amplicons. The results are shown in **Supplementary Figure S3.**



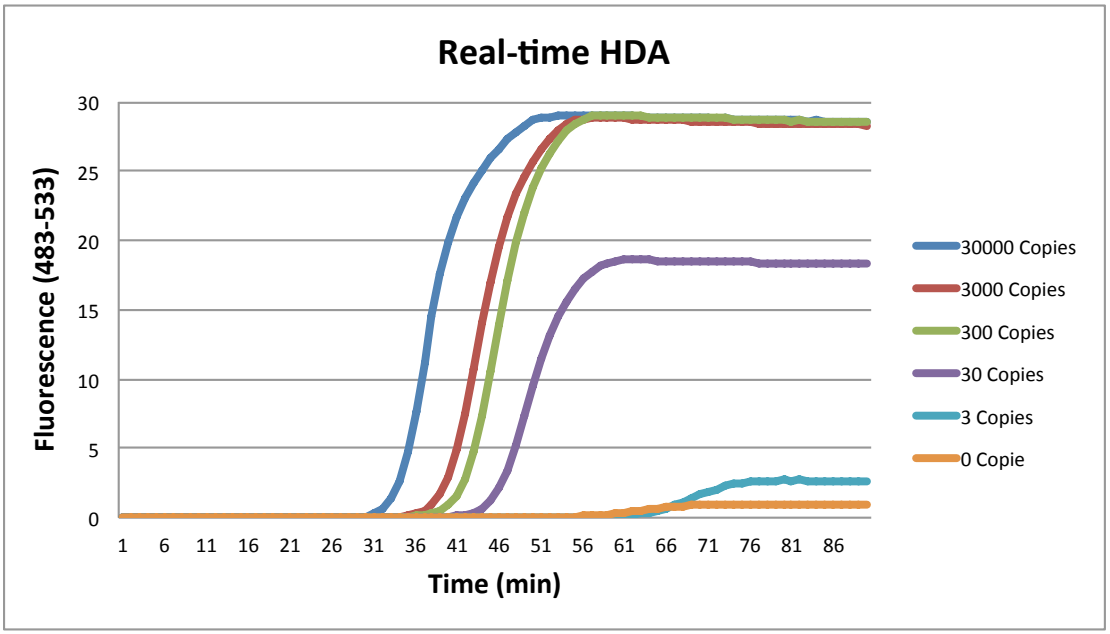
Supplementary Figure S3. Optimization of additives in HDA using SAMRS-containing primers (KIT-F-SAMRS4 and KIT-R-SAMRS4, Table 1) at various concentrations of $MgSO_4$ and betaine. Arrow indicates expected amplicon product (98 nts). These results show the importance of Mg^{2+} concentration, improved results with the Geobacillus (*Gsp*SSD) thermostable Family A polymerase, and no detectable formation of primer dimers over a range of conditions.

Additional *Bst*2.0 DNA polymerase (left) and *Gsp*SSD DNA polymerase (right) was added to SAMRS-HDA assay, respectively.
Lanes 1, 2, and 3: 4.0 mM, 4.5 mM, and 5.0 mM of $MgSO_4$ without betaine;
Lanes 4, 5, and 6: 4.0 mM, 4.5 mM, and 5.0 mM of $MgSO_4$ with 1 M of betaine;
Lanes 7, 8, and 9: 4.0 mM, 4.5 mM, and 5.0 mM of $MgSO_4$ without betaine;
Lanes 10, 11, and 12: 4.0 mM, 4.5 mM, and 5.0 mM of $MgSO_4$ with 1 M of betaine;
M indicates 25 bp DNA ladders.

The limit of detection (LOD) of HDA assay using SAMRS-containing primers for the KIT cancer gene in human genomic DNA.

Ten-fold serial dilutions of human genomic DNA (30,000, 3,000, 300, 30, 3, or 0 copies) were mixed with SAMRS-containing primers (KIT-F-SAMRS4 and KIT-R-SAMRS4, final 200 nM), and the mixed solutions were incubated at 65 °C for 3 min and then slowly cooled to 25 °C (0.1 °C / second). The HDA mixture consists of 1x annealing buffer II (BioHelix), $MgSO_4$ (final 5 mM), NaCl (final 40 mM), IsoAmp dNTPs (2 µL, BioHelix), IsoAmp enzyme mix II (2 µL, BioHelix), *Gsp*SSD DNA polymerase (2 µL, OptiGene), and 0.2x EvaGreen dye (Biotium). Genomic DNA and primers were mixed with amplification mixture at room temperature (final volume 20 µL). Three replicates were performed at 65 °C for 90 minutes. Amplification efficiency was monitored using the LightCycler 480 (Roche). Parallel HDA assays using standard primers (KIT-F-Std and KIT-R-Std) were performed under identical conditions. Each assay was analyzed by 3% agarose gel to resolve amplicons. The results are shown in **Figures 1 and Supplementary Figure S4.**

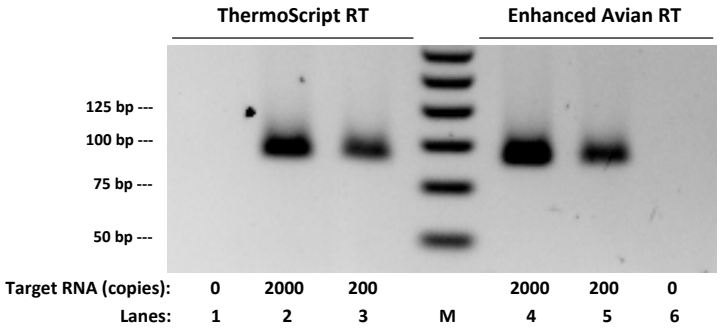
Supplementary Figure S4. Determining the limit of detection (LOD) for KIT cancer gene in HDA using SAMRS-containing primers. Amplification efficiency was monitored based on the fluorescence of EvaGreen dye using LightCycler 480 (Roche). All curves represent three replicates.



Screening thermostable reverse transcriptases (RT) for RT-SAMRS-HDA to amplify HIV-1 armored RNA.

To develop RT-SAMRS-HDA assays, a series of thermostable reverse transcriptases including ThermoScript RT (Invitrogen), Enhanced Avian RT (Sigma), AffinityScript RT (Agilent), SuperScript III RT (Invitrogen), Maxima RT (Fermentas), and RevertAid Premium RT (Fermentas) were tested in RT-SAMRS-HDA to amplify a conserved region in the *gag* gene among HIV-1 armored RNA (Asuragen) using SAMRS-containing primers. The RT-SAMRS-HDA reaction is dependent on the combination of two reaction mixtures, A and B. Mixture A contained the HIV-1 armored RNA (2000 copies/reaction or 200 copies/reaction) and SAMRS-containing primers (200 nM for each primer, Gag_92_F and Gag_92_R, Table S2) in nuclease free water in a volume of 10 µL, and the mixed solution A was incubated at 65 °C for 3 minutes and then slowly cooled down to 25 °C (at a rate of 0.1 °C / second). Mixture B contained annealing buffer II (2.5 µL, BioHelix), $MgSO_4$ (final 5 mM), NaCl (final 40 mM), IsoAmp dNTPs (2 µL, BioHelix), IsoAmp enzyme mix II (2 µL, BioHelix), *Gsp*SSD DNA polymerase (2 µL, OptiGene), RNase inhibitor (1 µL of RNaseOUT, Invitrogen), and 0.2 µL of each reverse transcriptase in a volume of 15 µL. Mixture A and B were combined at room temperature and incubated at 65 °C for 90 minutes. Amplified products were separated on 3% agarose gels and stained with ethidium bromide. Efficiency and specificity of each reverse transcriptase in RT-SAMRS-HDA was assayed based on the intensity and amplicon size of the correct product band. The results are shown in **Supplementary Figure S5.**

Supplementary Figure S5. Screening thermostable reverse transcriptases (RT) for RT-SAMRS-HDA to amplify HIV-1 armored RNA.



Target RNA (2000 or 200 copies of HIV-1 armored RNA; Asuragen) was amplified using SAMRS-containing primers (Gag_92_F and Gag_92_R, Table S2) in the HDA assays with ThermoScript RT (left) or Enhanced Avian RT (right).

Comparison RT-HDA using standard primers with SAMRS-containing primers to amplify complete genomic HIV-1 vira RNA.

Six standard primer pairs or six SAMRS-containing primer pairs (Table S2), 1_Gag_119, 2_Pol_110, 3_Env_94, 4_Env_101, 5_Gag_103, and 6_Pol_109, were tested in RT-HDA assay to amplify various genes in complete HIV-1 viral RNA (SeraCare).

The RT-HDA reaction was composed of two reaction mixtures, A and B. Mixture A consists of the target HIV-1 viral RNA (20000 copies/reaction) and each standard or SAMRS-containing primer pair (200 nM for each primer) in nuclease free water in a volume of 10 µL. The mixed solution A was incubated at 65 °C for 3 minutes and then slowly cooled down to 25 °C (at a rate of 0.1 °C / second). Mixture B contained annealing buffer II (2.5 µL, BioHelix), $MgSO_4$ (final 5 mM), NaCl (final 40 mM), IsoAmp dNTPs (2 µL, BioHelix), IsoAmp enzyme mix II or III (2 µL, BioHelix), with or without additional *Gsp*SSD DNA polymerase (2 µL, OptiGene), RNase inhibitor (1 µL of RNaseOUT, Invitrogen), and 0.2 µl of Enhanced Avian RT (Sigma) in a volume of 15 µL. Mixture A and B were combined at room temperature and incubated at 65 °C for 90 minutes. Amplicons were separated on 3% agarose gels and stained with ethidium bromide. The results are shown in **Figure 3, and S6.**

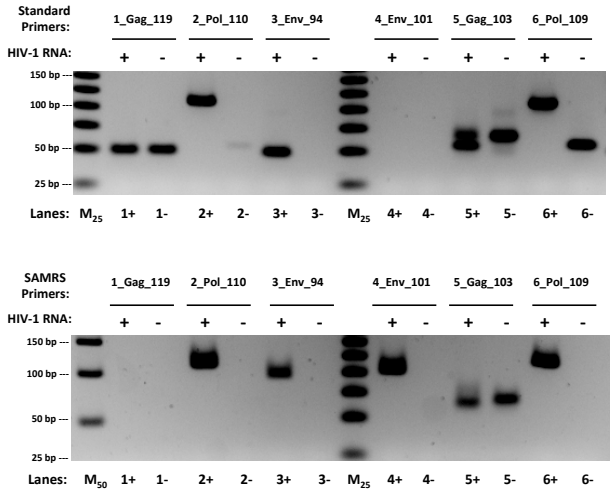


Figure S6. RT-HDA assays using standard primers (top) and SAMRS primers (bottom) to amplify *gag*, *pol*, and *env* genes in HIV-1 viral RNA. Standard primers generate significant amount of artifacts even using "hot start" IsoAmp enzyme mix III. SAMRS-containing primers have less artifacts using "hot start" IsoAmp enzyme mix III and *GspSSD*. M_{50} and M_{25} are 50 bp and 25 bp ladders.

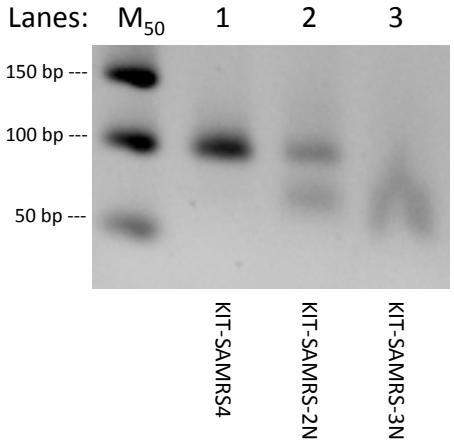
Amplification of HIV-1 viral RNA and rpoB DNA using twofold multiplexed RT-SAMRS-HDA assays

Two sets of standard primers or SAMRS-containing primers, 2_Pol_110 and rpoB-II (each primer was at 0.1 µM of final concentration), were mixed with various amount of rpoB DNA, or purified HIV-1 virus RNA (SeraCare), or both targets in nuclease free water in a volume of 10 µL. This mixed solution was incubated at 65 °C for 3 minutes and then slowly cooled down to 25 °C (at a rate of 0.1 °C / second). Another mixture contained annealing buffer II (2.5 µL, BioHelix), $MgSO_4$ (final 5 mM), NaCl (final 40 mM), IsoAmp dNTPs (2.4 µL, BioHelix), IsoAmp enzyme mix II, or III (2 µL, BioHelix for standard primers), *GspSSD* DNA polymerase (2 µL, OptiGene, for SAMRS-containing primers), additional 0.2 µL of Helicase (BioHelix, 500ng/mL), RNase inhibitor (1 µL of RNaseOUT, Invitrogen), and 0.2 µl of Enhanced Avian RT (Sigma) in a volume of 15 µL. Two mixtures were combined at room temperature and incubated at 65 °C for 180 minutes. Amplicons were separated on 3% agarose gels and stained with ethidium bromide. The results are shown in **Figure 4**.

Table S3: Primers with SAMRS components at different positions for **Supplementary Figure S7**.

Name	Sequence
KIT-F-SAMRS4:	5`-ACAAAGATTTGTGATTTTGGTCTA**GCCA**G-3`
KIT-R-SAMRS4:	5`-GGACTGTCAAGCAGAGAATGGGTA**CTCA**C-3`
KIT-F-SAMRS-2N:	5`-ACAAAGATTTGTGATTTTGGTCT**AGCC**AG-3`
KIT-R-SAMRS-2N:	5`-GGACTGTCAAGCAGAGAATGGGT**ACTC**AC-3`
KIT-F-SAMRS-3N:	5`-ACAAAGATTTGTGATTTTGGTC**TAGC**CAG-3`
KIT-R-SAMRS-3N:	5`-GGACTGTCAAGCAGAGAATGGG**TACT**CAC-3`

Four SAMRS components are in bold and underlined. 2N and 3N indicate two and three standard nucleotides are followed SAMRS components at the 3`-end of primers.



Supplementary Figure S7. Determining the positional effect of SAMRS components in SAMRS-HDA.
Lane 1: KIT-SAMRS4 primers, with just one standard nucleotide at 3'-end of primer, preceded by four SAMRS components (KIT-F-SAMRS4 and KIT-R-SAMRS4), gave desired amplicon.
Lane 2: KIT-SAMRS-2N primers, with two standard nucleotides at 3`-end of primer, preceded by four SAMRS components (KIT-F-SAMRS-2N and KIT-R-SAMRS-2N), gave both amplicon and artifact.
Lane 3: KIT-SAMRS-3N primers, with three standard nucleotides are placed at 3`-end of primer, preceded by four SAMRS components (KIT-F-SAMRS-3N and KIT-R-SAMRS-3N), gave only artifact.
M_{50}: 50bp DNA ladders.