

substitution affecting either of the first two bases at codon 12 results in loss of this restriction site and thus cleaves only at the 3' site to produce a 116 bp fragment. A codon 13 mutation creates an *HphI* recognition site. Digestion of the 135 bp amplified fragment with this enzyme thus leads to cleavage of mutant

DNA at a 5' and a 3' site to produce a 75 bp fragment, while normal sequence is digested at only the 3' position to produce a fragment of 117 bp. For both enzymes, the 3' site is always cleaved and serves as a control for the digestion (Figure 1) ⁽⁶⁾.

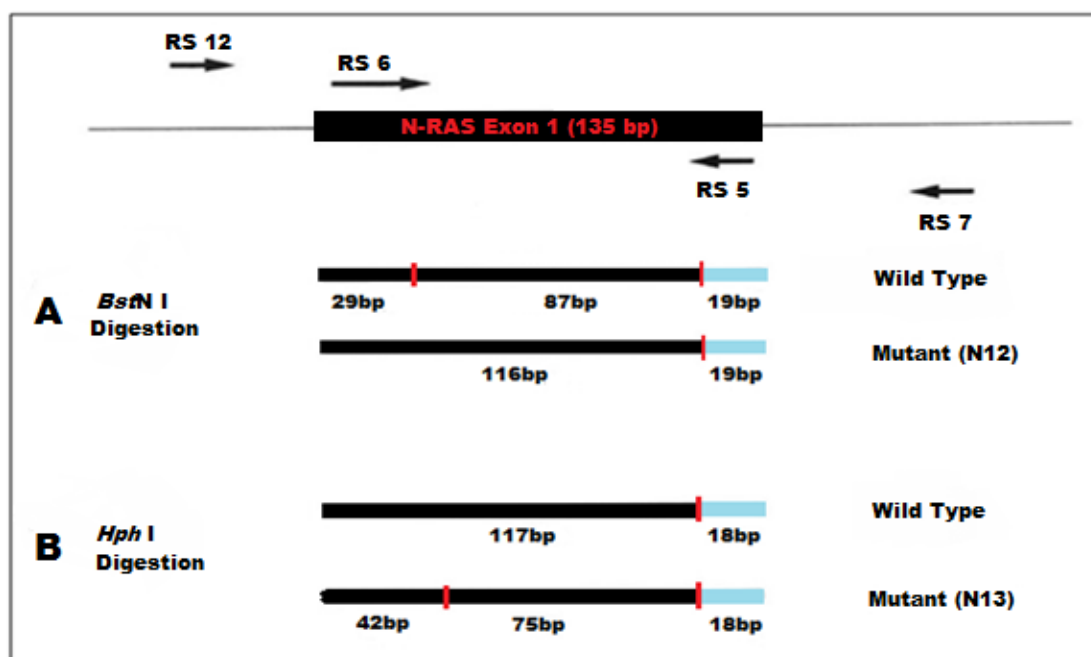


Fig. 1. Schematic illustration of the PCR-based MSDA used for the detection of codon 12 and 13 mutations. The positions of the first round primers for exon 1 (RS12 and RS7) and the second round nested primers (RS6 and RS5) are shown. (A) *BstNI* digestion of amplified sequence for codon 12 mutations. (B) *HphI* digestion of amplified DNA for codon 13 asp mutations ⁽⁶⁾.

PCR products were digested directly after amplification, for codon N12 detection, 10 µl of PCR reaction mixture (about 0.1-0.5 µg of DNA), 7 µl of nuclease free water, 2 µl of NE Buffer 2 (10X) and 1 µl of *BstNI* were mixed gently for a few seconds. Then incubated at 60°C for 2 hours and mixture were subjected to electrophoresis in 2% agarose gels containing ethidium bromide. For codon N13 detection, 10 µl of PCR reaction mixture, 7 µl of nuclease free water, 2 µl of NE Buffer 4 (10X) and 1 µl of *HphI* were mixed gently for a few seconds. Then incubated at 37°C for 2 hours and mixture were subjected to electrophoresis in 2% agarose gels containing ethidium bromide (Figure 2, 3 and 4) ^(6,7).

Induction Therapy

The primary objective in treating patients with AML is to induce remission and thereafter prevent relapse. Complete remission (CR) was defined morphologically as no circulatory blasts, with absolute neutrophil count (ANC) $>1.5 \times 10^9/L$, and platelet count $>100 \times 10^9/L$ and cellular marrow with blasts $<5\%$ and absence of extra medullary involvement ⁽⁸⁾. Failure of induction was defined as less than 50% reduction in marrow blast percent from that at presentation. Patients neither in complete remission nor in failure regarded as partial response. Treatment is conventionally divided into two phases: induction and post induction ⁽⁸⁾.