Rare allele enrichment and detection by allele-specific PCR, competitive probe blocking and melting analysis

PROTOCOL FOR:

Rare allele enrichment and detection by allele-specific PCR, competitive probe blocking, and melting analysis

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See full protocol online: <https://benchtalk.biotechniques.com/users/8521-biotechniques-editors/posts/11649-protocol-for-rare-allele-enrichment-and-detection-by-allele-specific-pcr-competitive-probe-blocking-and-melting-analysis>

Protocol Summary: We have combined allele-specific PCR, competitive probe blocking, asymmetric PCR, and melting analysis to enhance rare allele detection in a homogeneous system. Unlabeled, dual hybridization or molecular beacon probes were used for competitive blocking of the wild-type allele at a concentration 10 times that of the allele-specific primer. In each case, rare alleles were detected by probe melting analysis at a sensitivity of >0.001% (1 variant copy within 100,000 wild-type copies), providing single copy detection in typical PCRs. Ninety-one thyroid biopsies were tested for the BRAF mutation p.V600E (c.1799 T > A) by both dual hybridization probes without enrichment and an allele-specific, competitive blocking melting analysis with unlabeled probes. Eighty-seven samples were concordant between methods (43 positive, 44 negative), while 4 samples that were negative by direct analysis became positive after enrichment. Probes that both block wild-type amplification and detect rare variants by melting analysis improve the detection sensitivity of allele-specific PCR for rare alleles. In particular, melting analysis using unlabeled probes and amplification by rapid-cycle PCR provides cost-effective and fast enrichment and detection of rare alleles.

Protocol Schematic: Derivative melting curves obtained after PCR in the presence of unlabeled blocking probes that detect the BRAF c.1799T > A mutation. The melting curve resulting from 100% wild-type DNA is shown in black, while the 100% variant DNA is shown in gray. The thin black line is the no-template control. For both DNA templates, the resulting amplicon peak is at 83°C. The melting peak of the probe/amplicon duplex is 66°C for the matched wild-type allele and 62°C for the mismatched mutant allele. The detection sensitivity for the 1799A variant depends on the annealing/extension temperature during PCR. Sensitivity is maximal when the annealing/extension temperature is between the T_m s of the wild-type and mutant duplex peaks (top).