

B cell Gene Rearrangement Analysis

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This test is indicated for the accurate differentiation of malignant vs. benign lymphoproliferative disorders and for establishing B tumor cell lineage. Clonal proliferations of B-lymphocytes can be detected by the identification of specific DNA rearrangements in the immunoglobulin gene. The majority of lymphocytic leukemias and non-Hodgkin's lymphomas arise from a clonal proliferation of a single lymphoid cell that has become aberrant. B cells normally differentiate from stem (precursor) cells in a highly specific and sequential manner. The malignant transformation may take place at any stage in the maturation process and when it occurs the resulting malignant clone bears the characteristics of the originally transformed cell and is called monoclonal. Some of the early events in the maturation process of lymphoid cells involve specific nucleic acid rearrangements within the immunoglobulin genes in B cells.

To detect B cell gene rearrangements using PCR, primers are constructed to amplify the DNA between the conserved framework (FR) and joining (J) regions. These conserved regions lie on either side of an area within the V-J region where programmed genetic rearrangements occur during maturation. In the germline configuration found in somatic cells, these areas are widely separated (by more than 70KB) making PCR amplification across the area impossible. The sequence alteration brought about by gene rearrangement brings these regions into close proximity, making the area of amplifiable length. Each B cell has a single productive V-J rearrangement that is unique in both length and sequence. The template-free incorporation of nucleotides (N-regions) between the spliced V, D, and J gene segments also adds uniqueness to the PCR product. Therefore, when this region is amplified using DNA primers that flank this region, a clonal population of cells yields one or two prominent amplified products (amplicons) within the expected size ranges. Two products are produced in cases when the initial rearrangement was non-productive and was followed by rearrangement of the other homologous chromosome. In contrast, DNA from a normal or polyclonal population produces a bell-shaped curve (or Gaussian distribution) of amplicon products that reflects the heterogeneous population of V-J region rearrangements.

Since the antigen receptor genes are highly polymorphic, it is difficult to employ a single set of DNA primer sequences to target all of the conserved flanking regions around the V-J rearrangement. N-region diversity and somatic mutation further scramble the DNA sequences in these regions. Therefore multiplex master mixes, which target several FR regions, are required to identify the majority of clonal rearrangements. As indicated, clonal rearrangements are identified as prominent single-sized products within the smear of different sized amplicon products that form a Gaussian distribution around a statistically favored, average-sized rearrangement. As expected, primers that amplify from the different FR regions, which are located at three distinct regions along the heavy chain gene, produce a correspondingly different size range of products.

A powerful detection method for the amplicons produced in the multiplex reaction involves capillary electrophoresis and differential fluorescence detection of the amplified products. The primers used in the master mixes are labeled with different fluorescent dyes, each corresponding to a different target region. Reaction products from different master mixes can be pooled fractionated using capillary electrophoresis and detected simultaneously. This detection system results in unsurpassed sensitivity, single base resolution, differential product detection and relative quantitation. The limit of detection of this assay has been determined to be approximately 1 clonal cell in 100 normal cells. The inter-assay and intra-assay reproducibility in size determination using capillary electrophoresis is approximately 1-2 base pairs. This reproducibility and sensitivity allows monitoring and tracking of individual tumors.

B-cell immunoglobulin gene rearrangement testing can be used to determine the presence of a monoclonal B cell population in a submitted sample, indicative of a neoplastic lymphoproliferative disorder.

Figures 1 & 2: Example of B cell Gene Rearrangement Analysis using normal polyclonal DNA (1) and Monoclonal B cell population (2)

