MONOCLONALITY IDENTIFICATION OF CELL B LYMPHOMA

Ampli Lymphoma B — Cat. n. 1.400

The functional rearrangement of IGH gene, first DH to JH and then V to DH-JH, is follwed by the antibody expression, characteristic of mature B cells. These rearrangements can be used as clonal markers in the lymphoproliferative diseases. The AMPLI lymphoma-B kit allows to identify, trough the Polymerase Chain Reaction (PCR), the rearrangements of the heavy chain (IgH). The IGH gene is located on 14q32.3 chromosome in an area of 1250 Kb roughly. In all 46-52 VH functional segments have been identified; they can be grouped according to their homology in six or seven groups VH
The segments contain three "framework - (FRI-II-III)" regions and two "complementarity-determining - (CDRs)" regions. The FRs are characterized by their similarity between different VH segment, while the CDRs differ even within the same VH family. Furthermore the CDRs are the somatic hyper-mutations favorite target sequences during the central germinal reaction, increasing the variability within these regions. Nucleotide substitutions can take place within the Frs, especially in the B cells during a great mutational state.
Every primers' set consist in six or seven oligonucleotides able to recognize the corresponding VH (VH1–VH7) segments with no mismatches for the most and one or two mismatches for some rare segments. These primers' sets have been used together with a single primer consent for JH, designed to join to the six JH segments. All the primers work with high efficiency and sensitivity (at least $1x10^{-2}$) and they allow a detection rate of 99%.
The combined use of standardized primers in three different FRs, helps to decrease significantly the percentage of false negative, result of hyper-mutations in the binding sites of the involved VH segments.

Principle of assay:
A) extraction of genomic DNA
B) amplification
c) enzymatic digestion
D) detection on agarose gel
Applicability: genomic DNA exctracted by fresh and purified from whole blood samples.
Number of tests: 45.

REAGENTS AND STORAGE

AMPLIFICATION	
Mix PCR Fr1	-20°C
Mix PCR Fr2	-20°C
Mix PCR Fr3	-20°C
H_2O DNase/RNase-free	-20°C
Taq Polymerase (5U/µl)	-20°C
DNA Control	-20°C

Stability: over 18 months if correctly stored.

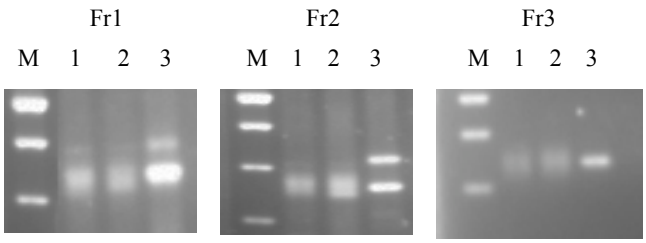
References:

Aithal GP, et al., Lancet (1999) 353: 717-19
Klein TE, et al., N Engl J Med (2009) 360:753-64
Rieder MJ, et al, N Engl J Med. (2005) 352:2285-93
Limdi NA, et al., Blood (2010) 115:3827-34

ANALYSIS OF RESULTS

Presence of poly-clonality= normal condition → the samples produce a smear amplified of a size of a range:
310 – 360 bp for Fr1
250 – 295 bp for Fr2
100 – 170 bp for Fr3

Presence of mono-clonality= =LYMPHOPROLIFERATIVE DISEASE → the samples produce one or two fragments of a range:
310 – 360 bp per Fr1
250 – 295 bp per Fr2
100 – 170 bp per Fr3



Samples 1 and 2 show poly-clonality for Fr1-2-3
Sample 3 show monoclonality for Fr1-2-3.

The kit has been updated to the european guidelines establish by the cooperative study BIOMED-2.