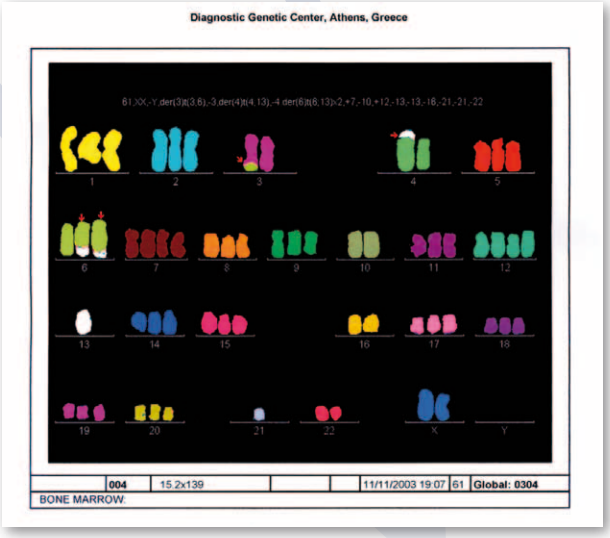
InterGenetics
DIAGNOSTIC GENETIC CENTER

HEMATOLOGICAL MALIGNANCIES



- ✓ *particular experience in cytogenetic analysis of leukemias (>10,000 cases)*
- ✓ *in-depth investigation of cases utilizing molecular cytogenetic techniques (FISH)*
- ✓ *simultaneous detection of characteristic microdeletions and microduplications of 12 chromosomal regions: 2p24, 5q33-35, 6q25-26, 7p12.2, 8q24, 9p21.3, 10q23, 11q22.3, 12p13.2, 13q14, 17p13, 21q22.1, as well as the parallel detection of the JAK2 V617F mutation, using a specially designed MLPA approach*

A few words about hematological malignancies

Hematological malignancies are a type of cancer that affects blood, bone marrow and the lymph nodes. As the three are intimately connected through the immune system, a disease affecting one of the three will often affect the others as well, e.g. lymphoma is a disease of the lymph nodes and it often spreads to the bone marrow, affecting the blood.

The genetic tests that follow are useful for patients with hemopoietic disorders, for the diagnosis and/or confirmation of chronic or acute leukemia, for evaluation of remission or relapse or even after bone marrow transplantation for evaluating recurrence of the disorder.

Bone marrow karyotype

It is a routine test applied to the investigation of leukemia or other hematological diseases. The test includes the analysis of at least 20 metaphases cultured bone marrow cells. It is necessary to analyze the chromosomes with at least one banding technique, e.g. RHG, at an analytical level of 300-350 bands (QAS 3). For patients under treatment, with known chromosomal abnormalities, 50-100 metaphases are analyzed.

Through the classical karyotype analysis of hematological malignancies we can detect most of the characteristic numerical as well as structural chromosomal abnormalities which may occur.

Analysis of chromosomal abnormalities using FISH technique

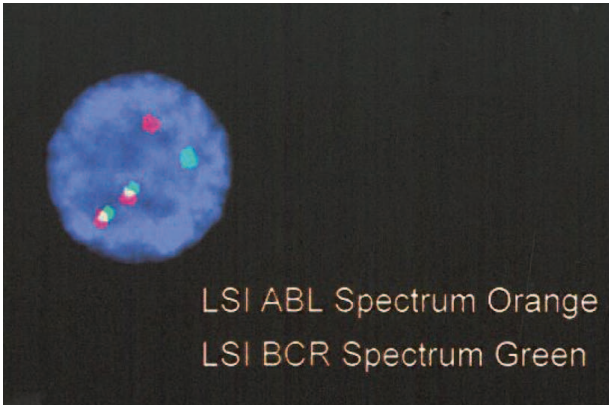
The technique of fluorescent in situ hybridization (FISH), is a valuable method for analyzing chromosomal rearrangements, such as microdeletions and microduplications, and it contributes not only to the qualitative analysis and diagnosis, but also in the quantitative evaluation of genetic abnormalities associated with hematological diseases. InterGenetics is among the first laboratories which have applied this technique to the study of hematological malignancies.

Molecular detection of multiple chromosomal rearrangements using MLPA technique

Classic karyotype analysis, as applied in hematological malignancies, will reveal most characteristic numerical and structural chromosomal abnormalities. However, it will not detect submicroscopic microdeletions or microduplications, occurring in various types of leukemias and which may correlate with disease diagnosis or even prognosis.

Moreover, it is not possible to apply the FISH technique as a routine method of screening, especially in patients being investigated for the first time, for the existence of possible genetic aberrations associated with the malignancy.

In order to compensate for this, we developed and apply a novel approach for the parallel detection of a number of characteristic microdeletions and microduplications involving 12 chromosomal regions: 2p24, 5q33-35, 6q25-26, 7p12.2, 8q24, 9p21.3, 10q23, 11q22.3, 12p13.2, 13q14, 17p13, 21q22.1 as well as the parallel detection of the JAK2 V617F mutation. Detection is based on a custom designed MLPA technique, using multiple probes for the regions under investigation.



Translocation t(9;22) – BCR/ABL
The precise genetic abnormality responsible for the typical "Philadelphia chromosome" is the translocation t(9;22)(q34;q11). The result of this translocation is the formation of the fusion-hybrid gene bcr/abl. This translocation is evident in ~90% of individuals suffering from chronic myeloid leukemia (CML) and 35% of those suffering from acute lymphocytic leukemia (ALL).

Detection may be achieved by classic karyotype analysis. The translocation is also detected by the FISH technique, using specific custom-labeled DNA probes, from at least 100 bone marrow cell nuclei.

A semi-quantitative RT-PCR method is also offered, from a peripheral blood or bone marrow sample, allowing the detection of at least 10 RNA copies of the M-, m-, or µ- BCR/ABL subtypes.

Translocation t(15;17) – PML/RARa
The result of the t(15;17)(p;22;q22) translocation is the formation of the hybrid gene pml/rara and is an indication of acute promyelocytic leukemia (APL). Detection may be achieved by classic karyotype analysis. The translocation is also detected by the FISH technique, using specific custom-labeled DNA probes, from at least 100 bone marrow cell nuclei.

A semi-quantitative RT-PCR method is also offered, from a peripheral blood or bone marrow sample, allowing the detection of at least 10 RNA copies of PML/RARa RNA.

Translocation t(8;21) – AML/ETO
The result of the t(8;21)(p;22;q22) translocation is the formation of the hybrid gene aml1/eto and is an indication of acute monocytic leukemia.

Detection may be achieved by classic karyotype analysis. The translocation is also detected by the FISH technique, using specific custom-labeled DNA probes, from at least 100 bone marrow cell nuclei.

A semi-quantitative RT-PCR method is also offered, from a peripheral blood or bone marrow sample, allowing the detection of at least 10 RNA copies of AML1/ETO RNA.

Inversion, inv(16)
Pericentric inversion of chromosome 16, between loci p13q22, is a clear indication of acute myelomonocytic leukemia (AMML). The inversion leads to formation of the hybrid gene CBFB/PEBP2B-MHY11, the function of which is analogous to that of oncogenes and is directly related to the pathology of this disorder.

Detection may be achieved by classic karyotype analysis. The inversion is also detected by the FISH technique, using specific custom-labeled DNA probes, from at least 100 bone marrow cell nuclei.

Translocation t(1;19)
The result of the t(1;19) translocation is the formation of the hybrid PBX1/E2A gene, an indication of acute lymphocytic leukemia (ALL) which is observed in 6% of all cases.

Detection may be achieved by classic karyotype analysis. The translocation is also detected by the FISH technique, using specific custom labeled NDA probes, from at least 100 bone marrow cell nuclei.

Translocation t(2;5)

The result of the t(2;5) translocation is the formation of the NPM/ALK hybrid gene which is an indication of the non-Hodgkin type lymphoma (NHL). It is observed in over 50% of adult patients suffering with NHL and in 10% of children and teenagers with an average age of 16 years old.

Detection may be achieved by classic karyotype analysis. The translocation is also detected by the FISH technique, using specific custom-labeled DNA probes, from at least 100 bone marrow cell nuclei.

Translocation t(4;11)

Rearrangements occurring at the chromosomal region 11q23 (MLL breakapart) are usually due to the t(4;11) translocation and involve the AF4 and MLL genes. The translocation is observed in chronic lymphocytic leukemia (CLL), in acute lymphocytic leukemia (ALL), in acute myeloid leukemia (AML) and in myelodysplastic syndromes (MDS).

Detection may be achieved by classic karyotype analysis. The translocation is also detected by the FISH technique, using specific custom-labeled DNA probes, from at least 100 bone marrow cell nuclei.

Translocation t(4;14)

The result of the t(4;14) translocation is the formation of the hybrid gene IGH/FGF3, an indication of multiple myeloma.

Detection may be achieved by classic karyotype analysis. The translocation is also detected by the

FISH technique, using specific custom-labeled DNA probes, from at least 100 bone marrow cell nuclei.

Translocation t(8;14)

The result of the t(8;14) translocation is the formation of the hybrid gene IGH/MYC, an indication of acute lymphocytic leukemia (ALL), non-Hodgkin lymphoma (NHL) or Burkitt's lymphoma and is observed in 75-85% of all cases.

Detection may be achieved by classic karyotype analysis. The translocation is also detected by the FISH technique, using specific custom-labeled DNA probes, from at least 100 bone marrow cell nuclei.

Translocation t(11;14)

The t(11;14) translocation results in the formation of the IGH/CCND1 hybrid gene, an indication of B-chronic lymphocytic leukemia, non-Hodgkin lymphoma (NHL) and multiple myeloma.

Detection may be achieved by classic karyotype analysis. The translocation is also detected by the FISH technique, using specific custom-labeled DNA probes, from at least 100 bone marrow cell nuclei.

Translocation t(11;18)
The t(11;18) translocation leads to the formation of the MALT1/API2 hybrid gene, a clear indication of MALT lymphoma.

Detection may be achieved by classic karyotype analysis. The translocation is also detected by the FISH technique, using specific custom-labeled DNA probes, from at least 100 bone marrow cell nuclei.

Translocation t(12;21)
The t(12;21) translocation leads to the formation of the TEL(ETV6)/AML1(RUNX1) hybrid gene and provides an indication of acute lymphocytic leukemia (ALL). It is the most common chromosomal aberration in childhood B-ALL (21% of cases) and has generally a good prognosis. This is a cryptic translocation and a difficult one to detect by means of classic karyotype analysis.

It is only detected by the FISH technique, using specifically labeled probes, in at least 100 bone marrow cell nuclei.

Translocation t(14;16)
The result of the t(14;16) translocation is the formation of the IGH/MAF hybrid gene, an indication of multiple myeloma.
Detection may be achieved by classic karyotype analysis. The translocation is also detected by the FISH technique, using specific custom-labeled DNA probes, from at least 100 bone marrow cell nuclei.

Translocation t(14;18)
The t(14;18) translocation leads to the formation of the IGH/BCL2 hybrid gene, and is an indication of non-Hodgkin lymphoma (NHL). It is also the second most common translocation of the IGH gene in chronic lymphocytic leukemia (CLL).

Detection may be achieved by classic karyotype analysis. The translocation is also detected by the FISH technique, using specific custom-labeled DNA probes, from at least 100 bone marrow cell nuclei.

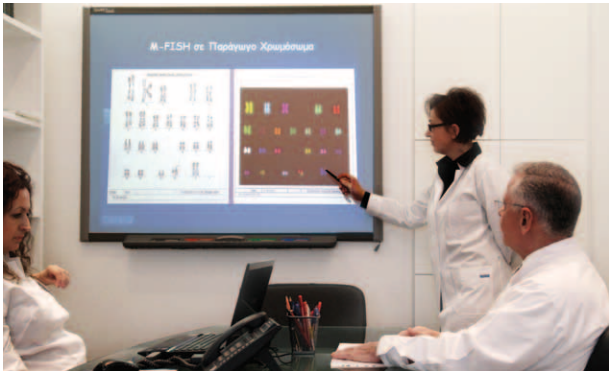
Duplication 2p24
Duplication of the MYCN gene at 2p24 occurs in many neoplasias and also in cases of chronic lymphocytic leukemia (CLL).

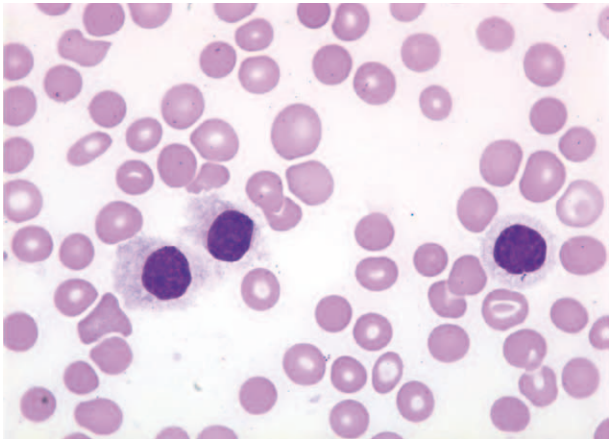
The detection is based on a custom MLPA technique, using multiple probes in the region under investigation.

Deletion 3q27
Deletion 3q27 of chromosome 3 is often related with rearrangements of the BCL6 gene, located in this particular region and occurs in non-Hodgkin lymphomas (NHL).

The deletion is detected through FISH, using specific custom-labeled DNA probes, from at least 100 bone marrow cell nuclei.

Deletion 5q31-q35
Deletions occurring on the long arm of chromosome 5 represent the most common abnormality in myelodysplastic syndrome (MDS)





and acute myeloid leukemia (AML). Aberrations involving chromosome 5 are observed in 42% of patients undergoing treatment for MDS. Deletions are usually large, with breakpoints located between 5q31-5q34, including the EGR1 and CSF1R genes.

The detection is based on a custom MLPA technique, using multiple probes in the region under investigation.

Deletion 6q23-26

Deletion of the long arm of chromosome 6 is frequently observed in acute lymphocytic leukemia (ALL), in chronic lymphocytic leukemia (CLL), in prolymphocytic leukemia and non-Hodgkin lymphomas. The deletion often includes the MYB gene.

The detection is based on a custom MLPA technique, using multiple probes in the region under investigation.

Deletion 7p12.2

Deletions of the IKZF1 gene (Ikaros), on chromosome 7p12.2 are detected in almost all acute lymphoblastic leukemia (ALL) patients, and especially in the cases which also bear the hybrid BCR-ABL. IKZF1 deletions in ALL cases have generally been associated with recurrence and poor clinical outcome. Deletions of IKZF1 may also participate in other hematological malignancies, since Ikaros proteins are active throughout the differentiation of B-lymphocytes.

The detection is based on a custom MLPA technique, using multiple probes in the region under investigation.

Deletion 7q22-q36

Deletions of the long arm of chromosome 7 occur frequently in myelodysplastic syndromes (MDS), in acute myeloid leukemia (AML), in a percentage of 5-10% for *de novo* AML cases, in ~15% of adults affected with MDS and in ~40% of children and ~50% of adult patients treated for MDS/AML. The two most frequent deletions are the ones located at the 7q22.1 and 7q31 regions.

The detection is based on a custom MLPA technique, using multiple probes in the region under investigation.

Deletion/duplication 8q24

Copy number aberrations at chromosomal region 8q24 occur frequently in lymphomas and typically include the MYC gene.

The detection is based on a custom MLPA technique, using multiple probes in the region under investigation.

Deletion 9p21.3

Deletion of the 9p21.3 region of chromosome 9 occur in acute lymphocytic leukemia (ALL), with a frequency of ~10% and is associated with CDKN2A-2B genes.

The detection is based on a custom MLPA technique, using multiple probes in the region under investigation.

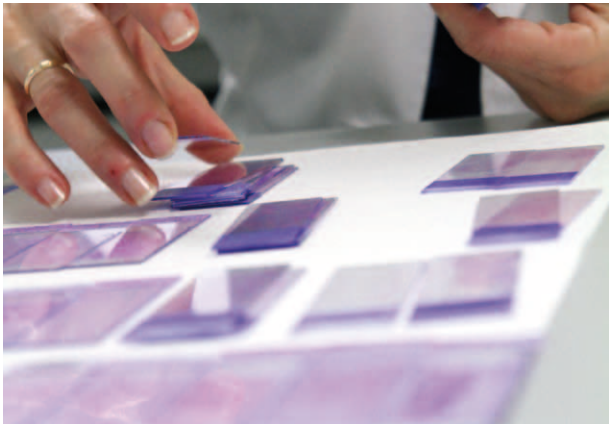
Deletion 10q23
Deletion 10q23 on chromosome 10 occurs typically in chronic lymphocytic leukemia (CLL) and is related to the PTEN tumor suppressor gene.

The detection is based on a custom MLPA technique, using multiple probes in the region under investigation.

Deletion 11q22.3
Deletions in the broader chromosomal region 11q22.3, which include the ATM gene, occur in B-chronic lymphocytic leukemia (B-CLL) with a frequency of ~14%.

The detection is based on a custom MLPA technique, using multiple probes in the region under investigation.

Deletion 13q14
Deletions at chromosome 13q14 include the RB1 gene and occur often in multiple myeloma, in



20% of cases of B-chronic lymphocytic leukemia (B-CLL) as well as in non-Hodgkin lymphoma.

The detection is based on a custom MLPA technique, using multiple probes in the region under investigation.

Deletion 17p13
Deletion of the 17q13 region of chromosome 17 includes the TP53 gene and is observed in 17% of all cases with B-chronic lymphocytic leukemia (B-CLL), in 3-4% of cases with acute myeloid leukemia (AML) and in myelodysplastic syndromes (MDS).
The detection is based on a custom MLPA technique, using multiple probes in the region under investigation.

Deletion 20q12
Deletion of the long arm of chromosome 20 occurs in approximately 4.1% and 1.5% of myelodysplastic syndrome (MDS) cases and acute myeloid leukemia (AML) cases, respectively. It is also observed in ~10% of cases with chronic myeloproliferative disorder (MPD).

The deletion is detected through FISH, using specific custom-labeled DNA probes, from at least 100 bone marrow cell nuclei.

Copy number changes of the 21q22.1 region
Changes in the number of copies (duplications - amplifications) involving the RUNX1 gene on chromosome 21q22.1 are primarily due to the intrachromosomal amplification of chromosome 21 (iAMP21). Amplifications have been reported in childhood leukemia and clinical studies have shown that all patients with acute lymphoblastic leukemia (ALL) and with an increased number of copies of RUNX1 are at an increased risk of

relapse and a lower survival compared to patients without this genetic aberration.

The detection is based on a custom MLPA technique, using multiple probes in the region under investigation.

Detection of the JAK2 V617F mutation

The 'activating' 1849G>T (V617F) mutation of the JAK2 gene (Janus kinase 2) is a relatively recently described mutation, present mainly (but not exclusively) in individuals with myelodysplasias and is closely related to disorders such as polycythemia vera, thrombocythemia και myelofibrosis.

A specially designed DNA minisiquencing technique is applied, from a peripheral blood or bone marrow sample, with a detection sensitivity of ≥5% of cells harboring the mutation.

Detection of the D835Y and ITD mutations of the FLT3 gene

The D835Y and ITD (internal tandem duplications) mutations of the FLT3 gene (FMS like tyrosine kinase 3) emerge independently and constitute the most common somatic mutations in acute myeloid leukemia (AML). Detection of the above mutations constitutes an important prognostic factor for patients being assessed for risk of possible disease relapse.

A specially designed DNA minisiquencing technique as well as a specially designed fluorescent PCR reaction is applied, from a peripheral blood or bone marrow sample, with a detection sensitivity of ≥5% of cells harboring the mutations.

NPM1 gene mutations

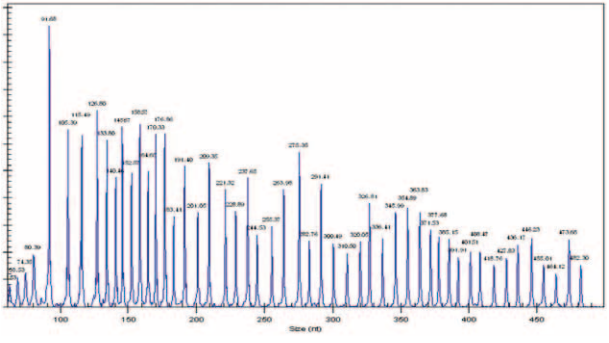
The NPM1 gene encodes a multi-functional phosphoprotein and gene mutations occurring at exon 12 of the gene are common in patients with acute myelogenous leukemia (AML) and a normal karyotype. NPM1 mutations have also been reported in 46-62% of patients with *de novo* AML and a normal karyotype. The mutations in exon 12 are typically frameshift mutations and lead to an anomalous cytoplasmic translocation of the protein.

We apply bidirectional automated fluorescent DNA sequencing of all of exon 12 of the NPM1 gene, for the accurate detection of all possible mutations.

Fanconi's Anaemia - Bone marrow cell/ peripheral blood lymphocyte karyotype analysis

Applied in cases where patients have a Fanconi's anaemia phenotype. Testing includes analysis of at least 300 metaphases of peripheral blood lymphocytes or bone marrow cells, following the application of a special protocol using mitostatic substances.

The test evaluates the percentage of chromosomes exhibiting fragility.



InterGenetics
DIAGNOSTIC GENETIC CENTER

120, Vas. Sofias Av.,
11526 Athens, Greece
T/ (+30) 2107705010 • 2107756588
T/ (+30) 2107705125 • 2104177919
F/ (+30) 2107705011

info@intergenetics.eu
www.intergenetics.eu