Hereditary Cancer in Clinical Practice 2008; 6(2) pp. 73-80

DNA and RNA analyses in detection of genetic predisposition to cancer

Joanna Matyjasik, Bartłomiej Masojć, Grzegorz Kurzawski

International Hereditary Cancer Centre, Department of Genetics and Pathology, Szczecin, Poland

Corresponding author: Grzegorz Kurzawski, International Hereditary Cancer Centre, Department of Genetics and Pathology, Pomeranian Medical University, Połabska 4, 70-115 Szczecin, Poland, e-mail: gkurz@sci.pam.szczecin.pl

Several genes have been identified which, if mutated, are associated with increased predisposition to tumours [1].

Carriers of mutations in these genes show a risk of cancer from a few percent to as high as 90%. Selected genes associated with high inherited predisposition to tumours and those most frequently examined in clinical practice are summarized in Table 1.

Several molecular methods have been devised aimed at detecting mutations. They can be subdivided into methods detecting:

- new mutations,
- known mutations/polymorphisms.

Detection of new mutations

Diagnostics of such mutations in cases appropriately preselected using pedigree and clinical data is justified in clinical practice, even though such techniques are still complex, time-consuming and expensive.

Techniques directly detecting constitutional changes.

DNA analyses

The main kinds of analyses:

- DNA isolation,
- amplification of gene fragments, usually of coding sequences,

Table 1. Genes associated with predisposition to cancer family syndromes. The table contains genes studied the mostly frequently in our centre

Gene localization	Predisposition to malignancies	Penetrance*	Number of exons Protein length
Rb[2] 13q14	**retinoblastoma**	about 90%	27 exons
BRCA1[3] 17q21	**cancers of the breast** **ovaries** prostate colon	about 80%	24 exons, 2 transcripts differing by first exon, 1863 amino acids
BRCA2 13q			27 exons 3418 amino acids
VHL[4] 3p25-26	haemangioblastoma of the cerebellum and retina kidney cancer pheochromocytoma	about 80%	3 exons 213 amino acids
MSH2[5] 2p22	**colon cancer** endometrial cancer	about 90% for male	16 exons 934 amino acids
MLH1 3p22-23	cancer of the stomach, small bowel, biliary tract, ovary	about 70% for female[6]	19 exons 756 amino acids
MSH6 2p16			10 exons 1361 amino acids

*Probability of malignancy during lifetime among mutation carriers

- preliminary detection of changes within amplification products using screening techniques,
- sequencing,
- southern method and multiplex ligation-dependent probe amplification.

DNA isolation

Material for DNA isolation is usually taken from blood leukocytes or less frequently from other tissues. Analyses allow detection of mutations which are constitutional, which means present in all cells of patients. It is especially effective if material for analyses is collected immediately before isolation. However, good results can be achieved even after a few days of storage of blood at room temperature or even storage for a few years at temperature below zero. If fresh tissue is not available, DNA isolation can be performed from tissues fixed in formalin and embedded in paraffin blocks, although achievement of unequivocal results using such material is more difficult and sometimes even impossible. DNA isolation leads to elimination of proteins from cellular lysate. Using the phenol-chloroform method this is achieved by digestion with proteinase K and extraction in a mixture of phenol and chloroform. Finally, nuclear acids are extracted using ethyl- or isopropyl- alcohols. At present, the above technique is in use only exceptionally despite it giving clean and non-degraded DNA (in practice it is used only for DNA isolation from paraffin blocks). Instead, other techniques are used which are less laborious and easier for automation. They are based on selected labelling of DNA with template (e.g. with dyne beads) and then washing in order to liberate DNA into solution.

Amplification of gene fragments

In this analysis DNA fragments are amplified using polymerase chain reaction. The reaction mixture includes: DNA template (usually genomic DNA), DNA polymerase, a pair of specific primers, deoxyribonucleotide triphosphate and the reaction buffer. This mixture is exposed to cyclic changes of temperature. Each cycle includes: denaturation, starters annealing and synthesis. After 22 cycles, assuming 100% effectiveness, the copy number of the amplified fragment is increased one million-fold.

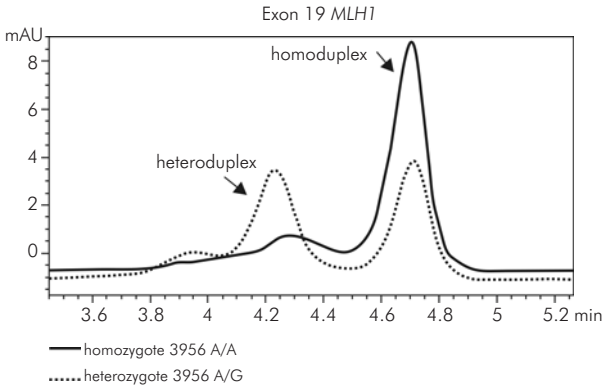

Figure 1. DHPLC elution profile characteristic for A→G mutation in exon 19 of *MLH1* gene

Preliminary detection of changes within amplification products using screening techniques

DNA-SSCP (single strand conformational polymorphism) was in the past the most popular technique of initial detection of changes in amplification products [2].

Other techniques of this kind include heteroduplex analyses (HET) [3], chemical mismatch cleavage (CMC) [4], denaturing high-performance liquid chromatography (DHPLC) [5] and denaturing gradient gel electrophoresis (DGGE) [6].

DHPLC – denaturing high-performance liquid chromatography

At present, the best and the most frequently applied technique of initial detection of changes is DHPLC [5, 7-10]. This is a kind of HET based on high resolution of modern chromatographic columns. Analysed DNA fragments are separated in a gradient of denaturing agent. (The key to DHPLC is the solid phase, which has differential affinity for single and double-stranded DNA.) Under sub-denaturing conditions, heteroduplexes show lower affinity than homoduplexes to the solid phase of the column and it is easier to elute them. Separation is monitored by UV absorption measured at 260 nm. Elution profile (Figure 1) is characteristic and replicable for a given change and allows differentiation between new changes and mutations or polymorphisms known earlier.

Based on literature data [11] and our own experience [12] we can say that DHPLC combines the advantages of several methods. Its sensitivity is close to 100% [5, 9, 10]. At the same time the cost is relatively low (cost of reagents per sample is 5-10 euro). The method is quick, and if an autosampler is used, it allows 200 samples to be analyzed per day.

Sequencing

Sequencing is the most sensitive technique for detection of changes in genomic material, allowing at the same time their full characterization.

Recently, significant progress in sequencing technologies has been achieved by application of automated machines, which function based on fluorescence induced by laser. Each nucleotide (A, C, G, T) can be labelled with different fluorescent dye

(Figure 2). The most convenient technique is the cyclic sequencing method [13].

During analyses the sequences of PCR products for both DNA stands are assessed. The real change is detected in both DNA strands (Figure 3). The sequencing procedure comprises several stages:

- preparative PCR – based on amplification of a chosen fragment of the gene using pairs of specific starters,
- asymmetric PCR – separate amplification with each of the starters using fluorescent dye-labelled dideoxynucleotides,
- electrophoresis in denaturing polyacrylamide gel with simultaneous detection and registration of products,
- analyses of achieved results using computer programs.

During asymmetric PCR all possible oligonucleotides of different length, complementary to the template and containing fluorochromes at the 3'-end are created. They are separated during electrophoresis and the order of coloured nucleotides can be read as the sequence complementary to the template. The detected DNA sequence is compared with the wild sequence available in databases such as GenBank and EMBL. The type of change is described precisely by comparison with wild type sequences (Figures 3, 4).

Currently, the leading companies are offering modern automated DNA sequencing instruments (DNA sequencers) allowing simultaneous sequencing of 96 samples based on capillary electrophoresis of products achieved by the cyclic method using fluorescent dye-labelled dideoxynucleosides. Progress in this discipline has been based recently not only on increasing the number of simultaneously analysed samples but also on improved "chemistry", and gels' composition enabled accurate sequencing of almost one thousand bases of one fragment.

New sequencers ("GSFLX machines 2007") are offered based on real time sequencing by simultaneous synthesis of many short DNA fragments (around two hundred base pairs in length).

They apply pyrosequencing with detection of luminescence occurring during ATP degradation. These machines allow analyses of 100 mln base pairs during one day. Pyrosequencing [14] uses a one strand DNA fragment as the template on which synthesis of a complementary strand is performed through addition of 4 different deoxynucleotides triphosphate (dNTPs). Addition of each base is associated with liberation of pyrophosphate which is transformed into ATP using sulfurylase and adenosine-5'-phosphosulfate. ATP is used by luciferase for transformation of luciferase into oxyluciferin. During this reaction the light is created in an amount corresponding to the amount of pyrophosphate produced early. This light is registered by CCD and transformed into peaks on a diagram. The same

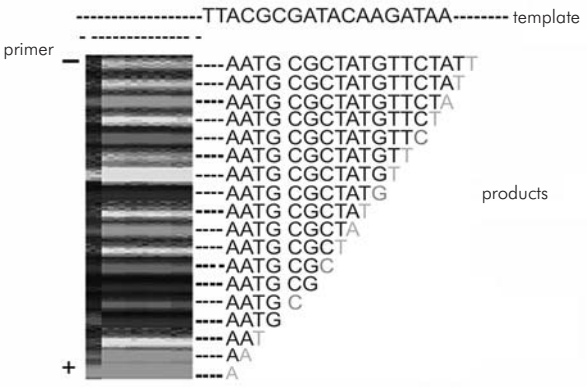

Figure 2. View of sequencing gel – result of resolution of asymmetric PCR products

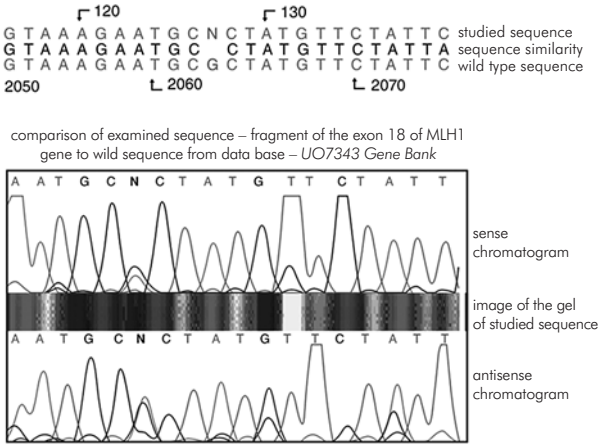

Figure 3. Preliminary analyzes of sequence with mutation in exon 18 of *MLH1* gene

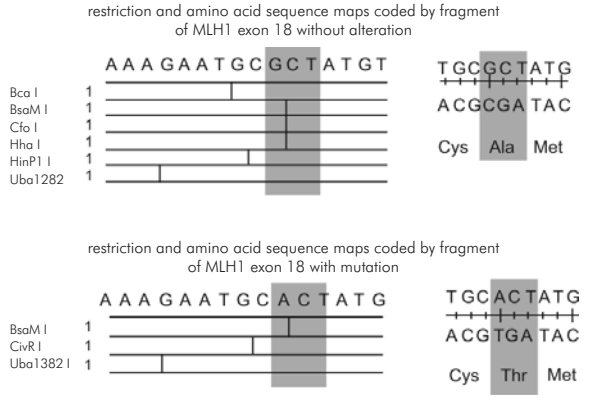

Figure 4. Changes of restriction map and amino acid sequence in protein caused by mutation in exon 18 of MLH1 gene. Mutation leads to lost of restriction place for Bca I, Cfo I, Hha I, HinP I and creates restriction place for Cvi R I, which can be easily used for detection

reaction scheme is valuable for different dNTPs. If added nucleotide is not complementary to the template it is not included in the newly synthesized strand and pyrophosphate is not created. Only the presence of a light signal is a basis for registration of a new nucleotide in a sequence.

DNA mutation covering the sites of primers annealing or other fragments outside of amplified ones are not detected using DNA tests based on analyses described above. Some of such changes are large rearrangements.

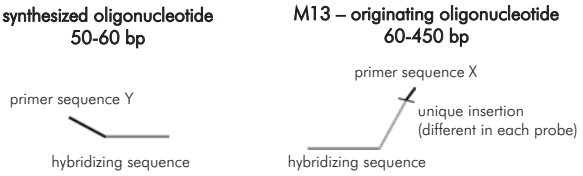

Figure 5. MLPA construction

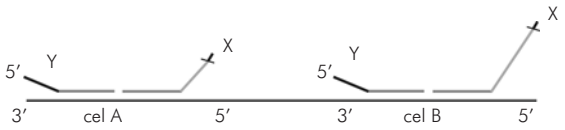

Figure 6. Probe hybridization and ligation

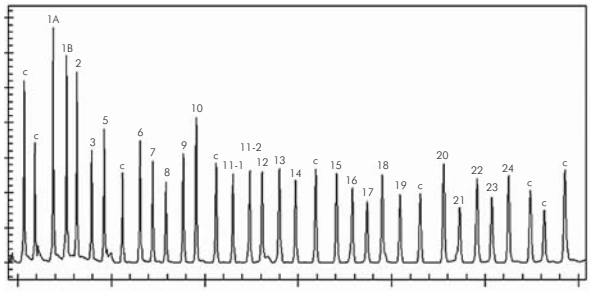

Figure 7. Result of electrophoresis for controls

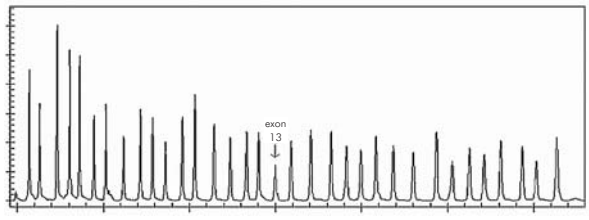

Figure 8. Result of electrophoresis of sample with deletion of exon 13

Southern method and multiplex ligation-dependent probe amplification

A technique very popular in the past for detection of large rearrangements was Southern blotting, described for the first time by E. M. Southern in 1975.

At present the method which has almost completely replaced detection of DNA rearrangements by Southern blotting is multiplex ligation-dependent probe amplification – MLPA [15]. This technique is based on the reaction of ligation of specific probes and the reaction of amplification and allows an assessment of the exon copy numbers to be made. On this basis, conclusions can be formed concerning deletions or duplications of gene fragments or of whole genes.

Many probes in "one tube" simultaneously are used in this technique. Probes matching the sequences complementary to exon sequences also contain primer sequences and one of each pairs additionally a unique insertion sequence called a stuffer sequence (Figure 5).

Hybridizing sequences of each pair of probes match neighbouring DNA fragments and only if hybridization is complete can ligation take place (Figure 6).

After probe hybridization to the template, they are ligated, then denatured. The dissociated ligated probe containing primer sequences is then amplified using PCR. The presence of different length stuffers allows differentiation of products labelling different targets, and the amount of product is proportional to copy number in the template. Each peak corresponds to a product of amplification of specific ligated pairs of probes (Figure 7).

Relative differences in the height or area of the peak indicate quantitative (sometimes qualitative) changes of a target sequence for the probe (Figure 8).

The advantages of this technique are that a small amount of DNA is necessary to perform analyses and the possibility that even degraded genetic material can give efficiently reproducible results.

Commercially available probes include those for the most important genes associated with a high risk of tumours, such as: *ATM*, *BRCA1*, *BRCA2*, *CHEK1*, *MLH1*, *MSH2*, *MSH6*, *PMS2*, *APC*, *FANCA*, *FANCD2*, *PTCH*, *BMPR1A*, *SMAD4*, *TP53*, *CDH1*, *MEN1*, *NF1*, *NF2*, *STK11*, *SMARCB1*, *RB1*, *CDKN2A-CDKN2B*, *WT1*.

RNA analyses

The advantages of RNA analyses are related mainly to the possibility of mutation detection using a lower number of reactions (this is related to the shorter length of RNA in comparison to DNA). So far the main disadvantages of these techniques have included difficulties in achieving reproducible results, lower stability of RNA with mutations and troubles in

interpretation associated with occurrence of RNA alternative splicing.

RNA analyses include a few stages:

- RNA isolation,
- amplification of coding parts of the genes,
- detection of changes in amplification products.

RNA isolation

In the majority of laboratories, RNA is isolated from peripheral blood lymphocytes. RNA isolation is performed similarly to DNA isolation. Due to widespread occurrence of thermostable RNAse in tissues, RNA isolation has to be performed more carefully. It is very popular to use the isolation method by Chomczyński [16] from cellular lysates in a solution of guanidine thiocyanate (RNA inhibitors) followed by extraction in a mixture of phenol and chloroform. Slightly acidic pH of phenol leads to extraction of not only proteins but also of DNA, which under such conditions is practically insoluble.

Reverse transcription PCR

RNA can be transcribed into cDNA (complementary DNA) using reverse transcriptase and then amplified using PCR. RNA does not include introns, so only a few pairs of starters usually allow amplification of the whole coding parts of selected genes. Analysing cDNA on regular agarose gels usually allows detection of RNA abnormalities which are caused by deletions or insertions of over a few dozen base pairs.

Detection of alterations in amplification products

Products of the RT/PCR reaction can be analysed using all of the techniques mentioned above and also by using the in vitro transcription translation (IVTT) assay, also called the protein truncation test (PTT) [17, 18]. RT-PCR is the first stage of PTT. In PTT one primer includes sequences not only initiating transcription into cDNA but also sequences initiating translation – it is protein synthesis "in vitro" on a cDNA template.

After electrophoresis and transmission into membranes the length of synthesized protein is assessed, which is changed not only if within RNA there are large deletions or insertions but also when single nucleotide mutations lead to stop coding (TGA, TAA or TAG) or splicing mutations. The disadvantage of PTT is limitation in detection of mutations of missense type. It is estimated that even if we use all known tests of direct detection of changes their sensitivity does not exceed 70-80%, mainly due to the lack of diagnosis of alterations in unknown sequences regulating functioning.

Detection of known mutations

More and more is known about the type and frequency of mutations predisposing to tumours and characteristic for different populations, including about founder mutations and changes recurrently occurring in many families of a given ethnic group. DNA tests aimed at detecting all known mutations are more and more valuable due to the unusually high economical effectiveness. Such genes as *BRCA1*, *MLH1*, *MSH2* and *VHL* have been studied intensively in many populations and due to this it is known which mutations can be studied first and in which fragments of genes [19-21]. The most frequently applied DNA tests detecting known mutations use the following techniques:

RFLP/PCR (restriction fragment-length polymorphism/PCR)

Restriction enzymes identifying specific sequences of the PCR products are described. This is why they are used for detection of all mutations which lead to loss or creation of restriction sites. Amplified products containing a particular change are digested by restriction enzymes and then separated on agarose (example – Figure 8) or polyacrylamide gels.

ASA – (allele-specific amplification) – detection of mutations using specific oligonucleotides

In the most popular variant of this technique, using agarose electrophoresis, not only flanking primers but also a primer fully complementary to the allele with a mutation or a primer of one which is complementary to the allele with a mutation and another to the wild allele are used. Primers are localized in such a way that different PCR products are of different length depending on the genotype of the examined DNA sample (Figure 9). The modern version of this technique uses short allele-specific probes and machines of real time PCR [22, 23]. This allows very fast analysis of many DNA samples. Technology using a template with oligonucleotides

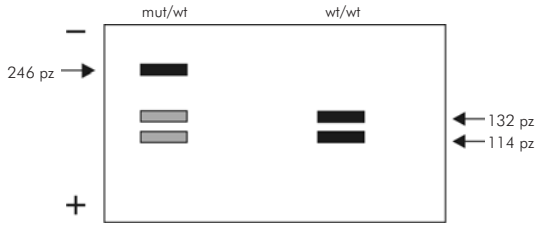

Figure 9. Principle of RLFP/PCR. View of gel after electroforesis – only variant of gene with G of PCR product will be digests by Cfo I

immobilized on a solid phase can be considered as a modern version of ASA. A big advantage of this technology is automation and the possibility to analyse even a few thousand known mutations. In many countries the use of such technology is limited due to high costs.

Real time PCR

One of the most modern and more frequently applied techniques in molecular biology is real time PCR allowing monitoring of the quantity of PCR products in each cycle. A modification of this technique based on application of fluorescent probes and complementary to sequences of examined DNA fragments is applied also for identification of known genetic changes.

There are several systems based on this technique which differ in the type of probe used for detection of analysed changes. Among them, systems applying TaqMan and SimpleProbes can be distinguished.

TaqMan probes

The probe specific to the amplified fragment used in this system is labelled at the 5′ end by reporter dye: FAM (6-carboxy-fluorescein), HEX (hexachloro-6-carboxy-fluorescein), TET (tetrachloro-6-carboxy-fluorescein) or JOE (2,7-dimethoxy-4,5-dichloro-6-6-carboxy-fluorescein) and at the 3′ end by quenching dye: TAMRA (6-carboxy-tetramethyl-rhodamine) or DABCYL (4-(4′-dimethylaminophenylazo)benzoic acid). The short distance between dyes within the same probe leads to quenching of the fluorescence. During the PCR reaction at the stage of primers annealing a labelled probe is linked specifically to a specific template between sites of primer hybridizations. Its 3′ end is unavailable, which means that in the next stage – primers extension – this probe cannot be elongated as with primers. Polymerase used in this system showing 5′-3′ activity degrades the probe during DNA strand building, which leads to release of the reported type from the quenching dye and causes increased fluorescence. This process occurs during each cycle, causing an increase of the fluorescence signal from each cycle, which allows signal detection at each moment of the reaction. Probes used in this system are 20-40 nucleotides in length. The number of G+C pairs in their sequence is 40-60%. Probes should not include single nucleotide repeats, particularly of guanine. Also the sequence of the probe should not be complementary to primer sequences or to sequences of the template at sites of the annealing primers. It is important that the probe does not include the base G at the 5′ end, because its presence quenches reporter dye even after separation of it from quenching. Modification of this system can be done by applying a TaqMan probe of MGB type (Minor Groove Binder), in which the group MGB is fixed to the 3′ end. It protects stabilization of probe annealing by matching the complex resulting from probe and template DNA. Interaction of the MGB group with the probe-template complex increases the temperature of probe melting by 15-30°C, which allows application of probes of much shorter sequence (14-18 nucleotides). This is valuable during analyses of single nucleotide polymorphisms because it is easier to destabilize short probes under the influence of nucleotide changes in the examined sequence.

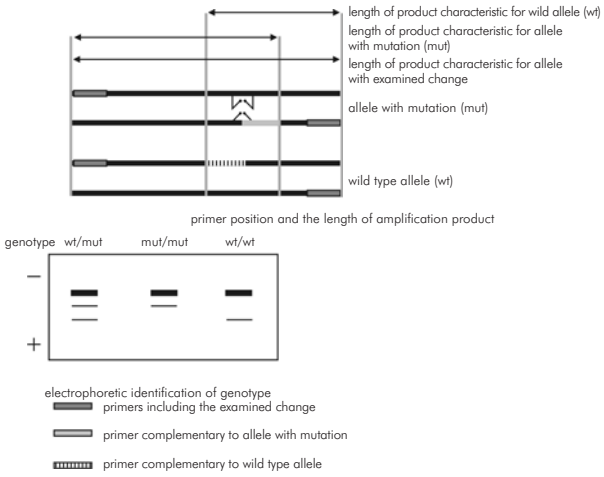

Figure 10. Principle of ASA

Simple probes (guanine quenching probes)

Guanine shows features of particles quenching fluorescence such as FAM or JOE. In this technique we use a short one-strand DNA fragment of 20-30 nucleotides in length (molecular probe) with a sequence complementary to the examined DNA containing the change/mutation labelled at 5′ or 3′ by fluorescent dye (FAM or JOE). This technique allows identification of heterozygote and homozygote variants of change/mutation by measuring the increase in fluorescence achieved in the temperature gradient. The probe hybridizing with the examined sequence is usually of higher melting temperature if it hybridizes with the fully complementary strand and lower melting temperature if an unpaired nucleotide is situated under the probe. Reading of fluorescence level during increase of temperature in the range 40-80°C allows the specific

variant of the examined DNA change to be identified. Application of complementary fluorescent probes has several advantages. They include: high sensitivity, short time and full automation of analyses and low risk of contamination by performing all stages in closed wells. Disadvantages of this technique include the necessity of projecting probes for particular sequences and low generality of experimental conditions.

MALDI-TOF (matrix assisted laser desorption/ionization time of flight)

MALDI-TOF is one of the techniques of mass spectrophotometry, applied for detection of changes within examined DNA fragments. Most frequently it is used for analyses of polymorphisms of single nucleotides (SNP). Analyses are preceded by stages based on PCR reaction. The first leads to amplification of selected fragments (in multiplex version) containing examined SNP. The second is asymmetric (one primer complementary to the sequence close to the polymorphic site), similarly to sequencing with application of dideoxy-nucleotides. After cleaning using ion exchange resin the samples are placed on Spectrochip and finally stimulated using a laser impulse for ion excitation.

Total analysis is performed in vacuum conditions, which means that the ion mobility is not disturbed by colliding gas particles. Speed of movement of exited ions from examined probes is analysed by a detector of the time of ion flight. Ions from longer mass reach the detector more slowly than ions from a smaller mass. Nucleotide differences occurring in examined probes are correlated with mass, which allows their differentiation. Separation of analyzed particles is performed based on the ratio of ion mass to their electric charge. This techniques is characterised by high sensitivity and permits a short time of analysis. Sequenome MassARRAY (www.sequenom.com) based on MALDI-TOF allows up to 76 thousand genotypes in one day. Only the high costs of the machine cause the low popularity of this technique.

Summary

The introduction of liquid handling robots made possible their application in DNA or RNA isolation, normalization of samples' concentration, PCR preparation, etc. Parallel development of software and hardware enabled complete automatic management of large sample series in genetic testing, including data transfer without any user intervention. Nowadays leading companies offer capillary sequencers analyzing simultaneously up to 96 samples amplified by means of cycling sequencing with fluorescent dyes. Improved "chemistry" and gels' composition enabled accurate sequencing of fragments of 1000 bp in length. Another system (GSFLX machine 2007), based on massive parallel sequencing by cyclic technology, can generate 100 Mb of genomic sequence in a single run (about 4.5 hours). Real-time PCR techniques with TaqMan® probes (or TaqMan® MGB probes) have become commonly used in laboratory practice. MLPA technique used in detection of rearrangements in genes associated with hereditary cancers allows the determination of exon copy number. The presence of deletions or duplications of exons or whole genes can be analyzed by that method. Commercial kits are available for genes with a well-documented association with hereditary cancers: *ATM*, *BRCA1*, *BRCA2*, *CHECK1*, *MLH1*, *MSH2*, *MSH6*, *PMS2*, *APC*, *FANCA*, *FANCD2*, *PTCH*, *BMPR1A*, *SMAD4*, *TP53*, *RB1*, *CDKN2A-CDKN2B*, *WT1*, *CDH1*, *MEN1*, *NF1*, *NF2*, *STK11*, *SMARCB1*.

References

1. Lubiński J, Górski B, Kurzawski G, Jakubowska A, Cybulski C, Suchy J, Debniak T, Grabowska E, Lener M, Nej K. Molecular basis of inherited predispositions for tumors. Acta Biochim Pol 2002; 49: 571-581.
2. Orita M, Iwahana H, Kanazawa H, Hayashi K, Sekiya T. Detection of polimorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. Proc Natl Acad Sci USA 1989; 86: 2766-2770.
3. Nagamine CM, Chan K, Lau YFCA. A PCR artifact: generation of heteroduplexes. Am J Hum Genet 1989; 45: 337-339.
4. Cotton RG, Rodrigues NR, Campbell RD. Reactivity of cytosine and thymine in single-base-pair mismatches with hydroxylamine and osmium tetroxide and its application to the study of mutations. Proc Natl Acad Sci USA 1988; 85: 4397-4401.
5. O'Donovan MC, Oefner PJ, Roberts SC, Austin J, Hoogendoorn B, Guy C, Speight G, Upadhyaya M, Sommer SS, McGuffin P. Blind analysis of denaturing high-performance liquid chromatography as a tool for mutation detection. Genomics 1998; 52: 44-49.
6. Myers RM, Maniatis T, Lerman LS. Detection and localization of single base changes by denaturing gradient gel electrophoresis. Methods Enzymol 1987; 155: 501-527.
7. Liu W, Smith DI, Rechtzigel KJ, Thibodeau SN, James CD. Denaturing high performance liquid chromatography (DHPLC) used in the detection of germline and somatic mutations. Nucleic Acids Res 1998; 26: 1396-1400.
8. Jones AC, Austin J, Hansen N, Hoogendoorn B, Oefner PJ, Cheadle JP, O'Donovan MC. Optimal temperature selection for mutation detection by denaturing HPLC and comparison to single-stranded conformation polymorphism and heteroduplex analysis. Clin Chem 1999; 45: 1133-1140.
9. Arnold N, Gross E, Schwarz-Boeger U, Pfisterer J, Jonat W, Kiechle M. A highly sensitive, fast and economical technique for mutation analysis in hereditary breast and ovarian cancers. Hum Mutat 1999; 14: 333-339.
10. Gross E, Arnold N, Goette J, Schwarz-Boeger U, Kiechle M. A comparison of BRCA1 mutations analysis by direct sequencing, SSCP and DHPLC. Hum Genet 1999; 105: 72-78.

11. Xiao W, Oefner PJ. Denaturing high-performance liquid chromatography: A review. Hum Mutat 2001; 17: 439-474.
12. Kurzawski G, Safranow K, Suchy J, Chlubek D, Scott RJ, Lubiński J. Mutation analysis of MLH1 and MSH2 genes performed by denaturing high-performance liquid chromatography. J Biochem Biophys Methods 2002; 51: 89-100.
13. Rosenthal A, Charnock-Jones DS. New protocols for sequencing with dye terminators. DNA Seq 1992; 3: 61-64.
14. Ronaghi M, Karamohamed S, Pettersson B, Uhlén M, Nyrén P. Real-time DNA sequencing using detection of pyrophosphate release. Anal Biochem 1996; 242: 84-90.
15. Schouten JP, McElgunn CJ, Waaijer R, Zwijnenburg D, Diepvens F, Pals G. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. Nucleic Acids Res 2002; 30: e57.
16. Chomczynski P, Sacchi N. Single step method of RNA isolation by acid guanidinum thiocyanate-phenol-chloroform extraction. Anal Biochem 1987; 162: 156-159.
17. Luce MC, Marra G, Chauhan DP, Laghi L, Carethers JM, Cherian SP, Hawn M, Binnie CG, Kam-Morgan LNW, Cayouette MC, Koi M, Boland CR. In vitro transcription/translation assay for the screening of hMLH1 and hMSH2 mutations in familial colon cancer. Gastroenterology 1995; 109: 1368-1374.
18. Plumer SJ, Casey G. Are we closer to genetic testing for common malignances? Nat Med 1996; 2: 156-158.
19. Kurzawski G, Suchy J, Kładny J, Safranow K, Jakubowska A, Elsakov P, Kucinskas V, Gardovski J, Irmejs A, Sibul H, Huzarski T, Byrski T, Debniak T, Cybulski C, Gronwald J, Oszurek O, Clark J, Góźdź S, Niepsuj S, Słomski R, Pławski A, Łacka-Wojciechowska A, Rozmiarek A, Fiszer-Maliszewska Ł, Bebenek M, Sorokin D, Stawicka M, Godlewski D, Richter P, Brozek I, Wysocka B, Jawień A, Banaszkiewicz Z, Kowalczyk J, Czudowska D, Goretzki PE, Moeslein G, Lubiński J. Germline MSH2 and MLH1 mutational spectrum in HNPCC families from Poland and the Baltic States. J Med Genet 2002; 39: E65.
20. Cybulski C, Krzystolik K, Murgia A, Górski B, Dębniak T, Jakubowska A, Martella M, Kurzawski G, Prost M, Kojder I, Limon J, Nowacki P, Sagan L, Białas B, Kałuża J, Zdunek M, Omulecka A, Jaskólski D, Kostyk E, Koraszewska-Matuszewska B, Haus O, Janiszewska H, Pecold K, Starzycka M, Słomski R, Cwirko M, Sikorski A, Gliniewicz B, Cyrylowski L, Fiszer-Maliszewska L, Gronwald J, Tołoczko-Grabarek A, Zajączek S, Lubiński J. Germline mutations in the von Hippel-Lindau (VHL) gene in patients from Poland: disease presentation in patients with deletions of the entire VHL gene. J Med Genet 2002; 39: E38.
21. Górski B, Byrski T, Huzarski T, Jakubowska A, Menkiszak J, Gronwald J, Pluzanska A, Bębenek M, Fischer-Maliszewska L, Grzybowska E, Narod SA, Lubiński J. Founder mutations in the BRCA1 gene in Polish families with breast-ovarian cancer. Am J Hum Genet 2000; 66: 1963-1968.
22. Heied CA, Stevens J, Livak KJ, Williams PM. Real time quantitative PCR. Genome Res 1996; 6: 986-994.
23. Matsubara Y, Fujii K, Rinaldo P, Narisawa K. A fluorogenic allele-specific amplification method for DNA-based screening for inherited metabolic disorders. Acta Paediatr Suppl 1999; 88: 65-68.