

MINI REVIEW

# Laboratory techniques for the detection of mutations in DNA

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Mutations in DNA are the origin of all genetic variation, normal and abnormal. Of course, the determination whether a given mutation is advantageous, neutral or deleterious occurs at the phenotypic level, where selection operates. At the DNA level there is no value judgment on mutations. The same techniques can be used for the molecular characterization of genetic diseases and for the study of genetic polymorphisms. In both, we have the formidable problem of discerning a small molecular event, very often a single base change or deletion, in a background of the 3 billion base pairs that constitute the human genome. Of course DNA sequencing will detect all mutations. Unfortunately sequencing is still relatively slow and cumbersome, although it has become much friendlier after the introduction of automatic sequencers. Thus, in the past few years a myriad of new techniques have been developed for the detection of mutations in DNA, most of them based on the polymerase chain reaction (PCR).

While several useful technologies for point mutation detection exist (Cotton, 1997), no single method is applicable to all situations. Many different and often complementary technical approaches have been developed to solve this conundrum. The several techniques that have been developed form a menu, from which we can choose the one that optimally suits the specific problem at hand. In this review we will try to systematize this menu, discussing the suitable application of each option, as well as the advantages and disadvantages. Unfortunately, space does not allow us to enter

into exquisite details of each methodology, nor to be thoroughly comprehensive and discuss every technique or every point of view. The techniques will thus be presented in a highly telegraphic tabular form, with references for further reading for those interested in a particular technique (Tables I-V). Two recent books completely review this burgeoning field (Landegren, 1996; Taylor, 1997). One further point is that readers should be prepared to digest a large number of acronyms, which abound in the marketing conscious mutation detection domain. Indeed, the acronym IPA has been coined to describe this Incredible Proliferation of Acronyms (H. Ehrlich, personal communication).

We will separate the techniques for the detection of mutations into two broad classes. The first, which we will call 'screening methods', consists of methods capable of scanning DNA segments for unknown mutations. The second class comprehends techniques used to identify the absence or presence of previously known mutations in target fragments, which we will denominate 'diagnostic methods'. Obviously, some techniques may be used either as screening and/or diagnostic methods. An example is the technique of low-stringency single specific primer PCR (LSSP-PCR) which was developed by our research group (Pena *et al.*, 1994; Pena and Simpson, 1996; Simpson and Pena, 1997). LSSP-PCR is a simple but extremely potent PCR-based technique that detects single or multiple mutations in gene-sized fragments of lengths up to 1 Kb. When applied to a gel-purified DNA fragment LSSP-PCR translates the underlying DNA sequence into a unique and informative multiband 'gene signature'. Changes as small as single mutations alter significantly the signature, producing a profile that is diagnostic of the specific alteration. LSSP-PCR can be used either as screening or diagnostic method and it has been used for the detection of mutations in human genetic diseases (Pena *et al.*, 1994), for the study of

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sequence variation in human mitochondrial DNA (Barreto *et al.*, 1996) and for genetic typing of infectious agents such as Papilloma virus (Villa *et al.*, 1995), *Trypanosoma cruzi* (Vago *et al.*, 1996) and *Entamoeba histolytica* (Gomes *et al.*, in press).

The whole mutation detection field is undergoing a revolution with the recent development of techniques based on hybridization to oligonucleotides immobilized in silicon microchips called DNA chips or biochips (Editorial, 1996). On the surface of a microchip of only 1.28 x 1.28 cm it is possible to synthesize as many as 409,600 different oligonucleotides. The target DNA is fluorescently labeled and hybridized to the array, thus binding to sequence-specific constellations of oligonu-

cleotides. The binding is quantified in a robotic station by confocal fluorescent microscopy and the results are analyzed automatically by computer. With one specific biochip containing "only" 66,276 oligonucleotides it is possible to determine all the nucleotide sequences of the mitochondrial DNA of an individual in less than 10 minutes (Chee *et al.*, 1996)! Biochips are being developed to determine with absolute certainty all mutations in *BRCA1* (Hacia *et al.*, 1996) and to unravel gene expression patterns in human normal and tumor tissues (Shumaker *et al.*, 1996; DeRisi *et al.*, 1996). In the not too distant future we may be able, with just a few biochips, to scan whole human genomes, looking for mutations known to be associated with disease.

**Table I** - Screening methods - electrophoretic techniques.

Method	Acronym	Molecules	Size of fragments	Efficiency of detection	Precise localization	Advantages	Disadvantages	References
Single strand conformation polymorphism	SSCP	Single-stranded RNA or DNA	200-300 pb	Up to 90%	No	Rapid and simple; no special equipment needed	Low sensitivity; better for short fragments; conditions require careful selection	Orita, 1989; Hayashi, 1996
Restriction endonuclease fingerprinting	REF	Single-stranded DNA	Up to 1 Kb	Estimated at 100%	Yes	High sensitivity; high informative content	Use of radioactivity; use of several enzymes	Liu and Sommer, 1995
Dideoxy fingerprinting		Single-stranded RNA	150-500 pb	Estimated at 100%	Yes	High sensitivity; determines the nature and position of the mutations	Uses radioactivity; better for short fragments; requires <i>in vitro</i> transcription	Sarkar <i>et al.</i> , 1992
Low-stringency single specific primer PCR	LSSP-PCR	Double-stranded DNA	At least up to 1 Kb	Estimated at 100%	No	Rapid, simple, precludes special equipment	Work intensive; danger of contamination; difficult analysis	Pena <i>et al.</i> , 1994; Pena and Simpson, 1996; Simpson and Pena, 1997
Heteroduplex analysis	HA	Double-stranded heteroduplex DNA:DNA	200-300 pb	Up to 90%	No	Rapid and easy	Low sensitivity; better for short fragments	White <i>et al.</i> , 1992; Santos <i>et al.</i> , 1995
Universal heteroduplex generator	UHG	Double-stranded heteroduplex DNA:DNA	200-300 pb	Up to 90%	No	Rapid and easy	Low sensitivity; analysis of short fragments	Wood <i>et al.</i> , 1993; Wood and Bidwell, 1996
Denaturing high-performance liquid chromatography	DHPLC	Double-stranded heteroduplex DNA:DNA	ND	ND	No	High speed; detection of heterozygotes; subject to automation	Better for short fragments; requires special equipment (HPLC)	Underhill <i>et al.</i> , 1996
Denaturing gradient gel electrophoresis	DGGE	Double-stranded homoduplex DNA:DNA heteroduplex DNA:DNA	500 pb	Up to 90%	No	Easy application after initial setting up; detection of heterozygotes	Efforts required to standardize the technique; use of special primers	Myers <i>et al.</i> , 1985; Sheffield <i>et al.</i> , 1989; Sheffield <i>et al.</i> , 1991
Constant denaturation capillary electrophoresis	CDCE	Double-stranded heteroduplex DNA:DNA	100 pb	Estimated at 100%	No	Detection of rare mutants; subject to automation	Better for short fragments; use of special equipment	Khrapko <i>et al.</i> , 1994

ND, Not determined.

Table II - Screening methods - cleavage techniques.

Method	Acronym	Molecule	Size of fragments	Efficiency of detection	Precise localization	Advantages	Disadvantages	References
Chemical cleavage of mismatch	CCM	Double-stranded heteroduplex DNA:DNA	Up to 1-2 Kb	Estimated at 100%	Yes	High sensitivity; analysis of large fragments	Use of hazardous chemicals; multi-step and labor intensive	Grompe <i>et al.</i> , 1989; Cotton, 1989; Ramus and Cotton, 1996
Fluorescent assisted mismatched analysis by chemical cleavage	FAMA	Double-stranded heteroduplex DNA:DNA	Up to 1-2 Kb	Estimated at 100%	Yes	High sensitivity; rapid; subject to automation	Use of special equipment (automated DNA sequencer)	Verpy <i>et al.</i> , 1994
Multiplex solid phase fluorescent chemical cleavage	FCC	Double-stranded heteroduplex DNA:DNA DNA:RNA	Up to 1-2 Kb	Estimated at 100%	Yes	High sensitivity; simultaneous analysis of multiple loci; can be automated	Use of special equipment (automated DNA sequencer)	Harris, 1994; Green <i>et al.</i> , 1996
Ribonuclease cleavage	RNAse cleavage	Double-stranded heteroduplex RNA:RNA RNA:DNA	Up to 1 - 2 Kb	Up to 60%	Yes	Analysis of large fragments	Use of radioactivity; low sensitivity; multi-step method; requires a step of cloning	Prosser, 1995
Enzyme mismatch cleavage	EMC	Double-stranded heteroduplex DNA:DNA	Up to 1-2 Kb	ND	Yes	High specificity; detection of rare mutants	Use of radioactivity; analysis of short fragments	Youil and Cotton, 1996
Mismatch repair enzyme cleavage	MREC	Double-stranded heteroduplex DNA:DNA	100-200 pb	Estimated at 100%	Yes	High specificity; detection of rare mutants	Use of radioactivity; better for short fragments	Lu and Hsu, 1992; Lu, 1996
Protection assay with MutS		Double-stranded heteroduplex DNA:DNA	200-500 pb	ND	No	High specificity; detection of rare mutants and heterozygotes; can be automated	Instability of DNA - MutS complex	Ellis <i>et al.</i> , 1994; Lishanski <i>et al.</i> , 1994

ND, Not determined.

Table III - Diagnostic methods based on hybridization of oligonucleotides.

Method	Acronym	Molecule	Size of fragments	Advantages	Disadvantages	References
Allele specific oligonucleotide	ASO	Double-stranded DNA	Usually 200-1000 bp	Dot-blot: simultaneous analysis of a large number of samples; reverse dot-blot: simultaneous analysis of a great number of mutations	Use of radioactivity; effort required to standardize the hybridization conditions; possible false positive and negative results	Conner <i>et al.</i> , 1983; Gyllenstein and Allen, 1996
Oligonucleotide ligation assay	OLA	Double-stranded DNA	ND	Simultaneous analysis of a large number of samples; detection of heterozygotes; use of solid supports; can be automated	Use of special equipment; use of special protocol for probe labeling	Landegren <i>et al.</i> , 1988; Samiotaki <i>et al.</i> , 1994
Exonuclease amplification coupled capture technique	EXACCT	Double-stranded DNA	300 pb	Simultaneous analysis of a large number of samples; non-isotopic labeling; can be automated	Better for short fragments; rigorous control of the digestion with the exonuclease	Holloway <i>et al.</i> , 1993

ND, Not determined.

Table IV - Diagnostic methods based on amplification with oligonucleotides

Method	Acronym	Molecule	Size of fragments	Advantages	Disadvantages	References
PCR amplification of specific alleles; allele specific oligonucleotide-PCR; allele-specific amplification; amplification refractory mutation system	PASA, ASO-PCR, ASA, ARMS	Genomic DNA, mRNA, cDNA	Apparently unlimited	Non-isotopic, simple and rapid; detection of heterozygotes	Efforts for the optimization of PCR; use of several primers; analysis based in electrophoretic procedures	Sommer, 1996; Okayama <i>et al.</i> , 1989; Wu <i>et al.</i> , 1989; Newton <i>et al.</i> , 1989
Ligase chain reaction; ligase detection reaction	LCR, LDR	Genomic DNA	Apparently unlimited	Non-isotopic, simple and rapid; detection of heterozygotes; use of solid supports; analysis of a large number of samples; can be automated	Analysis based in electrophoretic procedures	Barany, 1991; Abravaya <i>et al.</i> , 1995; Wallace <i>et al.</i> , 1996

Table V - Diagnostic methods based on extension from oligonucleotides.

Method	Acronym	Molecule	Size of fragments	Advantages	Disadvantages	References
Single nucleotide primer extension	SNUPE	Double-stranded homoduplex DNA:DNA	200-400 pb	Determines the nature and the position of the mutations	Use of radioactivity; need of purification of PCR products	Kuppuswamy <i>et al.</i> , 1991; Lin and Lin, 1992
Solid-phase mini-sequencing		Double-stranded homoduplex DNA:DNA	ND	Detection of heterozygotes; use of solid supports; simultaneous analysis of a large number of samples; can be automated	Use of radioactivity	Ikonen <i>et al.</i> , 1992; Syvänen, 1996
Multiplex solid-phase fluorescent primer extension		Double-stranded homoduplex DNA:DNA	ND	Detection of heterozygotes; use of solid supports; simultaneous analysis of a large number of loci; can be automated	Use of special equipment (automated DNA sequencer)	Shumaker <i>et al.</i> , 1996

ND, Not determined.

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