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REVIEW ARTICLE

# HLA ALLELE DETECTION USING MOLECULAR TECHNIQUES

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## SUMMARY

There are now many molecular biological techniques available to define HLA class I and class II alleles. Some of these are also applicable to other human polymorphic genes, in particular to those non-HLA genes encoded within the Mhc.

The range of techniques available allows laboratories to choose those most suited to their purpose. The routine laboratory supporting solid organ transplants will need to type large numbers of potential recipients over a period of time, probably using PCR-SSOP while donors will be typed singly and rapidly using PCR-SSP with HLA allele compatibility determined by heteroduplex analysis. Laboratories supporting bone marrow transplantation, where time is less pressing, can choose from the whole range of techniques to determine accurately donor recipient Mhc compatibility.

For disease studies, techniques defining precise HLA allele sequence polymorphisms are needed and high sample numbers have to be accommodated. When an association is established allele sequencing has to be used.

In the near future, the precise role of HLA alleles in transplantation and disease susceptibility is likely to be established unambiguously.

KEY WORDS HLA typing PCR HLA and disease Histocompatibility

## INTRODUCTION

The human major histocompatibility complex (Mhc) codes for the HLA genes which are the most polymorphic known in man. In many respects, novel human genetic mechanisms have first been identified within the Mhc (Sinnot *et al.*, 1991; Collier *et al.*, 1990; Collier *et al.*, 1993). Our understanding of this genetic region has grown exponentially over a relatively short period of time. This is largely due to the world-wide collaborative efforts of immunogeneticists and transplantation biologists in organising International Histocompatibility Workshops (IHWs). More recently molecular biology and the cloning and sequencing of genes has had a dramatic impact and the invention of

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specific gene amplification by the polymerase chain reaction (PCR)(Saiki *et al.*, 1985; Mullis *et al.*, 1986; Mullis and Faloona, 1987) has revolutionised the routine identification of HLA alleles.

The crystallographic structure of both HLA class I and HLA class II molecules has been established, and with that has come an understanding of how they restrict the presentation of peptides to the T cell receptors. The fundamental role of HLA molecules in modelling the repertoire of T cell receptors driving thymic education is now clearly established. The importance of HLA alleles and specificities in both health and disease is unquestionable as is their relevance to clinical transplantation. Hence, there are few aspects of medicine where Mhc genetics does not have some involvement.

The molecular techniques employed in HLA allele typing began with the detection of linked restriction fragment length polymorphisms (RFLPs) to identify HLA-DR specificities at the gene sequence level. This approach was necessitated by difficulties in HLA-DR serological typing, due to the limited availability of suitable antisera and the often poor quality of target cell preparations. Many laboratories have introduced RFLP typing to their routine service but it provides only a limited increase in definition of specificities and it is a lengthy and tedious procedure. As such it is unsuited to prospective matching of organ donors to recipients. Furthermore as the restriction site is outside the HLA-DRB1 second exon hypervariable region (HVR), it is particularly important to establish RFLP patterns in reference panels before assigning HLA specificities. This is vital when studying non-caucasoid populations since HLA-DRB1 RFLPs were originally identified in caucasoids. In just a few years the use of RFLP typing has been superseded by more sophisticated, rapid and high resolution molecular methods. These have arisen as HLA alleles have been sequenced (Marsh *et al.*, 1992) and PCR based techniques introduced. A variety of PCR based techniques have been developed for HLA allele typing but an aim of this review is to highlight those which are most widely used.

## OVERVIEW OF TECHNIQUES

The PCR permits selective *in vitro* amplification of specific DNA sequences. The reaction involves repeated cycles of heat denaturation of double stranded DNA, annealing of primers designed to hybridise specifically to regions flanking the DNA sequence of interest and extension of the annealed primers with the enzyme DNA polymerase. New DNA sequence and primer binding sites are generated allowing further cycles of amplification. The outcome is an exponential accumulation of specific target sequences. The PCR allows specific amplification of HLA gene HVRs and subsequently identification of sequence polymorphisms or alleles in a number of ways.

One widely adopted strategy is PCR amplification of HLA HVR sequence followed by hybridisation on nylon membrane with a series of sequence specific oligonucleotide probes (SSOPs). The SSOPs are designed to detect the different sequences coded within the allele HVRs. Washing conditions can be designed to ensure that even single base pair mismatches between the PCR product and the SSOP can be detected. Typing for HLA alleles by PCR-SSOP is a relatively cheap, rapid and reliable method allowing for processing of large numbers of samples but it is unsuited to typing single cadaveric organ donors where there are time constraints.

The techniques of PCR amplification of alleles using sequence specific primers (PCR-SSP) (Olerup and Zetterquist, 1992) or amplification refactory mutation system (ARMS)

(Newton *et al.*, 1989) lend themselves to rapid testing of single individuals. The PCR-SSP method is based on the principle that a PCR primer having a 3' end mismatch with respect to the target DNA template is not elongated in the presence of Taq polymerase as the enzyme lacks 3' to 5' proof-reading and exonuclease activity. Amplification only occurs when primers and template fully match. Using the HLA HVR sequence variation, specific primers have been designed to provide HLA-A, -DRB1, -DQA and -DQB allele typing methods.

## HLA CLASS II GENE POLYMORPHISMS

*a) HLA-DRB1 alleles*

Differences between HLA-DRB1 alleles can be identified using one of a number of techniques (see table 1) although to date most frequently used have been SSOP methods (Angelini *et al.*, 1986; Bugawan *et al.*, 1988; Savage *et al.*, 1993). These were largely stimulated by the XIth IHW when sets of oligonucleotide probes for HLA-DR, -DQ and -DP alleles were produced. Such SSOP reagents have also been designed and distributed within professional groupings such as the British Society for Histocompatibility and Immunogenetics (BSHI) giving the advantage of thorough collaborative testing and standardisation of SSOP kits.

Allele typing with SSOPs involves amplification of the required gene sequence by the PCR; subsequently the amplified products are immobilised onto a nylon membrane, usually in a dot or slot blot format and finally a series of labelled oligonucleotide probes are hybridised to the amplified target (figure 1). Membrane washing can be greatly simplified if all the probes are designed to be the same length so all washes can be performed in tetramethyl ammonium chloride (TMAC) based washing buffers at the same temperature. The reporter system used to label the SSOP is also important. Until recently, many protocols relied on the use of radioactive labelling techniques; there are now large numbers of non-radioactive alternatives available, including horseradish peroxidase (Saiki *et al.*, 1988; Scharf *et al.*, 1991), biotin and digoxygenin labelled probes (Holtke *et al.*, 1990; Kessler *et al.*, 1990; Seibl *et al.*, 1990). Some labels are detected using an antibody and enzyme catalysed chemiluminescent reaction and positive identification of labelled SSOP bound to amplified product is captured by exposure of filter to photographic film. These methods provide a simple, time-efficient and inexpensive technique particularly suited to allele typing on a batch basis but they are not suited to identification of alleles in single samples. An alternative approach is the "reverse dot-blot" technique, the principles of which are the same as for dot blots set out above except that the SSOPs are immobilised onto the membrane and subsequently hybridised with the labelled PCR product (Saiki *et al.*, 1989). Again the PCR products can be labelled using a non-radioactive moiety and subsequently detected using an antibody and enzymatic conversion of a chromogenic or chemiluminescent substrate.

Although SSOP allele typing is a popular method of choice for dealing with large numbers of samples, routine laboratories will ultimately require alternatives which allow greater sample through-put and robustness of technique. Recently, we have successfully converted the dot blot format to an ELISA plate method which lends itself to automation and increased sample through-put. The method we have chosen is based on a gene quantitation technique described by Kohsaka *et al.*, (1993)(figure 2). Briefly the method uses PCR amplification of the required gene sequence using two primers, one of which

Table 1. Molecular methods for identifying HLA polymorphisms.

| METHOD | RESOLUTION | ADVANTAGES | DISADVANTAGES |
|---|---|---|---|
| RFLP | Better than serology. | Identifies HLA-DR and DQ in the same test. Can be done non-radio actively. Inexpensive. | Slow (4–10 days). Relies on linkage disequilibrium for some alleles. Does not identify all subtypes or coding region polymorphisms. Difficult to identify errors in processing. Samples need to be batch processed. |
| PCR-SSOP | High definition. | Applicable to large numbers of samples. Relatively fast (1–2 days depending on the numbers of probes used). Can be done non-radioactively. | Multiple probing required. Not cost-effective for small numbers of samples. Definition of alleles based on patterns of reactivity. |
| PCR-SSOP Reverse dotblot | Same as for SSOP. | Can be used for single samples. | Not suitable for large numbers of samples or typing cadaveric donors. |
| PCR-SSOP by ELISA | Same as for SSOP | Can be automated and suitable for large numbers of samples. | Same as for PCR-SSOP. |
| PCR-SSOP oligo capture | Same as for SSOP | Can be automated and suitable for single samples. | Same as for SSOP. |
| PCR-hetro duplex analysis | Cannot be used to define alleles — only difference between individuals. | Can be used to rapidly (5 hrs) eliminate potential donors. Inexpensive. | Not suitable for HLA typing. |
| PCR-SSP | Presently designed for broad antigen definition similar to serology but can be adopted for high resolution typing. | Relatively fast (3–4 hrs), simple and non-radioactive. | Many PCR reactions involved. Expensive in terms of primers and Taq polymerase. Fast and high capacity thermal cyclers required. Not suitable for large numbers of samples. |
| DNA sequencing based typing | Definition of alleles at sequence level. | Can be used to define new alleles. Can be automated and performed non-radioactively. | Not suitable for large numbers of samples. |

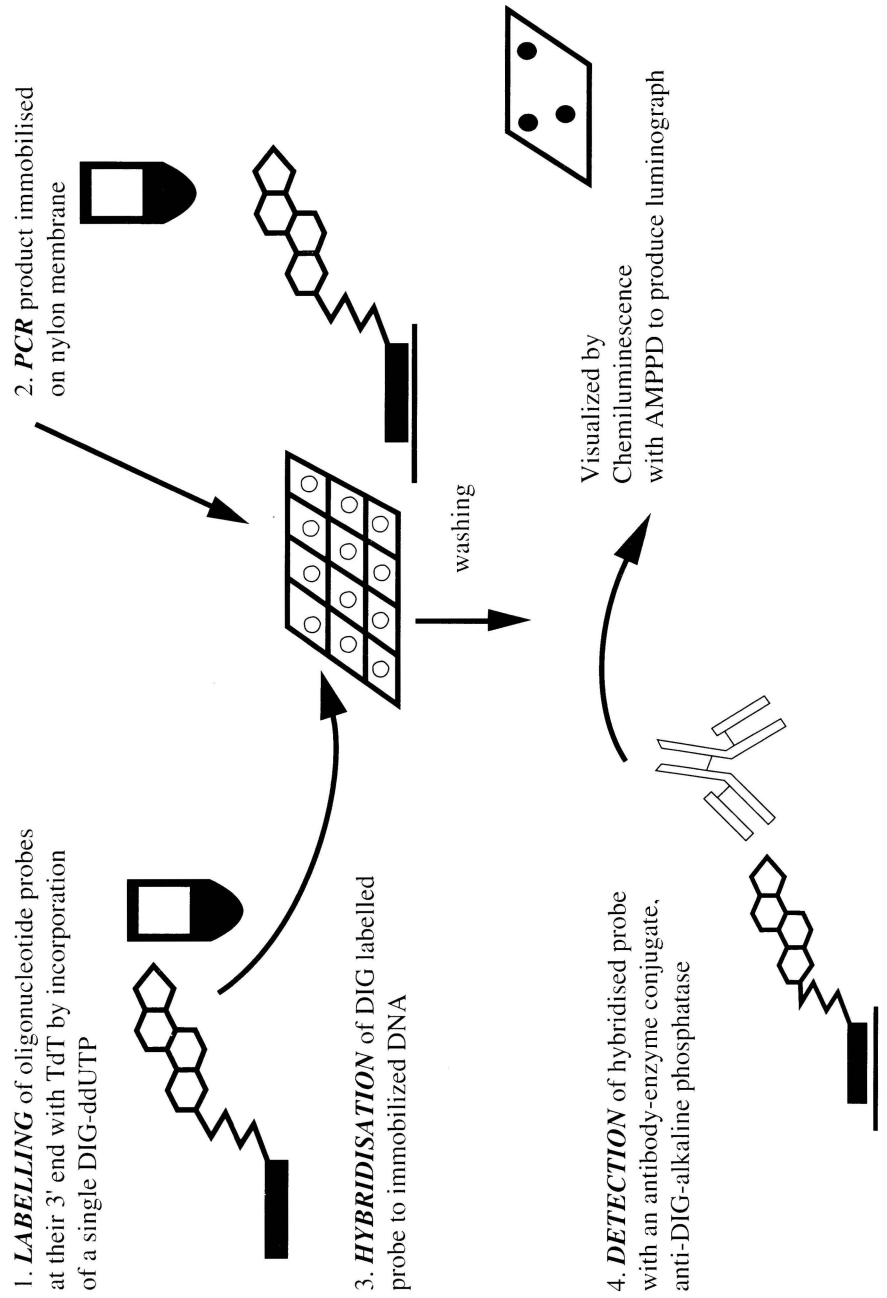


Figure 1. Procedure for PCR/SSOP typing of HLA alleles. From: Thomson and Ollier, 1992.

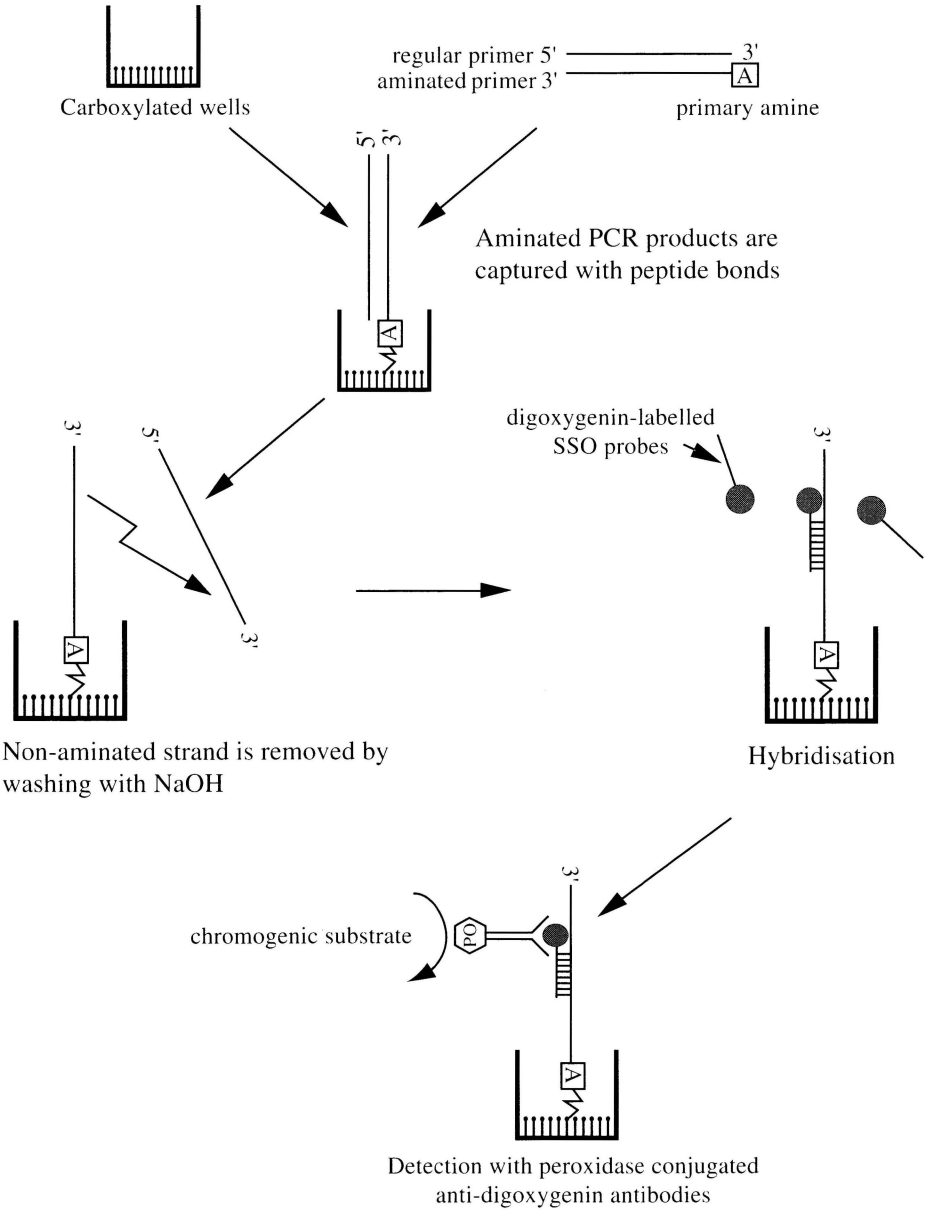


Figure 2. Procedure for ELISA detection of SSOP bound to PCR products for HLA allele typing.

is aminated at the 5' end. The PCR product can then be captured onto carboxylated wells in a microtitre (ELISA) plate through peptide bond formation. The plates are then treated with alkali so that the non-animated strand can be washed away, leaving the aminated strand to be hybridised with digoxygenin (DIG) labelled probes. Biotinylated probes are a less sensitive alternative. The washing of plates is a stage which lends itself easily to automation and bound probes are detected by a DIG specific antibody conjugated to peroxidase and chromogen. Optical densities are recorded using a conventional ELISA plate reader allowing greater standardisation of results. This method will be particularly useful when large numbers of samples need to be tested. It is not cost effective for rapid typing of a single sample and an alternative microtitre plate equivalent of the "reverse dot blot" has been described, the oligocapture technique (Cros *et al.* 1992; Lazaro *et al.*, 1993). In this method the SSOPs are captured on the ELISA plate by passive absorption; the denatured PCR product is hybridised with the captured probe along with an invariant horseradish peroxidase labelled "detection probe." The plates are then washed and positive signals detected via a chromogenic substrate. For these ELISA techniques automation of many of the steps, including washes and automatic plate reading makes them simple and rapid to perform whilst being accurate and relatively inexpensive.

*b) HLA-DR4 subtyping*

To date, fifteen different alleles of the HLA-DR4 specificity have been identified by DNA sequencing (Marsh *et al.*, 1992; Petersdorf *et al.*, 1992, Pile *et al.*, 1992; Tiercy *et al.*, 1993). Some of these alleles are most frequent in specific populations and are associated with autoimmune diseases (Ollier and Thomson, 1992) making precise identification of HLA-DR4 alleles highly relevant. Until recently, a drawback was that in individuals possessing two different HLA-DR4 alleles, some combinations of alleles (e.g. HLA-DRB1*0403/0404, 0407/0408) could not be differentiated using existing HLA-DR4 subtyping techniques (Gao *et al.*,1990; Ju *et al.*,1991; Olerup *et al.*,1992; Lanchbury *et al.*,1990; Sorrentino *et al.*, 1992). Two approaches to resolve this problem are "multiplex ARMS-RFLP" (Jawaheer *et al.*, 1993) and single strand conformation polymorphism (SSCP) typing (Young and Darke, 1993) which allow unambiguous identification of all HLA-DR4 alleles.

The multiplex ARMS-RFLP technique involves two separate ARMS reactions. Primers for human growth hormone may be included as positive controls. We have optimised the PCR cycles further since the technique was developed and they now consist of 2-step cycles: 94° for 20 sec. and 55° for 40 sec. instead of the original 3-step PCR cycles thus reducing the PCR amplification time by a third. The PCR products are visualised by ethidium bromide staining after agarose gel electrophoresis. From the patterns of bands obtained in the two reactions, each sample can be assigned one or two HLA-DR4 alleles.In three instances (HLA-DRB1*0403/0404, -DRB1*0410/0411 and -DRB1*0405/0407/0408) the alleles yield products of the same size and a restriction enzyme digest step is required to differentiate them. A *Sac II* digest is used for HLA-DRB1*0403/0404 and -DRB1*0410/0411 whereas the -DRB1*0405/0407/0408 alleles require a *Hae II/Sac II* double digest.

This multiplex ARMS-RFLP technique has a number of advantages over existing methods of HLA-DR4 subtyping; it is rapid, simple, inexpensive, robust and it can identify unambiguously any of the HLA-DR4 allele sequences in all homozygous or heterozygous combinations.

*c) Typing by allele specific PCR*

An elegant strategy for HLA-DRB1 allele typing has been developed by several groups independently (Olerup *et al.*, 1992; Bein *et al.*, 1992) and for HLA-DQA, -DQB allele typing (Olerup *et al.*, 1993; Bunce *et al.*, 1993a). These approaches involve the design of specific oligonucleotide primers for PCR amplification which will amplify exclusively a single allele sequence. Techniques have been described which permit detection of the serologially defined specificities HLA-DR1 to -DR18 solely by the detection of PCR products in reaction mixtures. These are identified by presence or absence of bands after agarose gel electrophoresis. A relatively pure genomic DNA sample can rapidly be extracted from a blood or mouthwash sample and then be subjected to a single PCR amplification step employing a set of 18 allele or group specific primer pairs. These systems also require an internal positive control in order to confirm the PCR integrity. The amplified products are detected in agarose gels with ethidium bromide staining. The extraction, amplification (using the latest second generation of thermal cyclers) and electrophoresis steps can be carried out within 3 hours comparing favourably with conventional serological testing. The technique has already proved suitable for typing of cadaveric organ donors for HLA-DR matching in transplantation and overcomes many of the limitations of serology.

*d) HLA class II PCR fingerprinting*

The technique of "PCR fingerprinting" has the dual advantages of being both technically simple and rapid. It allows HLA-DR, -DQ or -DP matching of two or more individuals by PCR amplification of HLA class II gene hypervariable regions followed by non-denaturing polyacrylamide gel electrophoresis (Bidwell and Hui, 1990; Bidwell *et al.*, 1993). Satellite bands of DNA generated in the PCR originate by heteroduplex formation between heterologous single stranded DNA molecules which are co-amplified using a single PCR primer set. The number and sequence differences of the amplified HLA class II gene second exon products depends on the contribution of each haplotype with both intra- and interlocus heteroduplex formation generating the diverse array of fingerprint patterns observed. Matching is achieved by visual comparison of the haplotype-specific patterns (PCR fingerprints) on acrylamide gels. Some patterns show marked similarities and in order to discriminate between them a pre-PCR spiking modification to this technique has been described (Wood *et al.*,1991). Unique PCR fingerprints have been observed for all combinations of HLA class II haplotypes examined to date. This technique has advantages over conventional HLA allele typing methods as it can be performed in less than 8 hours and there are no restriction enzyme digests or hybridisation steps. For allele typing purposes a reference database of PCR fingerprints can be generated by amplification of mixed genomic DNA from HTCs. The obvious clinical application of this technique is the rapid screening of HLA class I identical individuals for HLA class II compatibility in the selection of matched unrelated donors (MUD) for bone marrow transplantation since registeries often record HLA class I specifities only.

## HLA CLASS I GENE POLYMORPHISMS

With the increasing availability of HLA class I allele sequences it is also now possible to design allele typing methods for HLA-A, B and Cw using strategies similar to those

first developed for HLA class II alleles. A review of HLA class I allele sequences shows exons 2 and 3 to be the major HVRs and consequently class I typing systems recently developed have concentrated on identifying sequence differences within these exons. Initially, molecular based class I allele typing methods were designed to be supplementary to traditional serological assays to overcome some of their limitations. Systems using SSOPs were developed to provide positive identification of alleles belonging to cross-reactive groups such as the HLA-A2 -A68 -A69 group (Fernandez-Vina *et al.*, 1992). They have also been used to identify the alleles of a single specificity such as those of HLA-B27 (Hill *et al.*, 1991; Dominguez *et al.*, 1992) and some HLA-Cw specificities which are difficult to identify by serology (Sakkas *et al.*, 1991). Complete HLA class I SSOP systems are being developed for identification of HLA-A and -B alleles (Oh *et al.*, 1993; Yoshida *et al.*, 1992). However, while it is possible to use SSOPs to obtain unambiguous results when typing homozygous individuals, problems arise in the interpretation of reaction patterns generated for some heterozygotes. To overcome these problems it will be necessary to extend the panel of SSOPs used.

It is also possible to establish HLA class I types in individual samples using SSOP in a reverse dot blot or ELISA approach. A more suitable alternative is a SSP amplification system which to date has been established for HLA-A and HLA-Cw alleles (Browning *et al.*, 1993; Bunce *et al.*, 1993b) which offers allele typing systems giving equivalent or improved resolutions to serological assays. This technology should become available for HLA-B allele typing making complete HLA class I and class II allele typing possible using molecular biological techniques. In all probability this will be developed in diagnostic kit form opening the way for HLA typing to be used (or abused) in many laboratories.

In PCR/SSP methods the genotypes are visualised directly following electrophoresis of amplified products in agarose gel which negates the requirement for lengthy hybridisation and detection steps. Thus the method is ideal for typing individuals but the large number of PCR reactions required for each test means the method is costly and cumbersome when testing large numbers of samples.

## SOLID-PHASE SEQUENCING OF AMPLIFIED DNA

The ultimate typing method for definition of all known and novel HLA alleles is sequencing (Little and Parham, 1993). Direct sequencing of PCR products is preferable to sequencing of cloned PCR products since it is more rapid and simple. Solid-phase sequencing is the method of choice for direct sequencing (Kaneoka *et al.*, 1992). The DNA fragment to be sequenced must first be amplified using one biotinylated and one non-biotinylated primer, so that one strand of the PCR product is biotinylated. The PCR products are next mixed with streptavidin-coated magnetic beads which bind to the biotin. After purification and alkaline denaturation of the PCR products, the two DNA strands are separated and either strand can be sequenced using the appropriate sequencing primer. The procedure is outlined in figure 3. With the advent of automatic sequencers direct sequencing of HLA genes may well be the standard method used in future.

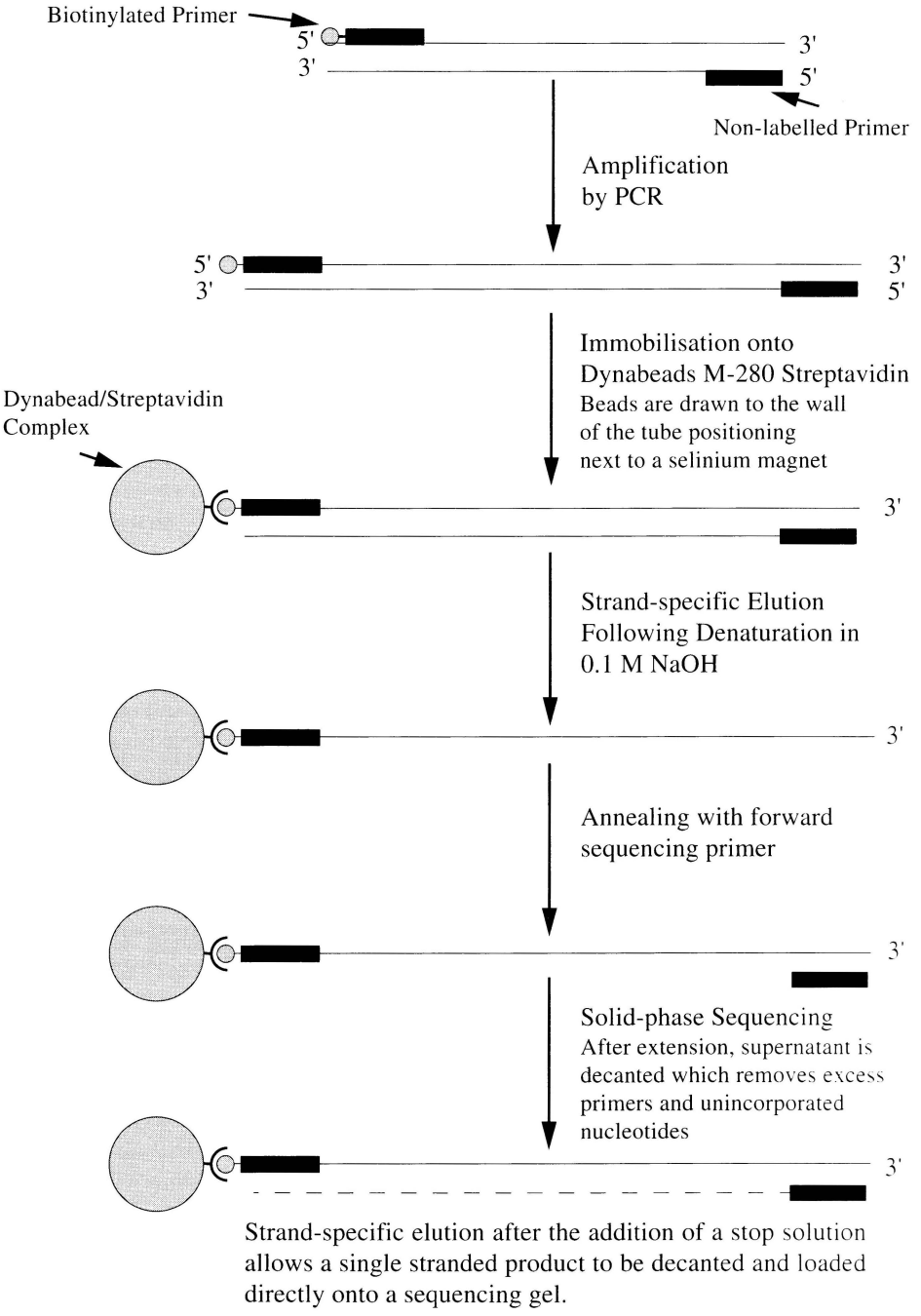


Figure 3. Direct sequencing of PCR products using streptavidin bound to magnetisable microspheres.

## IMPACT OF HLA ALLELE TYPING ON DISEASE STUDIES

Many diseases, especially those with an autoimmune component, are associated with particular HLA specificities (Tiwari and Terasaki, 1985). The explanation for these associations, however, remains unknown. The level of association with HLA specificity(s) varies considerably between diseases, although with the possible exceptions of narcolepsy (HLA-DR2) and ankylosing spondylitis (HLA-B27), it is never absolute. This may be due to both clinical and genetic heterogeneity, the latter being caused by a failure to determine polymorphisms at the DNA level or by the possibility that the HLA specificity or allele is strongly associated with the true disease susceptibility gene.

Advances in HLA allele typing have now started to make a major contribution towards our understanding of HLA associated disease susceptibility. This has been due primarily to the increased resolution in HLA allele definition. Serological and cellular techniques can only define specificities and epitopes within cell surface expressed HLA glycoproteins and whilst this represents important immunological information, it remains only a crude representation of the allele encoded by the DNA sequence and in most cases masks underlying genetic heterogeneity.

The use of RFLP techniques to define HLA types (chiefly HLA-DRB1) has done much to increase the confidence of antigen assignment, however it has provided only limited additional information regarding the level of genetic heterogeneity. Furthermore, any association between disease and a particular RFLP does not indicate whether the polymorphisms resides in the coding or non-coding regions of the gene.

The introduction of sequencing of HLA alleles and the development of PCR based techniques such as SSOP typing to define polymorphisms in the coding regions have already made a major impact in some disease studies. Two clear examples are insulin dependent diabetes mellitus (IDDM) and rheumatoid arthritis (RA) although a number of others have now been shown. In IDDM, identification of HLA class II sequences in patients has revealed that amino acid position 57 of the HLA-DQB1 molecule is of particular importance in determining resistance or susceptibility to this disease. Resistance is correlated with the presence of an aspartic acid at this position whereas possession of any other amino acid is correlated with disease susceptibility (Todd *et al.*, 1987).

Several HLA specificities are associated with RA susceptibility. These include HLA-DR4, -DR1, -DR10 and a subgroup of -DR6. Sequencing and SSOP studies have now confirmed that HLA-DRB1*0401, -DRB1*0404 and -DRB1*0405 alleles of -DR4 are associated with RA whereas -DRB1*0402 is not. Furthermore they have also demonstrated that the HLA-DBR1*1402 variant of -DR6 is associated with RA. From these studies it has become clear that RA susceptibility is associated with a conserved sequence of amino acids (QKRRA/QRRRA) within the third hypervariable region of the HLA-DRB1 molecule (Gregersen *et al.*, 1987).

The introduction of molecular based typing methods has had a major effect on the study design of investigations into HLA and disease associations. Serological techniques require significant quantities of viable lymphocytes which can be difficult to achieve for patients with chronic or childhood diseases. Problems also exist where there is a significant delay between sample collection and delivery to the laboratory. The latter may apply to studies collecting samples in areas remote from the site of testing.

These problems are largely solved by molecular biological methods where only small samples of blood or other tissue are required and these can be stored simply and long term

before testing. These features have now "opened up" new areas of study; for example in a recent study of malaria in W. Africa, molecular typing has demonstrated an association between HLA-B53 and increased resistance to this condition (Hill *et al.*, 1992).

Typing of HLA alleles by molecule techniques for disease studies also has a major advantage over serological techniques in that samples from large numbers of patients and controls can be batch processed with relative ease and at a reduced cost. This has already made an impact on epidemiological studies where base line HLA frequency data on large populations can be obtained and used in important longitudinal studies of disease development (Thomson *et al.*, 1993). The combination of molecular based HLA techniques in an epidemiological framework represents an exciting advance in disease studies.

## APPLICATIONS TO ORGAN TRANSPLANTATION

Whilst there are clear indications in favour of matching donor HLA specificities to those of the recipient in experimental allogeneic transplantation, the relevance for clinical transplantation continues to be debated mainly because the essential use of immunosuppressive drugs masks the immune response of the recipient. No doubt multiple and high dose drug use can overcome Mhc mismatching but the consequences of such long-term therapy are now known to include increased rates of malignancy and therefore must be balanced against exchange of organs between centres to achieve good Mhc matching.

In Europe HLA-A, -B and -DR specificity matching has been used for allocation of cadaveric kidneys for many years (Dyer *et al.*, 1989; Thorogood *et al.*, 1990) and such studies of prospective matching report great benefit with at least 25% increase in transplant survival rates at ten years for matched compared with unmatched cases, even when serological techniques are used.

A report from the respected multi-centre Collaborative Transplant Study (CTS) noted that in some centres RFLP typing for HLA-DRB1 revealed worrying discrepancies in HLA-DR serology (Opelz, 1991). In the extreme example, random allocation of organs would have achieved better matching by chance! However with the introduction of molecular based techniques, SSOP allele typing provides a method with a high level of resolution and accuracy. This can be used for batch testing of potential recipients of solid organ and bone marrow transplants. For the typing of cadaver organ donors, where low volume, high speed techniques are required, PCR/SSP typing is the method of choice. With the use of PCR/SSP for cadaveric donor typing the error rate in HLA-DR- and soon HLA-A, -B, -Cw- allele typing will be reduced to that expected of any laboratory technique. Given that situation, the benefits of Mhc matching in kidney transplantation can be assessed accurately. Furthermore, the effect of accurate Mhc matching can now be addressed in liver and thoracic organ transplantation since currently few centres apply prospective matching.

With molecular typing techniques it is also possible to ask exactly which Mhc encoded genes and alleles are most relevant to solid organ transplant survival. For some time the hierarchy by serological typing has been shown to be HLA-DR > -B > -A but as molecular methods allow typing for many other Mhc encoded genes such as HLA-DP, TNF, HSP, LMP, and TAP then their role can also be assessed. By serology, some studies have

highlighted a role for individual specificities on transplant survival (Dyer *et al.*, 1985, Cook *et al.*, 1987); using allele typing these studies can now be extended.

Molecular genetics in the laboratory moves faster than its application to the clinic; in organ transplantation HLA allele typing could prove an exception because this clinical speciality has a good track record of putting innovations into practice. Histocompatibility laboratories should now be in a position to provide this support service for their clinical colleagues and objections to the matching of HLA alleles for organ allocation are no longer valid on the grounds of inaccurate or time consuming methodologies.

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