IV.1 Intellectual Property Rights in the Field of Molecular Marker Analysis

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1 Introduction

Intellectual property rights – and especially patents – become more and more important in biotechnology as there are many industrial applications with high economic value. The economic value of biotechnological inventions, especially in the field of agrobiotechnology, is increasing with the worldwide expansion of the cultivation of transgenic plants (Herrlinger et al. 2003). Beyond this, patents on plant-related inventions can influence the funding that is available for research, in particular in the private sector of biotechnology (Fleck and Baldock 2003). Conventional (non-transgenic) plant breeding and plant breeding research is also strongly influenced by biotechnological processes and methods. One tool that has found its way into conventional plant breeding is molecular marker analysis of significant traits (e.g. resistance against pathogens, yield) or DNA fingerprinting with the help of molecular markers to obtain information on the relationship between individual plants. Moreover, with the improvements in our understanding of the genomes and in our knowledge of the relations between genotype and phenotype in economically important crops, this tool will become more significant in practical plant breeding. The importance of molecular marker analysis for the different applications was recognized very early so that many patents have been filed in the last 10–15 years. For scientists in all institutions, public or private sector, an understanding of intellectual property rights (IPRs) is fundamental in both research and development (Kowalski et al. 2002), but many scientists are still not aware of the rising number of patents in this field. This chapter will give an overview of patents for methods and applications in the field of microsatellite markers or simple sequence repeat (SSR) markers.

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2 What Is a Patent?

Patents are granted for inventions that are new, involve a creative step and can be applied in industry. A patent gives its owner the exclusive monopoly to use his invention, preventing others from using it without permission for a certain time (Shear and Kelley 2003). This means that if someone wants to use a patent-protected invention, the permission for use (license) must be obtained from the patent owner. One exception where the user of a protected invention does not need the permission of the patent owner is the so-called research exemption [e.g. §11 (2) of the German Patent Law]. It allows license-free activities concerning the improvement or testing of patent-protected inventions. Use of patent-protected inventions in research and development under the provisions of the patent, however, does not fall under the research exemption and is dependent on the permission of the patent owner. In most countries, patent specifications are published 18 months after the application date. The different patent offices, but also private companies, provide online patent databases that can be searched by different keywords. The results of such a patent investigation, in which different keywords concerning micro-satellite marker analysis were used, are provided here.

3 Microsatellite or Simple Sequence Repeat Markers

When considering a typical experiment regarding molecular marker analysis with microsatellite markers, one can divide this experiment into different steps (Fig. 1). Starting with the plant and the extraction of its DNA, in some experiments, the DNA is cut by restriction enzymes. After this step, specific primers are used to perform a PCR reaction. The resulting PCR fragments can be analysed by different methods like gel electrophoreses, mass spectrometry or micro-array analysis. This analysis will provide information on specific traits of different plants for marker-assisted selection or on their genetic relationship to each other (fingerprinting).

An investigation of the patent specifications that have been filed in this field shows that there are many patents claiming different steps of this typical marker experiment. Figure 1 shows some of these patents and indicates which step of the experiment is claimed.

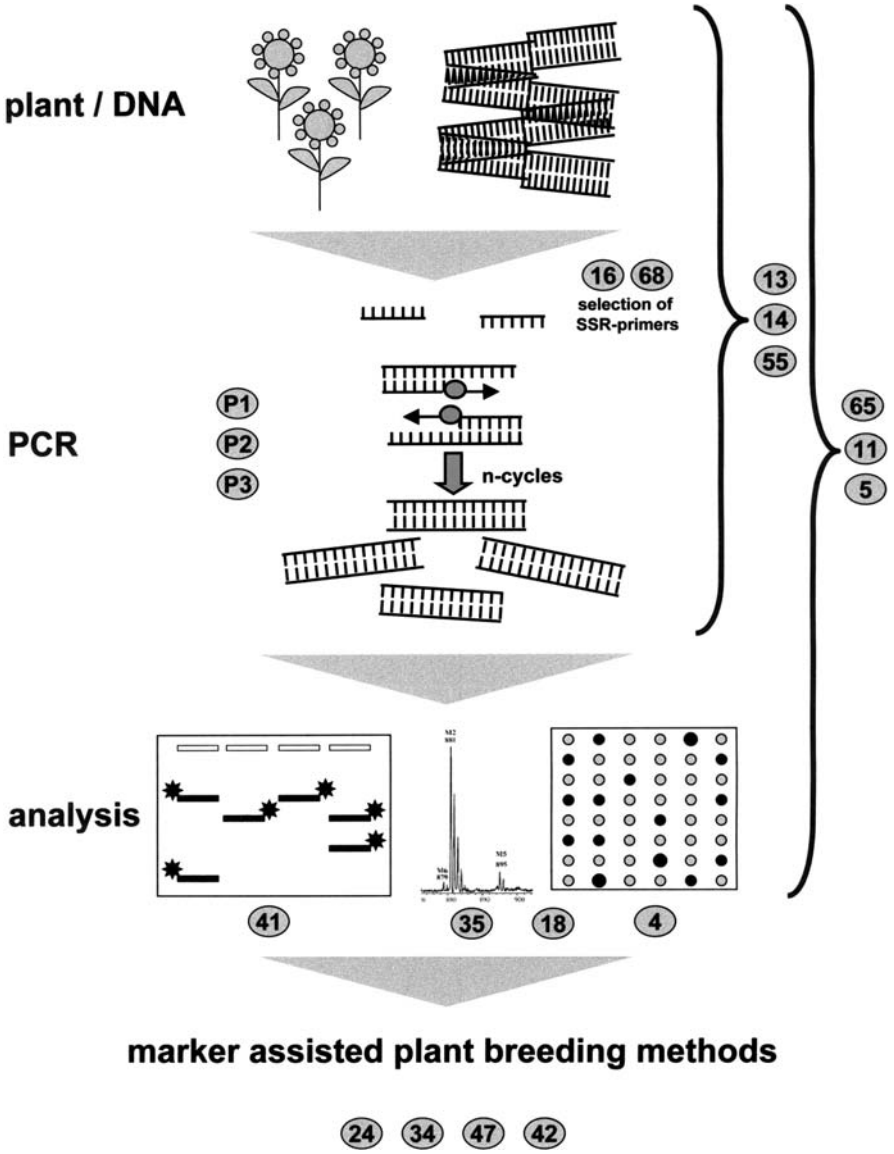

Fig. 1. An overview of a typical microsatellite marker experiment and some sample patents that are relevant for the different steps of such an experiment. Patents are indicated by *numbers*. The experiment is divided into different steps. Starting with the isolation of DNA of a plant, the DNA is sometimes cut by restriction enzymes. After the selection of specific SSR primers, a PCR reaction is carried out. There are different possible methods for the analysis of the resulting PCR fragments, here exemplified by gel electrophoresis (the fluorescent label of the PCR product is indicated by ✷), mass spectrometry and microarray analysis. Molecular marker analysis results in marker-assisted plant breeding. The *numbers* indicate patents that are relevant for the different steps of the experiment. Numbering of the patents is in accordance with the consecutive numbering of the patents in Table 1

4 The Selection of Microsatellite Primers and the PCR Reaction

Figure 1 shows two typical patents claiming primers for microsatellite marker analysis. Patent No. 16 (Röder et al. 1997) claims specific microsatellite markers from *Triticum aestivum*. Patents claiming specific primer sequences for marker analysis have become rare in the last few years. The problem is that patents are published 18 months after their registration (Art. 93, European Patent Convention). After publication, the owner of a patent has difficulty controlling whether someone unauthorized is using the patented primer sequences illegally because the plant that was analyzed by the primers does not show which primer was used for the analysis. As a consequence, primer sequences as specific as that are normally not patented and thereby published, but rather treated as a business secret that is licensed to users. This gives the inventor a controlling mechanism for the use of his invention. In contrast, patent No. 68 (Nagaraju 2003) claims a certain class of SSR primers, the inter-simple sequence repeat-PCR primers. Here, the scope of protection of the claim is broader, making it easier for the patent owner to control who is using the invention.

After selecting the primers, the PCR experiment follows. Most researchers are aware of patents concerning PCR methods. The basic patents on PCR were registered in 1985. Patents No. P1–P3 (Mullis 1992; Mullis et al. 1992, 1993) in Fig. 1 indicate these basic patents owned by Hoffmann La Roche. A license for PCR can be relatively easily obtained by buying a licensed polymerase and a licensed thermocycler. As there are also many cheaper non-licensed polymerases and thermocyclers on the market, the manufacturers indicate in their instructions for use that the product is not licensed for performing PCR reactions. Meanwhile, there are many other patents concerning registered PCR methods. These patents claim special polymerases or methods like RT-PCR and quantitative PCR. They are not listed in this context because their discussion would go beyond the scope of this chapter.

In the previous paragraphs, patent specifications claiming the primer sequences, on the one hand, and patent specifications claiming the PCR method, on the other, were discussed. However, if one has a closer look, one can also find patents claiming both steps, like patents No. 13 (Morgante and Vogel 1997), 14 (Kuiper et al. 1997) and 55 (van Eijk et al. 2001; Fig. 1).These patent specifications claim processes for detecting polymorphisms between different samples of DNA. The processes comprise the amplification of nucleic acid segments using defined primer sequences, sometimes starting with the previous restriction of the DNA sample by restriction endonucleases, and the ligation of certain adaptor sequences similar to the AFLP (amplified fragment length polymorphism) approach. Patent No. 55 (van Eijk et al. 2001) even claims a combined method between microsatellite and AFLP

marker analysis using special RAMP primers (random amplified microsatellite polymorphism primers) for the analysis of microsatellite sequences.

5 Analysis of PCR Products

Figure 1 shows three different methods for the analysis of the resulting PCR products. The most common one, the analysis by gel electrophoresis, can also be claimed by patents, if for example special fluorescent labels for detection are used. Such a method is claimed by patent No. 41 (Shuber and Pierceall 2002). The claimed method comprises the PCR reaction with fluorescent primers, the detection of the labelled extension products and the comparison of the PCR product size.

A second method of analysis, mass spectrometry, is claimed by patent No. 35 (Hillenkamp and Köster 1999). This patent generally claims the analysis of nucleic acids by mass spectrometry in general, and not just for microsatellite marker analysis. For high-throughput analysis of probes, the microarray technique is preferred. This method is protected by a patent of Affymetrix, patent No 4 (Fodor et al. 1998). This specification not only protects the detection of microsatellites by microarray analysis, but also the detection of nucleic acid sequences in general which comprises microsatellites. Meanwhile, there are other patents claiming further developments of this technique, but the discussion of these would also go beyond the scope of this chapter.

Another high-throughput technique described in patent No. 18 (Olek 1996) combines the method of mass spectrometric and microarray analysis of microsatellite markers.

Patent specifications No. 5 (Caskey and Edwards 1992), No. 11 (Perlin 1995) and No. 65 (Saint-Louis and Paquin 2003; Fig. 1) summarize the complete experimental process from DNA extraction to the analysis of the PCR products, in which different PCR methods are combined, for example, use of certain labelled nucleotide triphosphates and different analytical tools like mass spectrometry or computer analytical tools.

6 Marker-Assisted Breeding Methods

The most comprehensive patent specifications claim complete plant breeding methods in which molecular marker analysis is used. Examples are patent specifications No. 24 (Byrum and Reiter 1998), No. 34 (Beavis 1999), No. 47 (Jansen and Beavis 2001) and No. 42 (Openshaw and Bruce 2001; Fig. 1). These comprise the previously mentioned experimental steps in that they claim the association of the genotype with phenotypic traits of interest by

molecular marker analysis. The patents differ in the selection of plant populations that are the basis for the analysis, the statistical methods applied in the analysis and the integration of molecular biological techniques like expression profiling of genes. The claims of these patents are not restricted to microsatellite markers. They also comprise other well-known marker techniques like AFLPs, RFLPs (restriction fragment length polymorphisms) or RAPDs (random amplified polymorphic DNA). These patent specifications were filed in the late 1990s and are still in the process of examination in Europe. Details concerning the legal status of these patent applications are shown in Table 1.

7 Conclusions

Molecular marker analysis is one of the most powerful tools in modern plant breeding. However, as for most innovative applications, IPRs play an important role. As shown above, the implementation of microsatellite marker analysis for plants is also strongly dependent on IPRs. To identify these rights, a biotechnological process or method has to be dissected into its essential components and processes, with each part to be analysed under the IP microscope (Kowalski et al. 2002). This means that scientists must educate themselves on these issues so that they can make informed decisions regarding their research practices (Kimpel 1999). Beyond this, patents describe the latest inventions made by innovative researchers and companies and the publication of these patents guarantees their public availability. This, in turn, allows the further development and improvement of these innovative techniques.

Table 1. Results of a patent investigation concerning SSR-marker technology. The results are from a patent investigation in the Delphion (www.delphion.com) and Epoline (www.epoline.org) patent databases. Thought has been given to patent specifications in the field of microsatellite marker analysis. Column one comprises European (*EP*) and international (*WO*) patent publication numbers. Publication numbers of *US*, New Zealand (*NZ*) and Japanese (*JP*) patents are included when no EP or WO publications were available. The column "*main claim*" includes the first claim of a patent specification, if the respective patent is still in force. Comments on the legal status of the patent specifications were derived from the patent databases

Publication number and title	Publication date (V) and priority date (P)	Assignee	Main claims	Comment on legal status
1. EP0237362: Process for detecting specific nucleotide variations and genetic polymorphisms present in nucleic acids and kits thereof	V-16.09.87 P-13.03.86 P-22.06.86	Hoffmann La Roche	1. A process for detecting the presence of a specific nucleotide sequence in nucleic acid in a sample, which includes: (a) Treating the sample, together or sequentially, with four different nucleoside triphosphates, an agent for polymerization of the nucleoside triphosphates, and two oligonucleotide primers for said nucleic acid under hybridizing conditions such that a primer will hybridize to said nucleic acid and an extension product of the primer be synthesized which is complementary to said nucleic acid, wherein said primers are selected such that the extension product synthesized from one primer, when separated from its complement, can serve as a template for synthesis of the extension product of the other primer (b) Treating the sample under denaturing conditions to separate the primer extension products from their templates (c) Treating the sample, together or sequentially, with said four nucleoside triphosphates, an agent for polymerization of the nucleoside triphosphates, and oligonucleotide primers such that a primer extension product is synthesized using each of the single strands produced in step (b) as a template, wherein steps (b) and (c) are repeated a sufficient number of times exponentially to increase the amount of said nucleic acid and to result in detectable amplification thereof (d) Directly transferring, without gel fractionation, product derived from step (c) to a membrane	Granted EP-patent

Table 1. (Continue)

Publication number and title	Publication date (V) and priority date (P)	Assignee	Main claims	Comment on legal status
			(e) Treating the membrane from (d) under hybridization conditions with a labeled sequence-specific oligonucleotide probe capable of hybridizing with the amplified nucleic acid only if the sequence of the probe is complementary to a region of the amplified nucleic acid (f) Detecting whether the probe has hybridized to an amplified nucleic acid in the sample.	
2. WO9004651: Mapping quantitative traits using genetic markers	V-03.05.90 P-19.10.88	Whitehead Institute for biomedical research Cornell research foundation, Inc.		WO-application withdrawn
3. EP 0561796: Oligonucleotide constructs and methods for the generation of sequence signatures from nucleic acids	V-05.03.92 P-24.08.90	The University of Tennessee research corporation		Granted in EP, but lapsed, data supplied by contracting states AT, BE, CH, DE, FR, GB, IT, LI, NL, SE
4. EP 0834576: Detection of nucleic acid sequences	V-08.04.98 P-06.12.90	Affymetrix, Inc.	1. A method for detecting nucleic acid sequences in two or more collections of nucleic acids, comprising: (a) Providing an array comprising more than 100 different polynucleotide probes bound to a solid surface (b) Contacting said array of probes under hybridisation conditions with:	Granted in EP, opposition filed against

Table 1. (Continue)

Publication number and title	Publication date (V) and priority date (P)	Assignee	Main claims	Comment on legal status
			(i) a first collection of nucleic acids comprised of first-labelled nucleic acids having at least some sequences complementary to probes of said array (ii) at least a second collection of nucleic acids comprised of second-Iabelled nucleic acids having at least some sequences complementary to probes of said array, wherein said first and second labels are distinguishable from each other (c) Detecting hybridisation of first and second labelled complementary nucleic acids to probes of said array	
5. EP 0639228: DNA typing with short tandem repeat polymorphisms and identification of polymorphic short tandem repeats	V-20.08.92 P-31.01.91	Baylor College of Medicine	A DNA profiling assay for detecting polymorphisms in at least one short tandem repeat, comprising the steps of: extracting DNA from a sample to be tested; amplifying said at least one short tandem repeat in the extracted DNA, wherein the short tandem repeat sequence is characterized by the formula (Aw Gx Ty Cz)n wherein A,G,T, and C represent the nucleotides; w, x, y and z represent the number of each nucleotide and range from 0 to 7; the sum of w+x+y+z ranges from 4 to 7; and n represents the repeat number and ranges from about 5 to 50; and detecting said polymorphisms by identifying said amplified extension products for each different sequence, wherein each different sequence is differentially labelled.	In EP still under examination
6. US6455758: Process predicting the value of a phenotypic trait in a plant breeding program	V-24.09.02 P-19.02.91	Dekalb Genetics Corp.		US only, just maize

Table 1. (Continue)

Publication number and title	Publication date (V) and priority date (P)	Assignee	Main claims	Comment on legal status
7. EP 0552545: Detection of polymorphisms in simple sequence repeats using oligonucleotide ligation	V-28.07.93 P-17.01.92	Pioneer Hi-Bred International, Inc.		Refusal of application 13.11 1996
8. US 5746023: Method to identify genetic markers that are linked to agronomically important genes	V-05.05.98 P-07.07.92 P-23.07.93 P-18.07.95	Du Pont	1. A method for identifying alleles associated with agronomic fitness of crop plants, comprising: (a) Selecting a sample of current-day elite lines of a given crop to form an elite population; (b) Selecting the predominant and earliest known ancestral lines of said elite lines by considering the pedigrees of said elite lines (c) Conducting a genetic marker survey to determine the genotype of said elite lines and said ancestral lines (d) Using the pedigrees of said elite lines and genotypes of said ancestral lines to calculate the probability of each elite line inheriting each allele from said ancestral lines (e) Calculating the expected allele frequency of each allele within said elite population by averaging the probabilities calculated in step d) for each elite line (f) Calculating the observed allele frequency within said elite population; (g) Comparing said observed allele frequency with said expected allele frequency for each said allele in said elite population to identify alleles at each locus that have been inherited more frequently than expected	US only

Table 1. (Continue)

Publication number and title	Publication date (V) and priority date (P)	Assignee	Main claims	Comment on legal status
			(h) Producing crop plants with superior agronomic fitness; such that new crop plants with superior agronomic fitness can be efficiently identified with said genetic markers that are diagnostic of said alleles that have been inherited more frequently than expected	
9. EP 0733126: Immobilized mismatch binding protein for detection of mutations and polymorphisms, and allele identification	V-11.05.95 P-04.11.93	ValiGene Corporation	1. A method of detecting a mutation from a non-mutated sequence of a double stranded target DNA in a sample, the method comprising: (a) Denaturing any double stranded DNA in the sample into single strands and allowing the single strands to reanneal into duplexes (b) Incubating the denatured and reannealed duplexes of step (a) with a mismatch-binding protein immobilized by adsorption on a solid support, either (i) In the presence of a detectably labelled DNA having a mismatch and capable of binding to the mismatch binding protein; or (ii) Wherein the mismatch-binding protein was preincubated with and allowed to bind a detectably labelled DNA having a mismatch (c) Detecting the amount of detectably labelled DNA having a mismatch bound to the mismatch-binding protein, wherein the presence of a mutation in the double stranded target DNA of the sample results in a decrease in the binding of the detectably labelled DNA to the mismatch-binding protein	Granted in EP, but lapsed, data supplied by contracting states AT/ 24–04–2002 GR/ 24–04–2002 NL/ 24–04–2002 PT/ 24–07–2002 SE/ 24–07–2002 in DE in force
10. WO 9515400: Genotyping by simultaneous analysis of multiple microsatellite loci	V-08.06.95 P-03.12.93	The Johns Hopkins University		WO-application deemed to be withdrawn

Table 1. (Continue)

Publication number and title	Publication date (V) and priority date (P)	Assignee	Main claims	Comment on legal status
11. EP 0714537: Method and system for genotyping	V-28.12.95 P-17.06.94	Perlin, Mark W	1. A method for genotyping comprised of the steps: (a) Obtaining DNA or RNA material from a genome (b) Amplifying a location of the material, with the length of the location not exceeding 50 kb and the location containing a multinucleotide repeat region (c) Labelling the amplified material with labels (d) Converting the labels with a sensing device which produces a first electrical signa; (e) Removing a reproducible pattern of the amplification from the first electrical signal using a program residing in the memory of a computer to form a third electrical signal (f) Producing from the third electrical signal a genotype of the material at the location	In EP still under examination
12. EP0828853: Method for nucleotide sequence amplification	V-25.04.96 P-18.10.94	Genzyme Corporation		24.01.01 EP-application deemed to be withdrawn
13. EP0804618: Compound microsatellite primers for the detection of genetic polymorphisms	V-06.06.96 P-28.11.94	E.I. Du Pont de Nemours and Company	1. An improved method of detecting polymorphisms between two individual nucleic acid samples comprising amplifying segments of nucleic acid from each sample using primer-directed amplification and comparing said amplified segments to detect differences, the improvement comprising wherein at least one of the primers used in said amplification consists of a perfect compound simple sequence repeat in which two different repeating sequences are either directly adjacent or are separated by no more than three intervening bases	Granted in EP

Table 1. (Continue)

Publication number and title	Publication date (V) and priority date (P)	Assignee	Main claims	Comment on legal status
14. EP0721987 EP0805875 WO9622388: Amplification of simple sequence repeats	V-17.07.96 P-16.01.95 V-12.11.97 P-16.01.96	Keygene, N.V.	1. A Process for the selective amplification of restriction fragments comprising simple sequence repeats, comprising the following: (a) Digesting a starting DNA with two or more different restriction enzymes, at least one of these enzymes cleaving at or near its recognition nucleotide sequence overlapping or flanking with the simple sequence repeat (referred to as first restriction enzyme) and at least one of these enzymes cleaving the restriction fragments into amplifiable restriction fragments, in a preferably four-base sequence (referred to as second restriction enzyme, to obtain restriction fragments (b) Ligating an appropriate double stranded oligonucleotide adaptor to each of the ends of the restriction fragments produced by said restriction enzymes (c) Amplifying the restriction fragments of step (b) using two or more different amplification primers with the following general structure: one primer having a sequence at the V end matching the common sequence of the restriction fragments produced with the first restriction enzyme, or part thereof, and at the V end at least five nucleotides matching the sequence of the simple sequence repeat (referred to as primer one; one primer having a sequence at the V end matching the common sequence of the restriction fragments produced with the second restriction enzyme, or part thereof, and at its V end ranging from 0, 11 21 31 4 or more especially 0 to 3 randomly chosen nucleotides (referred to as primer two) (d) Recovering the amplified fragments	EP0805875 still under examination EP 0721987 deemed to be withdrawn

Table 1. (Continue)

Publication number and title	Publication date (V) and priority date (P)	Assignee	Main claims	Comment on legal status
15. EP 0815261: DNA diagnostics based on mass spectrometry	V-26.09.96 P-17.03.95	Sequenom, Inc.	1. A process for detecting one or more target nucleic acid sequences present in a biological sample, comprising the steps of: (a) Hybridizing one or more detector oligonucleotide with one or more nucleic acid molecules and removing unhybridized detector oligonucleotide (b) Ionizing and volatizing the product of step (a) (c) Analyzing the ionized and volatilized nucleic acid by mass spectrometry, wherein detection of the detector oligonucleotide by mass spectrometry indicates the presence of the target nucleic acid sequence in the biological sample	Granted in EP
16. EP 0835324: Microsatellite markers for plants of the species *Triticum aestivum* and tribe Triticeae and the use of said markers	V-16.01.97 P-28.06.95	Institut für Pflanzengenetik und Kulturpflanzenforschung	The patent claims 230 microsatellite markers for wheat	Granted in EP
17. WO9712059: Brown stern rot resistance in soybeans	V-03.04.97 P-26.09.95	Pioneer Hi-Bred International, Inc.		WO9712059 discontinued in Europe
18. EP 0870062: Genomic analysis process and agent	V-15.05.97 P-09.11.95	GAG Bioscience Zentrum für Umweltforschung und Technologie	1. Method for microsatellite analysis, wherein there exist – The fixation of microsatellite amplificates from genomic DNA samples separated into individual microsatellite markers before or after amplification to defined positions of a matrix – Evaporation of the individual positions in a mass spectrometer – Mass-spectrometry determination of the molecular weight	Granted in EP (PT, ES, DK. DE, AT)

Table 1. (Continue)

Publication number and title	Publication date (V) and priority date (P)	Assignee	Main claims	Comment on legal status
19. EP 1034307 EP 0815263 EP 1086247: Methods for the detection of nucleic acids	V-10.06.99 P-04.12.997 V-03.07.97 P-22.12.95 P-14.08.96 V-23.12.99 P-16.06.98	Exact Sciences Corporation	EP 0815263: 18. A method for detecting a nucleic acid sequence change in a target allele in a subpopulation of cells in a biological sample, comprising the steps of: (a) Determining (i) an amount of wild-type target allele in the biological sample (ii) an amount of a reference allele in the biological sample (b) Detecting a nucleic acid sequence change in the target allele in a subpopulation of cells in the biological sample, statistically significant difference in the amount wild-type target allele and the amount of reference allele obtained in said determining step being indicative of a nucleic acid sequence change	EP 1086247 application deemed to be withdrawn 07.08.02 EP1034307 claims fetal chromosomal abnormalities
20. EP 0912761: Detection of nucleic acid sequence differences using coupled ligase detection and polymerase chain reactions	V-04.12.97 P-29.05.96	Cornell Research Foundation, Inc.	1. A method for identifying one or more of a plurality of sequences differing by one or more single-base changes, insertions, deletions, or translocations in a plurality of target nucleotide sequences comprising: Providing a sample potentially containing one or more target nucleotide sequences with a plurality of sequence differences; providing one or more oligonucleotide probe sets, each set characterized by: (a) A first oligonucleotide probe, having a target-specific portion and a 5' upstream primer-specific portion (b) A second oligonucleotide probe, having a target-specific portion and a 3' downstream primer-specific portion, wherein the oligonucleotide probes in a particular set are suitable for ligation together when hybridized adjacent to one another on a corresponding target nucleotide sequence, but have a mismatch which interferes with such liga-	In EP still under examination

Table 1. (Continue)

Publication number and title	Publication date (V) and priority date (P)	Assignee	Main claims	Comment on legal status
			tion when hybridized to any other nucleotide sequence present in the sample; providing a ligase; blending the sample, the plurality of oligonucleotide probe sets, and the ligase to form a ligase detection reaction mixture; subjecting the ligase detection reaction mixture to one or more ligase detection reaction cycles comprising a denaturation treatment, wherein any hybridized oligonucleotides are separated from the target nucleotide sequences, and a hybridization treatment, wherein the oligonucleotide probe sets hybridize at adjacent positions in a base-specific manner to their respective target nucleotide sequences, if present in the sample, and ligate to one another to form a ligation product sequence containing (a) the 5' upstream primer-specific portion, (b) the target-specific portions connected together, and (c) the 3' downstream primer specific portion with the ligation product sequence for each set being distinguishable from other nucleic acids in the ligase detection reaction mixture, and, wherein the oligonucleotide probe sets may hybridize to nucleotide sequences in the sample other than their respective target nucleotide sequences, but do not ligate together due to a presence of one or more mismatches and individually separate during the denaturation treatment; providing one or a plurality of oligonucleotide primer sets, each set characterized by (a) an upstream primer containing the same sequence as the 5' upstream primer-specific portion of the ligation product sequence and (b) a downstream primer complementary to the 3' downstream primer-specific portion of the ligation product sequence, wherein one of the primers has a detectable reporter label; providing a polymerase; blending the ligase detection reaction mix-	

Table 1. (Continue)

Publication number and title	Publication date (V) and priority date (P)	Assignee	Main claims	Comment on legal status
			ture with the one or a plurality of oligonucleotide primer sets, and the polymerase to form a polymerase chain reaction mixture; subjecting the polymerase chain reaction mixture to one or more polymerase chain reaction cycles comprising a denaturation treatment, wherein hybridized nucleic acid sequences are separated, a hybridization treatment, wherein the primers hybridize to their complementary primer-specific portions of the ligation product sequence, and an extension treatment, wherein the hybridized primers are extended to form extension products complementary to the sequences to which the primers are hybridized, wherein, in a first cycle, the downstream primer hybridizes to the 3' downstream primer-specific portion of the ligation product sequence and extends to form an extension product complementary to the ligation product sequence, and, in subsequent cycles, the upstream primer hybridizes to the 5' upstream primer-specific portion of the extension product complementary to the ligation product sequence and the 3' downstream primer hybridizes to the 3' downstream portion of the ligation product sequence; detecting the reporter labels; and distinguishing the extension products to indicate the presence of one or more target nucleotide sequences in the sample	
21. US 5811239: Method for single base-pair DNA sequence variation detection	V-22.09.98 P-13.05.96	Frayne Consultants		US only

Table 1. (Continue)

Publication number and title	Publication date (V) and priority date (P)	Assignee	Main claims	Comment on legal status
22. EP 0943019: Sets of labelled energy transfer fluorescent primers and their use in multi component analysis	V-23.07.98 P-15.01.97	Incyte Pharmaceuticals, Inc.	1. A set of four fluorescently labelled oligonucleotide primers, wherein three of said oligonucleotide primers have a common donor fluorophore "A" and acceptor fluorophore "B" in energy transfer relationship and are separated by different distances so as to provide three distinguishable fluorescent signals, and the fourth primer has two donor fluorophores "A" so as to provide a fluorescent signal different from said three distinguishable fluorescent signals	Granted in EP (BE, CH, DE, FR, GB, L,I NL, AT)
23. EP 0986651: Polymerases for analyzing or typing polymorphic nucleic acid fragments and uses thereof	V-22.03.00 P-07.02.97 V-08.07.99 P-07.02.97 V-13.08.98 P-06.01.98	Life Technologies, Inc.	1. A method of identifying, analyzing or typing a polymorphic DNA fragment in a sample of DNA, said method comprising contacting said sample of DNA with one or more DNA polymerases substantially reduced in the ability to add one or more non-templated nucleotides to the 3' terminus of a DNA molecule, amplifying said polymorphic DNA fragment within said sample and analyzing said amplified polymorphic DNA fragment	In EP still under examination
24. EP0972076: A method for identifying genetic marker loci associated with trait loci	V-24.09.98 P-27.03.97	Asgrow seed Inc., Du Pont De Nemours and Co	1. A method for identifying a genetic marker locus associated with a trait locus from a crop species, the method comprising: (a) Creating a genotypic survey for a crop species using germplasm of multiple ancestry, the survey created using genetic markers, wherein individual entries of the germplasm survey are not members of a segregating population created for the purposes of the analysis (b) Comparing the genotypic survey to phenotypic data collected on the same entries used to create the genotypic survey or their progeny (c) Estimating the association between genetic marker loci and trait loci (d) Identifying a genetic marker locus that is associated with the trait locus	In EP still under examination

Table 1. (Continue)

Publication number and title	Publication date (V) and priority date (P)	Assignee	Main claims	Comment on legal status
25. EP1056528 EP1042503 EP1030933 EP1027121 EP1023463 EP1017841 EP1017466 EP1002137: Method of detecting mutant DNA by MIPC and PCR	V-14.05.99 V-15.04.99 V-22.04.99 V-05.11.98 V-22.04.99 V-25.02.99 V-05.11.98 V-18.02.99 P-25.04.97 P-23.09.97 P-17.10.97 P-27.10.97 P-30.10.97 P-05.12.97 P-05.01.98 P-13.03.98 P-10.04.98 P-04.08.98 P-06.10.98	Transgenomic Inc.	EP1056528: 1. A method for separating a mixture of polynucleotides comprising: (a) Flowing a mixture of polynucleotides having a target range of base pairs through a separation column containing a separation medium having a nonpolar separation surface (b) Separating said mixture by eluting said column using a mobile phase having a composition which remains essentially constant for the duration of the chromatographic separation EP1042503: 1. A method for detecting a putative mutant DNA in a sample of DNA, the method comprising the steps of: (a) Amplifying the sample of DNA using PCR (b) Hybridizing the amplified sample to form a mixture of homoduplexes and heteroduplexes (c) Separating the product of step (b) into fractions by denaturing matched ion polynucleotide chromatography (d) Blind collecting the fractions from step (c) at a retention time corresponding to the retention time of the heteroduplex EP1023463: 1. A method for enhancing the detection of a polynucleotide separated by matched ion polynucleotide chromatography comprising: (a) Covalently attaching a chemical tag to said polynucleotide to form a tagged polynucleotide (b) Applying said tagged polynucleotide to a separation medium having a non-polar surface	EP1030933 withdrawal of application 12–05–2003 EP1027121 Application deemed to be withdrawn 25–07–2003 EP1002137 withdrawal of application 09–07–2003 EP1056528, EP1042503, EP1023463, EP1017841 and EP1017466 in EP still under examination

Table 1. (Continue)

Publication number and title	Publication date (V) and priority date (P)	Assignee	Main claims	Comment on legal status
			(c) Eluting said tagged polynucleotide from said surface with a mobile phase containing a counterion agent and an organic solvent (d) Detecting said tagged polynucleotide, wherein said medium is characterized by having a DNA separation factor of at least 0.05 EP1017841: 1. A method for analyzing a sample of double stranded DNA to determine the presence of a mutation therein comprising: (a) Contacting said sample with a mutation site binding reagent (b) Chromatographically separating and detecting the product of step (a) EP1017466: 1. A method for separating a mixture of polynucleotides, comprising flowing a mixture of polynucleotides having up to 1500 bp through a separation column containing polymer beads having an average diameter of 0.5 to 100 microns, said beads having a surface composition essentially completely substituted with a moiety selected from the group consisting of unsubstituted, methyl, ethyl, hydrocarbon, and hydrocarbon polymer, and wherein said beads are characterized by having a DNA separation factor of at least 0.05; and separating said mixture of polynucleotides	
26. EP 0983383: Length determination of nucleic acid repeat sequences by discontinuous primer extension	V-03.12.98 P-27.05.97	PE Corporation (NY)	1. A method for determining the number of repeat units in a repeat region of a target nucleic acid comprising the steps of: (a) Annealing a primer-complementary portion of a target nucleic acid to a primer thereby forming a target-primer hybrid (b) Performing a first primer extension reaction using a first primer extension reagent	In EP still under examination

Table 1. (Continue)

Publication number and title	Publication date (V) and priority date (P)	Assignee	Main claims	Comment on legal status
			(c) Separating the target-primer hybrid and unreacted first primer extension reagent (d) Performing a second primer extension reaction using a second primer extension reagent, wherein at least one of the first or second primer extension reagents includes an extendible nucleotide having a label attached thereto (e) Separating the target-primer hybrid from unreacted second primer extension reagent (f) Measuring a signal produced by the label (g) Treating the label so as to render the label undetectable (h) Repeating a cycle of steps (a) through (g) until the signal is substantially less than a signal detected in a previous cycle (i) Determining a number of repeat units in a repeat region of the target nucleic acid	
27. EP 1002127: Method of determining the genotype of an organism using an allele specific oligonucleotide probe which hybridises to microsatellite flanking sequences	V-14.01.99 P-02.07.97 P-27.03.98 P-01.04.98	University of Bristol		EP-application deemed to be withdrawn
28. EP 1025262: Sequence-based screening	V-04.03.99 P-26.08.97	Diversa Corporation	1. A method for identifying a desired activity encoded by a genomic DNA population comprising: (a) Obtaining a single-stranded genomic DNA population (b) Contacting the single-stranded DNA population of (a) with a DNA probe bound to a ligand under conditions and for sufficient time to	In EP still under examination

Table 1. (Continue)

Publication number and title	Publication date (V) and priority date (P)	Assignee	Main claims	Comment on legal status
			allow hybridization and to produce a double-stranded complex of probe and members of the genomic DNA population which hybridize thereto (c) Contacting the double-stranded complex of (b) with a solid phase specific binding partner for said ligand so as to produce a solid phase complex (d) Separating the solid phase complex from the single-stranded DNA population of (b) (e) Releasing from the probe the members of the genomic population which had bound to the solid phase-bound probe (f) Forming double-stranded DNA from the members of the genomic population of (e) (g) Introducing the double-stranded DNA of (f) into a suitable host cell to produce an expression library containing a plurality of clones containing the selected DNA (h) Screening the expression library for the desired activity	
29. WO 9914376: Detection of aneuploidy and gene deletion by PCR-based gene-dose co-amplification of chromosome specific sequences with synthetic sequences with synthetic internal controls	V-30.03.99 P-19.09.97	Genaco Biomedical Products, Inc.		WO-application deemed to be withdrawn

Table 1. (Continue)

Publication number and title	Publication date (V) and priority date (P)	Assignee	Main claims	Comment on legal status
30. WO 9914375: DNA typing by mass spectrometry with polymorphic DNA repeat markers	V-25.03.99 P-19.09.97	Genetrace Systems, Inc.		EP-application deemed to be withdrawn
31. EP 1045927: Method for identifying genes underlying defined phenotypes	V-22.07.99 P-15.01.98	ValiGen, Inc.		EP-application deemed to be withdrawn
32. EP 1058727: Materials and methods for identifying and analyzing intermediate tandem repeat DNA markers	V-12.08.99 P-04.02.98	Promega Corporation	1. A method for detecting a target intermediate tandem repeat DNA sequence having a low incidence of stutter artifacts, comprising the steps of: (a) Providing a sample of DNA having at least one target intermediate tandem repeat sequence, wherein the target intermediate tandem repeat sequence is a region of the DNA containing at least one repeat unit consisting of a sequence of five (5), six (6), or seven (7) base pairs repeated in tandem at least two (2) times (b) Detecting the target intermediate tandem repeat sequence in the sample of DNA, wherein an average stutter artifact of no more than 2.4% is observed	In EP still under examination
33. WO 9946404: DNA Sequences and their use for the selection of cereals	V-16.09.99 P-10.03.98	Scottish Crop Research Institute		EP-application deemed to be withdrawn
34. EP1042507: QTL mapping in plant breeding populations	V-01.07.99 P-04.05.98	Pioneer HiBred	A method of identifying quantitative trait loci in a mixed defined plant population comprising multiple plant families, the method comprising:	In EP still under examination

Table 1. (Continue)

Publication number and title	Publication date (V) and priority date (P)	Assignee	Main claims	Comment on legal status
			(i) Quantifying a phenotypic trait across lines sampled from the mixed population, thereby providing a quantified population phenotype (ii) Identifying at least one genetic marker associated with the distribution of phenotypic trait by screening a set of markers for associations with the quantified population phenotype (iii) Identifying the quantitative trait loci based on the association of the phenotypic trait and genetic marker	
35. EP 1075545: Infrared matrix-assisted laser desorption/ionization mass spectrometric analysis of macro-molecules	V-11.11.99 P-07.05.98	Sequenom, Inc.	1. A process for performing matrix assisted laser desorption/ ionization (MALDI) of a nucleic acid in preparation for analysis by mass spectrometry, comprising the steps of: (a) Depositing a solution containing the nucleic acid and a liquid matrix on a substrate, thereby forming a homogeneous, thin layer of a nucleic acid/liquid matrix solution (b) Illuminating the substrate with infrared radiation, so that the nucleic acid in the solution is desorbed and ionized	In EP still under examination
36. US 6074831: Partitioning of polymorhpic DNAs	V-13.06.00 P-09.07.98	Agilent Technologies Inc.		In US only
37. EP1106687: Method for isolating satellite sequence	V-13.06.01 P-18.08.98	Japan as represented by Director General of Ministry of Agriculture, Forestry and Fisheries National Institute of Agrobiologica	1. An isolation method for satellite sequences, wherein a genomic DNA is cleaved by a nucleotide sequence-independent method, the isolation method comprising: (a) Obtaining randomly cleaved fragments of the genomic DNA (b) Selecting, from the fragments obtained in a), fragments comprising the satellite sequences	In EP still under examination

Table 1. (Continue)

Publication number and title	Publication date (V) and priority date (P)	Assignee	Main claims	Comment on legal status
38. WO 0017341: Myrtaceae microsatellites	V-30.03.00 P-23.09.98 P-16.02.99	Business and Research Management PTY Ltd.		Myrtaceae only, WO-application deemed to be withdrawn
39. WO 0042210: Microsatellite DNA markers and uses thereof	V-20.07.00 P-15.01.99	USA, the Secretary of Agriculture		Tree, forest only, WO-application deemed to be withdrawn
40. US 6573047: Detection of nucleotide sequence variation trough fluorescence resonance energy transfer label generation	V-03.06.03 P-13.04.99 P-11.04.00	DNA Sciences, Inc.		US only
41. EP 1203100: Methods for detecting nucleotide insertion or deletion using primer extension	V-15.02.01 P-11.08.99	Exact Sciences Corporation	1. A method for detecting a nucleic acid insertion or deletion the method comprising the steps of: (a) Selecting a nucleic acid having a known wild-type sequence and having a target region comprising a repeat sequence having at most three different types of nucleotide bases selected from the group consisting of dGTP, dATP, dTTP, and dCTP (b) Contacting a sample with an oligonucleotide primer that is complementary to a portion of said nucleic acid immediately upstream of said target region (c) Extending said primer in the presence of nucleotide bases that are complementary to the nucleotide bases of the target region, thereby to form a primer extension product	In EP still under examination

Table 1. (Continue)

Publication number and title	Publication date (V) and priority date (P)	Assignee	Main claims	Comment on legal status
			(d) Extending the primer extension product in the presence of a labelled nucleotide complementary to a nucleotide base downstream from the target region in said nucleic acid, wherein said labelled nucleotide is not complementary to any of the nucleotide bases of the target region, thereby to produce a labelled extension product comprising a sequence that is complementary to the entire target region (e) Detecting the labelled extension product (f) Comparing the size of the labelled extension product detected in step (e) to a standard, wherein a labelled extension product smaller than the standard is indicative of the presence of a deletion in the target region and a labelled extension product larger than the standard is indicative of the presence of an insertion in the target region Further comprising the step of terminating the primer extension product by incorporating a terminator nucleotide in said product that is complementary to a nucleotide downstream from the target region in a wild-type nucleic acid, wherein said terminator nucleotide is not complementary to any of the nucleotides of the target region, said step of terminating the primer extension product being performed simultaneously with or immediately after step (d).	
42. EP1230385: Marker-assisted identification of a gene associated with a phenotypic trait	V-19.04.01 P-08.10.99	Pioneer Hi Bred	1. A method of associating a gene with a phenotypic trait of interest, comprising: (a) Segregating members of a biological population by the presence or absence of at least one genetic marker in linkage disequilibrium with said phenotypic trait, wherein said phenotypic trait is statistically associated with more than one genetic locus (b) Expression profiling segregated members of (a) (c) Determining from expression profiles of (b) said gene associated with said phenotypic trait	In EP still under examination

Table 1. (Continue)

Publication number and title	Publication date (V) and priority date (P)	Assignee	Main claims	Comment on legal status
43. WO 0140512: Resistance gene	V-07.06.01 P-29.11.99	Plant Bioscience Limited		WO-application deemed to be withdrawn
44. EP 1250452: Methods for determining single nucleotide variations and genotyping	V-07.06.01 P-02.12.99	DNA Sciences, Inc.		EP-application withdrawn
45. NZ 0509194: Simple sequence repeats (microsatellites) in clover	V-25.05.01 P-24.12.99	Agriculture Victoria Services PTY Ltd.		AUH, NZ only
46. NZ 0509193: Molecular markers in ryegrass and fescues	V-25.05.01 P-24.12.99	State of South Australia as represented by South Australian Research and Development Institute		NZ only
47. EP 1265476: MQM mapping using haplotyped putative QTL-alleles: a simple approach for mapping QTLs in plant breeding populations	V-12.07.01 P-30.12.99	Pioneer HiBred	1. A method of mapping a phenotypic trait to a corresponding chromosomal location or region, the method comprising: (i) Providing a population of progeny, the progeny descending from a plurality of families resulting from related or unrelated crosses (ii) Assigning phenotypic values to at least one phenotypic trait segregating in the population of progeny (iii) Determining a genotype for at least one haplotype in the population of progeny, which at least one haplotype comprises a plurality of genetic markers	In EP still under examination

Table 1. (Continue)

Publication number and title	Publication date (V) and priority date (P)	Assignee	Main claims	Comment on legal status
			(iv) Applying a statistical model which evaluates correspondence between the haplotype and the assigned phenotypic value, thereby identifying a chromosomal location corresponding to the phenotypic trait	
48. WO 0151627: Soybean SSRs and methods of genotyping	V-19.07.01 P-07.01.00	–		EP-application deemed to be withdrawn
49. WO 0162967: A method that compares genomic sequences	V-30.08.01 P-22.02.00	Genena Ltd.		WO-application is deemed to be withdrawn
50. WO 0162966: Methods for characterizing polymorphisms	V-30.08.01 P-24.02.00	Gemini Inc		WO-application has not entered EP-Phase
51. JP 2000060559: Isolation of satellite sequence	V-29.02.00	Natl. Inst. of Agrobiological Resources		JP only
52. WO 0179482: Gene mapping method	V-25.10.01 P-13.04.00	Inoko, Hidetoshi		JP, US only

Table 1. (Continue)

Publication number and title	Publication date (V) and priority date (P)	Assignee	Main claims	Comment on legal status
53. EP 1278894: Identification of genetic markers	V-29.01.02 P-02.05.00	Centre National de la Recherche Scientifique, Institut National de la Santé et de la Recherche Médicale	1.A method for the identification of the presence of a genetic marker in a DNA sample comprising the following steps: (a) Selection of sequences specific of said genetic marker (b) Fixation of oligonucleotides comprising said specific sequences or the complementary sequences on a solid support (c) Addition of a mixture of DNA fragments representing the said DNA sample to the solid support in a way that hybridization is 1 0 possible (d) Detection of the presence of the genetic marker in the DNA sample by the presence of a signal corresponding to the hybridization of a fragment of the DNA sample to the specific oligonucleotide, 1 5 wherein said specific sequences are flanking sequences of said genetic marker and said DNA sample has been reduced in complexity	In EP still under examination
54. WO 0185988: Methods for detecting nucleic acid molecules having particular nucleotide sequences	V-15.11.01 P-09.05.00	Diatech Pty. Ltd.		WO-application is deemed to be withdrawn
55. EP 1282729: Microsatellite-AFLP	P-15.05.00 P-12.01.01 V-22.11.01	Keygene N.V.	1. Use of a RAMP primer and an AFLP primer in analysing a nucleic acid sequence, in particular in analysing a nucleic acid sequence for the presence polymorphisms associated with microsatellites	In EP still under examination
56. EP 1297181A2: Sample generation for genotyping by mass spectrometry	V-03.01.02 P-30.06.00	Centre National de Genotypage	1. A method for DNA genotyping by mass spectrometry, comprising the steps of: (a) Reduction of the complexity of the DNA sample (b) Generation of allele-specific products on the products generated in step (a), wherein the generation of allele specific products in step (b) is achieved by at least one method that uses (an) allele-specific oligonucleotide(s)	In EP still under examination

Table 1. (Continue)

Publication number and title	Publication date (V) and priority date (P)	Assignee	Main claims	Comment on legal status
			(c) Mass spectrometric analysis of the products generated in step b, 1 0 wherein the mass spectrometric analysis in step c. is performed on the products generated in step b. without purification or separation from the reaction mixture	
57. WO 0205628: Pollen polymyx plant breeding method utilizing molecular pedigree analysis	V-24.01.02 P-18.07.00	Weyerhaeuser Company		WO-application has not entered EP-Phase
58. WO 0238804: Method for marking samples containing DNA by means of oligonucleotides	V-16.05.02 P-08.11.00	Agrobiogen GmbH Biotechnologie	No English translation of the claims available 1. Verfahren zur Kennzeichnung von DNA-enthaltenden Proben, bei dem mindestens ein Kennzeichnungs-Oligonukleotid mit einer zu kennzeichnenden Probe zusammengebracht wird und die Probe zusammen mit dem Kennzeichnungs-Oligonukleotid einer Untersuchung unterworfen wird, wobei das Kennzeichnungs-Oligonukleotid ausgewählt ist aus der Gruppe bestehend aus artifiziellen Mikrosatelliten-Oligonukleotiden oder artifiziellen Single- Nukleotide-Polymorphismus-Oligonukleotiden	In EP still under examination
59. EP 1207210: Method for melting curve analysis of repetitive PCR products	V-09.07.02 P-15.11.00	F. Hoffmann La Roche AG	1. Method for analysis of a target nucleic acid consisting of repetitive and non repetitive sequences comprising: (a) Hybridization of at least one polynucleotide hybridization probe comprising a first segment which is complementary to a non repetitive region and a second segment which is complementary to an adjacent repetitive region, said second segment consisting of a defined number of repeats (b) Determination of the melting point temperature of the hybrid which has been formed between the target nucleic acid and the at least one hybridization probe	In EP still under examination

Table 1. (Continue)

Publication number and title	Publication date (V) and priority date (P)	Assignee	Main claims	Comment on legal status
60. WO 02086159: Method for genotyping Microsatellite DNA markers	V-31.10.02 P-23.04.01	Galileo Genomics	1. A method for genotyping different alleles of a microsatellite DNA locus by using combinations of at least three oligonucleotides for each allele on the locus comprising: (a) Providing a sample containing the microsatellite DNA (b) Selecting at least three oligonucleotides comprising: (i) A 5' primer which comprises at least a 5-base pair sequence that is complementary a flanking region of a repeat region of the microsatellite (ii) A central primer which is complementary to a repeated region of the microsatellite DNA (iii) A plurality of 3' primers which comprises: (a) A sequence that is complementary to the 5' flanking sequence of the repeated region of the microsatellite (b) A number (n) of repeat units at the 5' end of the plurality of 3' primers (c) Mixing the sample and primers such that the primers and microsatellite DNA hybridize (d) Adding a ligating reagent (e) Detecting the presence of ligation products that consist of all the oligonucleotide primers	
61. WO 02090563A1: Methods for the reduction of stutter in microsatellite amplification using sorbitol	V-14.11.02 P-07.05.01	PE Corporation (NY)	1. A method for reducing stutter in the amplification of a microsatellite comprising the steps of: (a) Providing a sample comprising a microsatellite of interest, said microsatellite having a G+C content of greater than 50% (b) Contacting said sample with at least one enzyme having nucleic acid polymerase activity	

Table 1. (Continue)

Publication number and title	Publication date (V) and priority date (P)	Assignee	Main claims	Comment on legal status
			(c) Incubating said sample with said enzyme for a time and under conditions sufficient to amplify said microsatellite; wherein said incubation is performed in the presence of an amount of sorbitol effective to reduce said stutter relative to the amount of stutter observed in the absence of sorbitol	
62. WO 02090562: Methods for the reduction of stutter in microsatellite amplification	V-14.11.02 P-07.05.01	Applied Biosystems Inc.		Belonging to WO02090562
63. WO 0185988: Methods for detecting nucleic acid molecules having particular nucleotide sequences	V-15.11.01 P-09.05.01	Diatech Pty. Ltd.		WO-application deemed to be withdrawn
64. WO 03023055: Method for detecting mutations, insertions, deletions and polymorphisms on DNA and the use thereof	V-20.03.03 P-12.09.01	Max-Delbrück-Centrum für Molekulare Medizin	No English translation of the claims available 1. Methode zum Nachweis von Mutationen, Insertionen, Deletionen und Polymorphismen auf der DNA, dadurch gekennzeichnet, dass – eine erste spezifische PCR-Reaktion durchgeführt wird mit einem Primerpaar, dass die zu untersuchende Stelle im Genom flankiert, wobei am Y-Ende jedes Primers zusätzlich eine jeweils unterschiedliche universelle Oligonukleotidsequenz hängt, die mit der zu untersuchenden DNA-Sequenz nicht komplementär ist; – eine zweite universelle PCR-Reaktion durchgeführt wird mit einem markierten, vorrangig fluoreszenzmarkierten oder biotinmarkierten, Primerpaar, dass komplementär zu den in der ersten PCR verwendeten 15 universellen Oligonukleotidsequenzen ist; – die PCR-Produkte durch Erhitzung denatu-	

Table 1. (Continue)

Publication number and title	Publication date (V) and priority date (P)	Assignee	Main claims	Comment on legal status
			riert und danach schnell wieder abgekühlt werden; – eine Auftrennung der PCR-Produkte erfolgt; und – die Detektion der Laufeigenschaften der PCR-Produkte, wobei die Laufeigenschaften abhängig sind von der speziellen Faltung und der Konformation der DNA-Einzelstränge und die spezielle Faltung wiederum von der DNA-Sequenz	
65. WO 03035906: Method for genotyping Microsatellite DNA markers by mass spectrometry	V-01.05.03 P-26.10.01	Galileo Genomics	1. A method for genotyping different alleles of a microsatellite DNA locus by using enzymatic and/or chemical agents that produce short, single stranded DNA fragments of a size suitable for mass spectrometry analysis, said method comprising: (a) Providing a genomic DNA sample containing the microsatellite DNA (b) Performing PCR amplification of a microsatellite DNA marker locus, using: (i) An appropriate combinations of oligonucleotides (ii) A dNTP mix in which the 2'-thymidine 5'-triphosphate is replaced by 2'-uridine 5'-triphosphate (iii) A thermostable DNA polymerase that is capable of incorporating uridine nucleotides at positions where thymidine nucleotides are usually incorporated (iv) An appropriate buffer (c) Treating the PCR fragment with uracyl-DNA-glycosylase (d) Treating further the UDG-treated DNA with an enzymatic or chemical agent that cleaves DNA at a basic site to yield single-stranded DNA products	

Table 1. (Continue)

Publication number and title	Publication date (V) and priority date (P)	Assignee	Main claims	Comment on legal status
66. WO 03040395: Universal nucleotides for nucleic acid analysis	V-26.06.03 P-07.11.01	Applera Corporation	1. A method of sequencing at least one target nucleic acid template comprising: (a) Forming a reaction composition comprising at least one target nucleic acid template, at least one primer, at least one polymerase, at least one universal nucleotide, and at least one specific terminator (b) Incubating the reaction composition under appropriate conditions to generate at least one primer extension product comprising at least one or more of the universal nucleotides and at least one or more of the specific terminators (c) Separating at least one or more of the primer extension products, wherein the separating comprises at least one mobility-dependent analysis technique (MDAT) (d) Detecting at least one or more of the primer extension products	
67. EP 1217079: Microsatellite markers from *triticum tauschii*	V-26.06.02 P-?	Institut National de la Recherche Agronomique (INRA)		EP-application deemed to be withdrawn
68. WO03085133: Novel FISSR-PCR primers and method of identifying genotyping diverse genomes of plant and animal systems including rice varieties, a kit thereof	V-16.10.03 P-08.04.02	Centre for DNA fingerprinting and diagnostics, India	1. A set of inter-simple sequence repeats (ISSR)-PCR primers of SEQ ID Nos. 1 to 37 for genotyping eukaryotes, (for example: SEQ ID NO. 1. GATGCTGATACACACACACACACA)	

Table 1. (Continue)

Publication number and title	Publication date (V) and priority date (P)	Assignee	Main claims	Comment on legal status
P1 EP 0509612: Process for amplifying and detecting nucleic acid sequences	V-21.10.92 P-28.03.85 P-25.10.85 P-07–02–86	F. Hoffmann La Roche Inc.	1. A first and second single-stranded oligonucleotide allowing amplification of a specific template nucleic acid sequence contained in a single- or double-stranded nucleic acid or in a mixture of such nucleic acids, wherein (a) One oligonucleotide of said oligonucleotides contains a part which is substantially complementary to said template nucleic acid sequence in said single-stranded nucleic acid or in one strand of said double-stranded nucleic acid (b) The other oligonucleotide of said oligonucleotides contains a part which is substantially complementary to a complement of said template nucleic acid sequence in said single-stranded nucleic acid or in said strand of said double-stranded nucleic acid (c) Said parts of oligonucleotides (a) and (b) have attached to their 5'-end a nucleotide sequence which is non-complementary to said template nucleic acid sequence and which comprises a restriction site; and wherein (d) The parts of said oligonucleotides of (a) and (b) that have substantial complementarity are different and define the termini of the specific template nucleic acid sequence to be amplified	Granted EP-patent, no opposition
P2 EP 0201184: Process for amplifying nucleic acid sequences	V-21.10.92 P-28.03.85 P-25.10.85	F. Hoffmann La Roche Inc.	1. A process for exponentially amplifying at least one specific double-stranded nucleic acid sequence contained in a nucleic acid or a mixture of nucleic acids wherein each nucleic acid consists of two complementary strands, of equal or unequal length, which process comprises:	Granted EP-patent, but opposition filed against

Table 1. (Continue)

Publication number and title	Publication date (V) and priority date (P)	Assignee	Main claims	Comment on legal status
			(a) Treating the strands with a molar excess of two oligonucleotide primers, one for each of the strands, under hybridizing conditions and in the presence of an inducing agent for polymerization and the different nucleotides, such that for each strand an extension product of the respective primer is synthesized which is complementary to the nucleic acid strand, wherein said primers are selected so that each is substantially complementary to one end of the sequence to be amplified on one of the strands such that an extension product can be synthesized from one primer which, when it is separated from its complement, can serve as a template for synthesis of an extension product of the other primer (b) Separating the primer extension products from the templates on which they were synthesized to produce single-stranded molecules (c) Treating the single-stranded molecules generated from step (b) with the primers of step (a) under hybridizing conditions and in the presence of an inducing agent for polymerisation and the different nucleotides such that a primer extension product is synthesized using each of the single-strands produced in step (b) as a template; and, if desired (d) Repeating steps (b) and (c) at least once; whereby the amount of the sequence to be amplified increases exponentially relative to the number of steps in which primer extension products are synthesized	

Table 1. (Continue)

Publication number and title	Publication date (V) and priority date (P)	Assignee	Main claims	Comment on legal status
P3 EP 0200362: Process for amplifying, detecting, and/or cloning nucleic acid sequences	V-20.01.93 P-28-03.85 P-25.10.85 P-07.02.86	F. Hoffmann La Roche Inc.	1. A process for detecting the presence or absence of at least one specific double-stranded nucleic acid sequence in a sample, or distinguishing between two different double-stranded nucleic acid sequences in said sample, which process comprises first exponentially amplifying the specific sequence or sequences (if present) by the following steps, and then detecting the thus-amplified sequence or sequences (if present): (a) Separating the nucleic acid strands in the sample and treating the sample with a molar excess of a pair of oligonucleotide primers for each different specific sequence being detected, one primer for each strand, under hybridizing conditions and in the presence of an inducing agent for polymerization and the different nucleoside triphosphates such that for each of said strands an extension product of the respective primer is synthesized which is complementary to the strand, wherein said primers are selected so that each is substantially complementary to one end of the sequence to be amplified on one of the strands such that the extension product synthesized from one primer, when it is separated from its complement, can serve as a template for synthesis of an extension product of the other primer of the pair (b) Treating the sample resulting from (a) under denaturing conditions to separate the primer extension products from their templates (c) Treating as in (a) the sample resulting from (b) with oligonucleotide primers such that a primer extension product is synthesized using each of the single strands produced in step (b) as a template; and, if desired,	Granted, but opposition filed against

Table 1. (Continue)

Publication number and title	Publication date (V) and priority date (P)	Assignee	Main claims	Comment on legal status
			(d) Repeating steps (b) and (c) at least once; whereby exponential amplification of the nucleic acid sequence or sequences, if present, results thus permitting detection thereof; and, if desired, (e) Adding to the product of step (c) or (d) a labelled oligonucleotide probe capable of hybridizing to said sequence to be detected; and (f) Determining whether said hybridization has occurred	
P4 EP 0497784: Quantitation of nucleic acids using the polymerase chain reaction	V-19.12.95 P-21.08.89 P-28.09.89	F. Hoffmann La Roche Inc.	1. Use of an internal standard for the quantitation of at least one target nucleic acid segment contained within a sample in an amplification method, said internal standard comprising on one strand a nucleic acid segment comprising a 5' sequence and a 3' sequence, which sequences provide an upstream primer hybridization site and the complement of a downstream primer hybridization site which are identical to an upstream primer hybridization site and the complement of a downstream primer hybridization site within said target nucleic acid segment, wherein said internal standard nucleic acid segment and said target nucleic acid segment are co-amplified using the same set of primers and wherein upon amplification said internal standard nucleic acid segment and said target nucleic acid segment can be distinguished	Granted EP-patent, but opposition filed against

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