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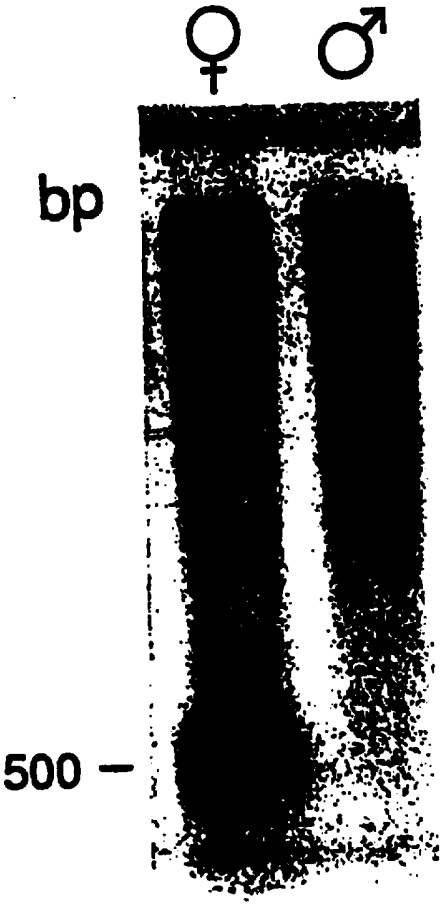
(54) Title: SEX-SPECIFIC DNA PROBE FOR PARROTS, METHODS AND KITS

(57) Abstract

Universal nucleotide sequences are used as DNA or RNA probes in a procedure for sex determination of parrots requiring only a few microliters of blood. The DNA or RNA probes of the invention are sex-specific but not parrot-species specific.



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SEX-SPECIFIC DNA PROBE FOR PARROTS, METHODS AND KITS

Related Applications

This is a continuation-in-part of application for U.S. patent assigned Serial No. 08/093,198 and filed on July 15, 1993.

Field of the Invention

The present invention relates to DNA or RNA probes for binding specifically to female DNA of parrots to determine the sex of parrots within a single day and methods and kits.

Background

Parrots is the collective name for approximately 350 species of birds scientifically known as the Psittacidae genus. Parrots include well-known species such as cockatoos, macaws, parakeets and amazones.

Parrots are among the most popular pet birds because of the ability of many species to mimic human speech and to develop strong bonds with their

caretakers. However, as a result of habitat loss and the pet bird trade, many species of parrots have become rare or endangered in the wild.

In order to protect populations of wild parrots, many countries have restricted or completely forbidden the exportation of parrots. Moreover, captive breeding has become an important procedure to prevent further depletion of wild populations and to satisfy the demand for parrots in the pet bird market.

For such breeding projects, male and female birds are needed. Therefore, the ability to determine the sex of individual birds is of major importance. Unfortunately, most species of parrots are monomorphic, meaning that there are no recognizable visible or audible differences between male and female birds. More specifically, virtually all juveniles and about 60% of the adults of all avian species are monomorphic.

Presently, there are basically five methods relied upon today to determine the sex of birds. Unfortunately, the methods are not without drawbacks. In one method, the external reproductive organs are physically examined to the extent possible. This method, called "vent-sexing," is applicable for a few bird families where the external reproductive organs are large enough that they can be observed with relative ease in the cloaca. This method is, in practice, only applicable to waterfowl, such as ducks, geese, and

swans, and gallinaceous birds, such as chickens, where the method can be applied at an early age. However, the difficulty in handling, as well as the difficulties in observing the external reproductive organs, make "vent-sexing" unsuitable for use with parrots, as well as other birds in which the external reproductive organs cannot be observed.

A second method, which is an invasive method, involves the examination of internal reproductive organs. According to this method, a bird must be placed under general anaesthesia and an incision made so that the internal reproductive organs can be observed with an endoscope. Although this is the most often used procedure, it causes great trauma to the bird and often results in infection. Moreover, the method cannot be used with very young birds, because of their sensitivity to surgery, and the insufficient development of the internal reproductive organs in such young birds.

A third method involves biochemical determinations of the concentrations of the steroid sex hormones, estradiol and testosterone, from blood and/or fecal matter. This procedure is based on the observation that male animals are characterized by a high concentration of testosterone in their blood, while females have high levels of estradiol. A disadvantage of this procedure is that it is technically very complicated and requires experienced personnel for its

operation. Another problem is that the procedure has no diagnostic value when used on young animals having low steroid hormone concentrations. Although the method can be applied to faecal matter rather than blood samples, it is often difficult to assign faecal samples to individual birds when more than a few individual birds are under observation.

The fourth method, which is also an invasive method, is based on the occurrence of a specific W chromosome in female birds. Male birds have two Z sex chromosomes, "ZZ," whereas female birds have one W sex chromosome and one Z sex chromosome ("ZW"). In many, but not all bird species, the W chromosome has some unique staining characteristics and can, therefore, be observed and distinguished by microscopic examination of bird chromosome preparations. A disadvantage is that expensive laboratory equipment is needed and highly qualified personnel are required for the performance of this procedure. As a further drawback associated with this method, in a number of bird species, including some parrots, an unambiguous identification of the W sex chromosome is not possible.

The fifth method available today, is a fingerprint based DNA analysis which involves the identification of sex specific DNA fragments by DNA fingerprinting. According to the method, the DNA of individual birds is degraded with specific enzymes,

resulting in the generation of specific fragments, which upon fractionation by electrophoresis forms a pattern specific for each individual bird, including its sex. The disadvantages of a fingerprint based DNA analysis is that it is technically very complicated and takes approximately one week to perform. Moreover, it requires isolation of very pure DNA, enzymatic digestion of the isolated DNA, fractionation by electrophoresis of the DNA fragments in the digest, and transfer of these fragments from the gel to a nylon membrane, followed by probing with a specific probe. Moreover, radioactive labelling of the probe is essential to the procedure, thus requiring the use of highly sophisticated equipment and facilities.

In view of the foregoing, it is evident that there is a demand for a simple, quick, and accurate method to determine the sex of birds, such as parrots.

Summary of the Invention

In brief, the present invention overcomes certain of the above-mentioned problems and shortcomings of the present state of the art through the discovery of novel nucleotide sequences derived from the W chromosome of a female parrot, the African grey parrot, Psittacus erithacus. Uniquely, the novel nucleotide sequence of the present invention are highly female specific for this species of parrot. Moreover, they are

believed to be highly female specific for almost all other species of parrot.

According to the present invention, the novel, universal nucleotide sequences are used as DNA or RNA probes in a quick and reliable procedure for sex determination of parrots. Unique to this procedure and as an advantage over prior invasive procedures utilized heretofore, the sex typing procedures of the present invention can be completed within 24 hours. Moreover, the procedures of the present invention are reliable because virtually all species of parrots contain a sex specific component or components on the W sex chromosome which is closely similar to the sex specific component, i.e., the nucleotide sequences, isolated from the African grey parrot in accordance with the present invention.

In carrying out the present invention, it requires only a few microliters of blood, which can be collected easily without anesthesia, from a wing vein or a toe of the parrot. As an alternative, the blood sample may be acquired from a blood feather such as a developing secondary or primary flight or tail feather.

To summarize a procedure of the present invention, a blood sample is first obtained from a parrot of choice, and DNA is the obtained from this blood sample (10 - 60 min). The DNA is denatured, bound to a nylon membrane and prehybridized (1-2 hrs). The

DNA bound to the nylon membrane is the hybridized with a radioactive or nonradioactive DNA probe solution (12 hrs) of the present invention, and washed to remove the non-specifically bound DNA probe (1 hr). The specifically bound DNA probe is the visualized with the appropriate procedure (2 hrs) to determine the sex of the parrot.

The DNA or RNA probes used in the present invention are sex specific but not parrot-species specific. Radioactively or nonradioactively (biotin) tagged DNA or RNA probes of the present invention are successful in determining the sex of many parrot species in avian collections. The advantages of using the DNA or RNA probes, methods and kits of the present invention for the determination of the sex of parrots include, for example, 1) rapid determination (24 hrs); 2) major surgical procedures are not required; 3) the use of blood as a readily available source of DNA; 4) the use of a safe, stable, highly sensitive and highly sex specific but not species-specific DNA probe; 5) the use of simple procedures utilizing standard clinical and research laboratory equipment which require minimal technical expertise for their operation; and 6) technical simplicity as compared to currently available procedures. Thus, the procedures of the present invention can be easily practiced by veterinarians and

breeders in their offices and by other qualified personnel.

Accordingly, it should be appreciated that the present invention is believed to provide a solution to the sex-typing parrot art that has long sought rapid and reliable methods for determining the sex of different species of parrots. This is accomplished by the present invention, as indicated above, through the identification of novel, universal nucleotide sequences which are useful as DNA or RNA probes that are complementary to DNA segments on the W chromosome which are characteristic for female parrots of the Psittacidae genus. Applications of the DNA or RNA probes contemplated by the present invention therefore include, for example, determination of the sex of parrots in captive breeding programs. Moreover, the applications of the probes include the determination of the sex of parrots in wild populations as part of, for example, research and ecological studies.

The above features and advantages will be better understood with reference to the FIGS., Detailed Description, and Examples set out hereinbelow. It will also be understood that the biological materials, methods and kits of this invention are exemplary only and are not to be regarded as limitations of this invention.

Brief Description of the FIGS

Reference is now made to the accompanying FIGS. in which are shown characteristics corresponding to the novel nucleotide sequences of the present invention from which certain of their features and advantages will be apparent:

FIG. 1 shows the identification of female specific DNA components in a restriction digest of DNA of the African grey parrot. A Southern blot of 2.5 μg DNA of male and female African grey parrot digested with MspI was probed with a genomic female specific DNA probe of the African grey parrot prepared as described under Example I.

FIG. 2 shows the cloning of female specific DNA components of the African grey parrot.

FIG. 3 shows the nucleotide sequence of the 628 bp female specific DNA component (PARsex) of the African grey parrot.

FIG. 3A shows the nucleotide sequences of the fragment between nucleotides 1 and 461 of two cloned genomic MspI DNA fragments (PARsex1 and PARsex 2)

FIG. 3B shows the nucleotide sequence of the fragment between nucleotides 462 and 628 (PARsex11 and PARsex12) determined after PCR amplification and cloning of the amplified fragments. CG dinucleotides are underlined. Nonconserved nucleotides in the second sequences (PARsex2 and PARsex12) are indicated, (-)

means the same base in PARsex2 as in PARsex1 and in PARsex12 as in PARsex11.

FIG. 4 shows the internal repeat structure of the PARsex repeating unit. Fragment number, position of the (starting) nucleotide preceding an oligo dT_{2-6} segment of a fragment, fragment sequence, fragment length and the length of the oligo dT_{2-6} segment of the fragments are indicated. A consensus sequence was generated from the individual fragment sequences using the nucleotides which were present in more than 50% of the positions in the individual fragments. R = purine, Y = pyrimidine, subscript indicates frequency of the nucleotide at the position in the fragment.

FIG 5. shows the genomic organization of PARsex-like material in DNA of the female African grey parrot. Fifteen μg of female African grey parrot DNA in 300 μl restriction buffer B was digested at 37°C with 4 units of the restriction endonuclease MspI. Twenty μl samples were removed at the times indicated. After 2 hours and additional 10 units of the enzyme was added to insure complete degradation. The samples were fractionated by electrophoresis through a 1% agarose gel. A Southern blot was made and probed with ^{32}P-labeled Parsex1.

FIG. 6 shows a gel electrophoresis of the PCR amplification products of a sex specific DNA fragment of the African grey parrot (*Psittacus erithacus*). Eight

µl of the reaction product of the PCR amplification reaction was applied to a 1.2% agarose gel. Lanes a: DNA ladder; b:female DNA; c: male DNA; d:no DNA.

FIG. 7. demonstrates the occurrence of PARsex-like sex specific DNA among the Psittacidae. Two µg (lane a), 1 µg (lane b) and 0.5 µg (lane c) of alkali denatured DNA of male and female *Lorius garrulus* (1), *Aratinga erythrogenys* (2), *Cacatua galerita* (3), *Amazona aestiva* (4), *Ara ararauna* (5) and *Psittacus erithacus* (6) was applied to a Nytran filter with a slot blot apparatus and probed with ^{32}P-labeled PARsex1.

FIG. 8. shows the pattern of PARsex-like restriction fragments in DNA of male and female *Electus roratus*. DNA (1.5 µg) of male and female *E. roratus* was digested with the restriction endonuclease TaqI, and a Southern blot was made and probed with PARsex.

Fig. 9. shows the hybridization of subfragments of PARsex with DNA of female and male Psittacidae. One µg of DNA of males or females of the species indicated was spotted on a Nylon membrane with a dot blot apparatus and hybridized with probes A-D. Probes (25-50 ng/ml) were labeled with ^{32}P by random primer labelling and α-dCTP^{32} (A,B,C) or at the 5' terminus with polynucleotide kinase and Y-ATP^{32} (D). Probes: A = 245 bp MspI - Sau3A fragment 1-245, B = 220 bp Sau3A - MspI fragment 241- 461, C = PCR fragment 461 - 628 (+ primers), and D = 37 nucleotide

fragment 265-301. Sex: female = a,c, and e; male = b, d, f. Psittacidae species = African grey parrot (1ab), blue-fronted amazon (2ab), budgerigar (3ab), moluccan cockatton (4ab), cherry-headed conure (1cd), eclectus parrot (2cd), chattering lory (3cd), blue and gold macaw (4cd) , Indian ringneck parrakeet (1ef), and isolated PCR fragment 461-628 (4f).

Detailed Description

By way of illustrating and providing a morecomplete appreciation of the present invention and many of the attendant advantages thereof, the following detailed description is provided concerning the novel nucleotide sequences, methods and kits.

The novel nucleotide sequences of this invention, preferably used as DNA or RNA probes, have been derived from the DNA of the female African grey parrot *Psittacus erithacus*. More particularly, they have been cloned as a fragment of 461 base pairs, obtained with exhaustive digestion of female African grey parrot DNA with the restriction nuclease MspI, in the vector pGEM3Z+ and the host *Eschericia coli* 31/17. Further they have been cloned as a fragment of 167 base pairs derived by PCR amplification of female parrot DNA using primers with the nucleotide sequence of the 5′ and 3′ terminal segments of the

461 base pair fragments. The complete 628 base pair PARsex element occurs as a tandemly repeated sex specific monomer and is made up of 58 subfragments in which oligo dT fragments of 2-6 thymidine residues are repeated at an average distance of 10.8 base pairs. More specifically, the 2-6 base pair long oligo T fragments are part of 43 subfragments of a minimum length of about 4 nucleotides (nts) and a maximum length of about 20 nts, and an average length of about 10.7 nts which together form the 461 nts long fragment which is cloned. The PCR amplified 167 nts fragment is formed of 15 subfragments of 2-6 base pair oligo T fragments with a minimum length of 8 nts and a maximum nts of 21 nts, and an average length of 11.1 nts. The complete 628 base pair PARsex element is tandemly repeated with a copy number of approximately 13,000 copies per female African grey parrot genome and forms a substantial part of the W chromosomal DNA of this species. The nucleotide sequence of the PARsex component is conserved among the DNA of females of many other species of parrot.

The novel procedure to demonstrate the presence of female W chromosome specific components in parrot DNA utilizes a probe comprising a trace amount of radio-labelled female parrot DNA and a large excess (4000 fold) of unlabelled male DNA in an

analysis of enzymatically digested and electrophoretically fractionated female parrot DNA. FOCUS (BRL) 14:106-108 (1992). The excess unlabelled male DNA acts to dilute the radio-label in the components which are common to male and female DNA, so that common sequences do not produce any signal in the analysis, and the only signal observed is that produced by female specific components. Utilizing this procedure with the restriction enzyme MspI, in a digest of the female African grey parrot, *Psittacus erithacus*, identification of a component of approximately 500 base pairs, which does not occur in the male DNA, is accomplished as demonstrated in FIG 1.

This fragment is isolated from the electrophoretically fractionated MspI digest and cloned in plasmid vector pGEM 3Z+ in the bacterium *Eschericia coli* 71/18 (FIG. 2). This cloned component has been used for the determination of the nucleotide sequence of the female specific component, its copy number and genomic organization, and the conservation among other species of parrots.

FIG. 3a shows that the sequence of this fragment of the PARsex component has a molecular length of 461 base pairs. FIG. 4 shows that the fragment has an internal repeat structure in which

groups of 2-6 thymidine residues are repeated within an average of 10.7 nucleotides. This sequence characteristic is typical for a rather unusual DNA structure, the so called "curved DNA element".

A curved DNA is a double stranded (native) DNA which most often has short runs of 2-6 adenine or thymidine residues at an average distance of 10.5 nts, which is just a complete winding of the DNA double helix. The curved nature of such DNA has been demonstrated by electronmicroscopy and circularization experiments. Curved DNA exhibits an anomalous, slow electrophoretic velocity in polyacrylamide gels. This latter characteristic is usually taken as diagnostic evidence for the curved structure. The curved structure is an inherent property of such DNA and should be distinguished from so called bent DNA. The latter owes its curvature to interaction with certain proteins.

DNA components can occur either as unique or as repeated sequence elements. A quantitative analysis shows that the sex specific component amounts to approximately 0.3% of the genome of the female African grey parrot, whereas it amounts to at most 0.005% of the male genome. See Table I. Hence, the female African grey parrot genome contains about 13,000 copies of the sex specific DNA component,

PARsex, with a length of 628 base pairs. These copies are tandemly organized in the genome. In situ hybridization to chromosomes of the African grey parrot shows strong hybridization of the DNA or RNA probes of the present invention to the W chromosome in the female whereas such hybridization is not observed in the male.

The DNA probes of the present invention can be produced by chemical synthesis, recombinant or cloning technology or any other methods available in the art so long as the methodology selected does not interfere with their utilities stated herein. Moreover, the DNA probes of the present invention may be modified by adding, deleting and/or substituting nucleotides to form DNA probes of varying lengths which are functionally equivalent to the nucleotide sequences set forth in FIGS. 3a and 3b. In addition, RNA probes with nucleotide sequences contemplated by the present invention may be substituted for the DNA probes. Therefore, it is to be understood by those versed in this art that any DNA or RNA nucleotide sequence, including equivalents and active segments if the nucleotide sequences depicted in FIGS. 3a and 3b., which are complimentary to a DNA segment on a W chromosome which is characteristic for a female parrot of the *Psittacidae* genus is contemplated by

the present invention. Examples of active nucleotide fragments in accordance with the present invention are those fragments derived by, for example, digesting the 461 base pair fragment with the enzyme Sau3A1 at a GATC site using digestion techniques known to those skilled in the art. When the 461 base pair fragment is digested with the enzyme Sau3A1, the 461 base pair fragment is cleaved at the GATC sites beginning with the nucleotide designated as 245 in the 5'-3' sequence and with the nucleotide designated as 213 in the 3'-5' sequence in FIG. 3a to generate active fragments having, for example, bases corresponding to bases 1-245 and 246-461 in the 5'-3' sequence. Furthermore, fragments derived from the PCR amplified 167 base pair segment of PARsex containing nucleotides 461-628 in the 5'-3' sequence may be used. Additionally, small chemically synthesized oligonucleotides of approximately 20-40 nucleotides in length may be used. For example, a 37 oligonucleotide equivalent to nucleotides 265-301 of the 5'-3' sequence of PARsex is quite effective as a female sex specific probe.

The DNA or RNA probes of the present invention may be formed into kits which can be easily used by, for instance, veterinarians, breeders, as well as other qualified personnel interested in sex-

typing parrots of the *Psittacidae* genus. A typical kit in accordance with this invention includes blood sample stabilizing Solution A; proteinase-K and sodium sarcosylate solutions, or a commercially available fast DNA isolation kit; DNA denaturing and neutralizing solutions; nylon membranes; biotin-labelled sex specific DNA or RNA probe; hybridization Solution B; wash Solutions C and D; and a detection system specific for the biotin tag so that all that is required to carry out the sex-typing methods of the present invention is standard laboratory equipment.

According to the present invention, blood is used as a source of DNA because avian erythrocytes are nucleated and contain DNA. Avian blood contains on average approximately 5 to 10 mg of DNA per ml. In carrying out the sex typing of parrots in accordance with the present invention, blood samples of approximately 25 to 100 μl are obtained by brachial (wing) puncture or by clipping of a toe nail or by removing a blood feather such as a secondary, or primary flight feather or a tail feather. Clotting of the blood is prevented by immediate dilution after collection, with an equal volume of a solution containing about 0.15M NaCl and about 0.05 M sodium EDTA, pH 7.5 (Solution A). In this solution, blood

samples can be stored for at least a week in a refrigerator at 4°C.

The major contaminant of the DNA in avian blood is protein, which can be removed from the sample by protease digestion followed by phenol or chloroform extraction in the presence of (cationic or anionic) detergents. Accordingly, 10 µl of the 1:1 diluted blood in Solution A (containing on average 50 µg of DNA is further diluted with 90 µl of Solution A and incubated for one hour at about 65°C with proteinase K (2 g/100 µl) and 0.2% sodium lauryl sarcosylate. The phenol or chloroform should be removed by for example centrifugation, followed by a final precipitation with ethanol, and dissolving the deproteinized DNA in TE buffer.

Alternatively, DNA can also be isolated with cationic detergents using kits obtained from Washington Biotechnology Inc. (Bethesda, MD, USA) or InVitrogen (San Diego, CA, USA), and a modification of the protocol recommended by the manufacturers for the isolation of mammalian DNA as follows: In this modification, 0.1 ml avian blood diluted with 0.1 ml 0.15M NaCl, 0.02M EDTA pH 8.0 is lysed in 2 ml of a solution containing 10% dodecyltrimetyl-ammonium bromide, 1.5M NaCl, 100 µM Tris-HCl buffer pH 8.8 and 60 µM EDTA, heated for 2 min at 68°C, cooled and

shaken with 2 ml chloroform. After centrifugation for 5 min at 3000g, the aqueous layer is collected, and the DNA precipitated by the addition of 4 ml of 1% cetyl trimetyl ammonium bromide and further purified by dissolving in 1.2M NaCl and precipitation with ethanol, followed by dissolving the DNA in TE buffer.

DNA samples thus obtained are then denatured with NaOH (final concentration = 0.5M) for 10 min and then the samples are neutralized with an equal volume of 1M NaH_2PO_4. Samples containing approximately 1-10 g of denatured parrot DNA are then loaded and immobilized onto nylon filters (0.2 μM pore size) with the aid of a slot or dot blot apparatus. The filters are dried in vacuo at about 80°C for about 1-2 hours to bind the DNA irreversibly to the filters.

Prior to hybridization with the probe, filters containing the DNA samples are prehybridized for about one hour at about 65°C in a solution containing about 0.9M NaCl, 0.1M Tris-HCl buffer pH 7.8, 0.05M Na_2EDTA, 0.2% sodium lauryl sulphate and 500 μg/ml heparin as a blocking agent (Solution B).

The sex specific DNA or RNA fragments obtained by cloning, PCR amplification, or chemical synthesis as described above and in the examples are tagged by enzymatic or nonenzymatic procedures with

radioactive ^{32}P, nonradioactive biotin, fluorescent dyes like fluorescein and rhodamine, or enzymes such as alkaline phosphatase using technologies available to those versed in the labelling art. DNA probes may be tagged with biotinylated dNTP or ^{32}P-dNTP using nick translation or random primer extension. As to RNA probes, they can be labeled with biotinylated NTP or ^{32}P-NTP using T7 RNA polymerase. The tagged, denatured DNA probe is then incubated for approximately 60-65°C and at a concentration of about 25 ng/ml of Solution B with the nylon filters containing the parrot DNA. Subsequent to incubation, the filters are washed first at room temperature with 1X SSC (0.15M NaCl, 0.015M sodium citrate pH 7.0; Solution C) five times for about five minutes with about 100 ml, and subsequently one time for about two minutes at about 55°C with approximately 100 ml 1X SSC containing about 0.1% sodium lauryl sulphate (Solution D). Binding of radioactively labelled probe can be visualized and quantified by autoradiography and/or a betascope. Bound probes labelled nonradioactively with biotin are visualized through commercially available detection processes which involve specific color reactions or chemiluminescence. The specific color reaction involves, e.g., the binding of a streptavidin or

avidin phosphatase conjugate to the biotin residue (streptavidin and avidine are proteins which have a very high specific affinity for biotin; phosphatase is an enzyme which hydrolyses phosphate esters). The subsequent hydrolysis of a so-called chromogenic substrate consists of an uncolored phosphate ester which becomes strongly colored after removal of the phosphate by the phosphatase. Examples of such esters are bromo-indoxyl phosphate which is uncolored, but which generates dark blue indigo after removal of the phosphate. Chemiluminescent detection systems make use of chemiluminescent substrates in a similar phosphatase catalyzed reaction, followed by photographic detection of the emitted light.

The present invention will now be further illustrated with reference to the following specific examples.

Example I: SEX SPECIFIC DNA FRAGMENT-ISOLATION AND CLONE SELECTION

To identify female parrot sex specific DNA fragments, male and female African grey parrot DNA (10ug) are separately digested in 100 µl of a buffer, containing 6 mM $MgCl_2$, 6 mM Tris-HCl buffer pH 7.5, and 50 mM NaCl, with 20 units of the restriction endonuclease MspI for 3 hours at 37°C. For identification purposes of sex specific DNA

fragments, 10 μl of each digest is fractionated by electrophoresis through a 1.2% agarose gel in TPE buffer (TPE buffer = 0.08M Tris-phosphate pH 7.5, 0.008M EDTA). A Southern blot of the gel is made and probed with a genomic DNA probe made from 50 ng radioactively (^{32}P) labelled female DNA, previously labelled by random primer extension (Feinberg, A.P. et al., Anal. Biochem. 132:6-13 (1983)) preannealed to 200 μg reiterated, denatured male African gray parrot Cot2 DNA (Cot indicates a degree of repetivity of the DNA sample; de Kloet, D.H. and S.R. de Kloet, Focus(BRL) 14:106-108 (1992)). As illustrated in Fig. 1, probing of a Southern blot of a complete digest of male and female African grey parrot DNA with ^{32}P labeled DNA female DNA reveals the presence of an approximately 500 base pair sex specific DNA fragment in the digested female DNA that is absent from digested male DNA.

After identification of the sec specific DNA fragment by autoradiography or with a betascope, a preparative (larger) gel is run and the gel slice containing an approximately 461 base pair sex specific fragment is cut out. The sex specific fragment is isolated from the gel by the powdered glass procedure, Vogelstein, B. et al., Proc. Natl. Acad. Sci. USA 76:615-619 (1979). The fragment is

ligated into the AccI site of the plasmid pGEM 3Z+ and the ligation product is used to transform *E. coli* 71/18 using the $CaCl_2$ procedure (Maniatis, T. et al. (1982) "Molecular cloning: a laboratory manual.", Cold Spring Harbor Laboratory. Cold Spring Harbor Laboratory, NY.) The transformed bacteria are spread on 20 ml agar plates containing LB medium (LB = 1% bacto-tryptone, 1% NaCl, 0.5% bacto yeast extract pH 7.4 containing per ml 50μg of penicillin, 150 μg bromo-indolyl-beta-galactoside and 75 μg isopropyl beta thiogalactoside). After growth overnight at 37°C, the white colonies (containing plasmids with inserts) are selected and transferred to fresh LB plates covered with a Nylon filter. Control plates without a filter are also inoculated with the colonies in the same pattern. After growth until the colonies are detectable, the nylon filter is removed from the plate and dried in a vacuum oven at 80°C. Colonies with a sex specific insert are identified by colony hybridization (Maniatis, T. et al. (1982) "Molecular cloning: a laboratory manual.", Cold Spring Harbor Laboratory. Cold Spring Harbor Laboratory, NY.) using the same genomic sex specific DNA probe as described above in this example. As illustrated in FIG. 2, positive colonies are identified by autoradiography and identified on the

control plates which are inoculated in identical patterns but without the nylon filter. The identified colonies are transferred from the control plates into LB medium broth containing 50 μg/ml penicillin, and incubated overnight at 37°C. The bacterial clones are collected by centrifugation and resuspended in LB + ampicillin containing 15% glycerol and stored at -80°C.

Example II: PARsex GENE ORGANIZATION AND SEQUENCE

Nucleotide sequences of the cloned sex specific DNA fragments are determined using standard methodology known to those skilled in the art with a commercial automatic sequencer (ABI, Foster City, CA, USA) using forward and reverse primers. As illustrated in FIG. 3a, the nucleotide sequence of one of the cloned fragments, PARsex1, shows that the actual length of the cloned sex specific fragment to be 461 base pairs. Sequence determination of a second clone, PARsex2 (also illustrated in FIG. 3a) also gives a length of 461 base pairs. The two clones of the sex specific DNA fragments differ by only 17 nucleotides or approximately 4% of the sequence.

Digestion of female African grey parrot genomic DNA with MspI with sampling of the digest over time allows one to follow the sex specific

component from partial digestion to complete digestion of the genomic DNA. Probing the digest mixtures with ^{32}P-PARsex1, as shown in FIG. 5, reveals that the sex specific component forms a transient ladder type pattern with a monomer length for the sex specific component of approximately 600 base pairs, indicative of a tandemly repeated organization. Exhaustive digestion results in a decrease of the 600 base pair component and the appearance of the 461 base pair PARsex product indicating that the cloned PARsex element is only part of the actual repeat sex specific monomer.

The nucleotide sequence of the remaining segment can be determined after PCR amplification, using primers with the nucleotide sequence of the 5' and 3' terminal segments of the 461 base pair PARsex1 or PARsex2 elements (5'-GGTTAGGAATGGACG(A)AAAA(G)CG-3' and 5'-GCTTGAAGACCTTTTTGCC-3') and female genomic African grey parrot DNA as template. DNA products are purified by agarose gel purification and the powered glass procedure. Products are ligated into dT extended EcoRV cut pGEM5 plasmid, cloned in *E. coli* 71/18, and positive clones identified by colony hybridization as described above. As illustrated in FIG. 6, only DNA of female but not DNA of male parrots can serve as the template. A sex specific

DNA fragment of approximately 200 base pairs is amplified. Sequencing of clones of the fragment (PARsex11 and PARsex12) shows (FIG. 3b) that the actual length between the primers is 167 nucleotides, yielding a total length for the repeat sex specific monomer (PARsex) of 628 nucleotides. The sequence of the two clones for this fragment of the monomer, like the 461 base pair piece, differs by about 4% between the two clones. Analysis of the sequence for the complete repeat monomer reveals an internal repeat structure with a consensus repeat sequence of $R_{0.74}T_{1.00}T_{1.00}T_{0.84}T_{0.55}T_{0.51}R_{0.82}C_{0.65}C_{0.61}Y_{0.65}$ and for which oligo dT_{2-6} segments are repeated at average intervals of approximately 10.8 nucleotides. See FIG. 4.

Example III: DETERMINATION OF THE SEX OF PARROTS WITH THE AFRICAN GREY PARROT SEX SPECIFIC DNA COMPONENT "PARsex 1"

In carrying out a procedure in accordance with the present invention to determine the sex of a parrot, 25 µl blood is removed from a wing vein or toe with a heparinized capillary and suspended in 0.5 ml of Solution A (a solution containing 0.05M Na_2EDTA and 0.15M NaCl (pH 7.4)). Of this suspension, 100 µl (corresponding to 5 µl of blood) is taken and diluted with 200 µl of a solution containing 8% sodium dodecyl trimethylammonium bromide, 1.5M NaCl, 100 mM

Tris-HCl buffer pH 8.6, and 50 mM EDTA. After heating for two minutes at 68°C, vortexing for two minutes with 300 µl of chloroform and centrifugation for two minutes in an Eppendorf centrifuge, the aqueous phase (top layer, 250 µl) is collected. Since avian blood contains approximately 5 µg DNA per µl, this aqueous phase contains approximately 25 µg of DNA per 250 µl, or 1 µg of DNA per 10 µl. Fifty µl of the aqueous phase is then taken (containing approximately 5 µg of DNA), diluted with 50 µl of TE (0.01M Tris-HCl buffer pH 8.0, 0.001M Na_2EDTA), and the DNA is denatured by the addition of 100 µl of 1M NaOH. After five minutes, the denatured DNA is neutralized with 200 µl 1M NaH_2PO_4 and 100 µl of 1.5M NaCl is added to a final volume of 500 µl. One hundred µl of this solution (containing approximately 1 µg of DNA) is applied to a Nylon filter with the aid of a slot blot or dot blot apparatus. The filter is dried and baked in an oven at 80°C. For hybridization, the filter with the bound denatured DNA is then preincubated for thirty minutes at 60°C with 0.2 ml per cm^2 of a solution containing 0.9M NaCl, 0.002M Na_2EDTA, 0.02M Tris-HCl pH 8.0, 0.1% sodium dodecyl sulphate and 500 µg per ml of heparin (as the blocking agent to prevent direct binding of the probe to the filter without binding to the DNA)

and 1000 μg per ml of sodium pyrophosphate. Heat denatured radioactively labelled parrot sex specific probe (40 - 100 ng per 10 ml buffer) is then added and the incubation continued overnight. Subsequently, the filters are washed with 1X SSC to remove the nonhybridized probe, and the bound radioactive label visualized by autoradiography or a betascope.

In the event the blood sample is taken from a blood feather, the procedure is as follows. A growing primary or secondary feather is collected and the feather pulp is squeezed out of the feather into 1 or 2 ml (depending on the size of the feather) of Solution A or any other stabilizing solution (e.g., Dulbecco's modified Eagles, etc.). To this suspension is added 75-150 μl of a solution of 10 Mg per ml collagenase (*Clostridium haemolyticum*) in water and the mixture is incubated for two hours at 37°C. After gentle homogenization DNA is prepared from the resulting cells as described above for blood DNA. After measuring the amount of DNA with a spectrophotometer, the DNA is again alkali denatured, applied to a nylon filter and the filter prehybridized and hybridized with a probe as described above.

As illustrated in Table I and FIG. 7, the PARsex1 fragment hybridizes in a sex specific manner with DNA of many psittacine species belonging to different subfamilies. Strong hybridization can be observed with DNA of females of *Alisterus amboinensis*, *Psittacus erithacus*, *Polytelis alexandrae*, *Platycercus eximus* and females of the *Cacatuiini*, with the exception of *Probosciger aterrimus*. A weaker but still highly sex specific signal can be observed with the *Ariini*, *Psittaculini* and *Loriini*. Further, PARsex hybridizes at 1.6 times as strongly to DNA of female as compared to DNA of male *Eclectus roratus*, but very little hybridization can be detected with DNA of the single male and female *Nestor notabilis* which were available for analysis. Similarly, very little hybridization could be detected with DNA of male and female chickens (*Gallus gallus*).

Despite the weak hybridization difference between female and male *Eclectus roratus*, FIG. 8 demonstrates that in *Eclectus roratus* distinct sex-specific PARsex-like components occur. When male and female *Eclectus roratus* DNA is digested with TaqI, electrophoretically separated and hybridized as above, although bands shared in common between male and female *Eclectus roratus* DNA are present,

hybridization with ^{32}P-labelled PARsex 1 labels distinct bands present in female *Eclectus roratus* DNA which are absent in male *Eclectus roratus* DNA.

TABLE I

Subfamily	Species	females	males
Ariini	Amazona aestiva (Blue-fronted amazone)	7.6 (3)	0.7 (3)
	Amazona ochracephala (Mexican double yellowhead)	8.4 (2)	0.7 (2)
	Amoropsittaca aymara (Aymara)	15.8 (3)	1.5 (2)
	Ara ararauna (Blue and gold macaw)	12.4 (4)	1.2 (2)
	Ara macao (Scarlet macaw)	14.7 (2)	0.9 (2)
	Ara severa (Severe macaw)	13.8 (1)	2.4 (2)
	Aratinga erthrogenys (Cherry-headed conure)	15.8 (3)	2.3 (3)
	Aratinga solstitalis (Sun conure)	17.2 (3)	2.7 (3)
	Pionus menstruus (Blue-headed pionus)	12.4 (1)	0.9 (1)
Cacatuini	Cacatua goffini (Goffins cockatoo)	30.3 (3)	0.9 (3)
	Cacatua moluccensis (Moluccan cockatoo)	35.0 (3)	1.3 (3)
	Cacatua galerita (Greater sulfur crested cockatoo)	33.7 (1)	1.1 (3)
	Callocephalon fimbriatum (Gang-gang cockatoo)	30.2 (2)	0.8 (1)
	Calyptorhyncus funereus (White-tailed black cockatoo)	38.3 (1)	0.7 (2)
	Calyptorhynchus magnificus (Red-tailed black cockatoo)	35.3 (1)	0.9 (1)
	Eolophus roseicapella (Rose-breasted cockatoo)	31.3 (3)	1.1 (2)

		Nymphicus hollandicus (Cockathiel)	34.5 (4)	0.6 (2)
		Probosciger aterrimus (Palm cockatoo)	12.7 (2)	0.7 (1)
	Loriini	Lorius garrulus (Chattering lory)	13.5 (2)	1.3 (1)
	Nestorini	Nestor notabilis (Kea)	0.5 (1)	0.3 (1)
	Platycercini	Melopsittacus undulatus (Budgerigar)	23.5 (3)	0.7 (2)
		Platycercus eximus (Eastern rosella)	25.6 (2)	0.4 (1)
	Psittacini	Psittacus erithacus (African grey parrot)	100.0 (3)	1.5 (2)
	Psittaculini	Agapornis personata (Black-masked love bird)	8.3 (2)	0.3 (2)
		Alisterus amboinensis (Amboina king parrot)	40.2 (2)	2.2 (1)
		Apromiscus erythropterus (Crimson wing)	16.4 (1)	1.3 (2)
		Eclectus roratus (Eclectus parrot)	7.0 (2)	4.8 (4)
		Polytelis alexandrae (Princess of Wales)	24.3 (2)	0.5 (2)
		Psittacula eutrapia (Alexander ringneck)	20.6 (2)	1.3 (3)
		Psittacula krameri (Indian ringneck)	30.3 (2)	2.1 (2)
	Psittrichasini	Psittrichas fulgidus (Pesquets parrot)	8.5 (1)	0.3 (1)
	Gallidae	Gallus gallus	0.2 (2)	0.2 (2)

Example IV: DETERMINATION OF THE SEX OF PARROTS WITH PARsex OLIGONUCLEOTIDES

Fragments of the sex specific monomer, PARsex, shorter than the 461 base pair PARsex1 or PARsex2 elements also are very effective in the determination of the sex of various psittacine species. The 461 base pair PARsex1 can be cut with the nuclease Sau3A, and the fragments purified as described above, to yield two shorter nucleotide probes equal to nucleotides 1-245 and nucleotides 241-461 of the monomer. Further the fragments produced by PCR, PARsex11 or PARsex12, can be used as probes. Additionally smaller oligonucleotides of 20-40 nucleotides whose sequence is based on the monomer's sequence can be chemically synthesized using commercial sources or commercially available equipment. An example of one of these oligonucleotides is the 37 nucleotide (nt) sequence 5'-TTAAGCCGTTTTGTCCATTTCTGACCCATCTTTGCC-3', the 5' terminus which corresponds to nucleotide 265 of the PARsex sequence. As illustrated in FIG. 9, hybridization of the above ^{32}P-labelled probes with male and female parrot genomic DNA of nine different psittacine species demonstrates that these smaller probes encompassing only a portion of the PARsex monomer sequence are very effective as sex specific probes. The best results are obtained using the

shorter 37 nt oligonucleotide which shows high specificity with all the parrot species tested. An additional advantages of the oligonucleotide are that a hybridization temperature of 50-55°C may be used and that a hybridization time of only an hour instead of overnight is required.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: de Kloet, Siwo R.

(ii) TITLE OF INVENTION: Sex-Specific DNA Probe For Parrots, Methods And Kits

(iii) NUMBER OF SEQUENCES: 64

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/376,946
(B) FILING DATE: 20-JAN-1995
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
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(C) REFERENCE/DOCKET NUMBER: FL20979-34

(ix) TELECOMMUNICATION INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 628 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GTTTTCGTCC ATTCCTAACC ACATTAAAGC CTATTTTTCA CCCATTTCCA ACCAATTTTA 60

AGCAATTTGT GGTCATTTCA AACACAGTTT TCACCACTTT GAACAAGCTT TAAGTCCTTT 120

TTGGTTGCTT GTAAATGATT TTTGGAGTTT TCTAACCCCT TTTGAGCCAT TTTTTCTGTT 180

TCTAACCCAT TTTTTCAACA GTTCTAGCTC GGTTTAAGTA GTTTTTGCTT TTTTCTAACC 240

CATTGATCCC ATGACTAATC AGTTTTAAGC CGTTTTTGTC CATTTCTGAC CCATCTTTGG 300

CCAGCTCTAG CTTTGTTTAA GCCGTTTTTC TCCATTTCTA ACCCGTTTCT AGCCCATTCC 360

TGACCTGTTT TAAGCCTATT CCTAACCCAT TTCCAACCCA TTTTTGGTCT TGTCAAATGC 420

ATTTTTCACC TCTTCTGACT CGCTTGAAGA CCTTTTTGCC GGTTTCTAAA GCATTTTTGG 480

CGTTTTCTAA CCCGGTTTAA TCCTTTTCTT GGCTCTTTCT CACCCATTTC TCACATTTCT 540

CGTCCATTTT AATCCATTTT TGGTCATTTC TAAACCATTT CTGCCCATGT GCAACCTGTT 600

TCAAGCAGGT TTTGGGGATT TCTAACCG 628

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 461 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GTTTTCGTCC ATTCCTAACC ACATTAAAGC CTATTTTTCA CCCATTTCCA ACCAATTTTA 60

AGCAATTTGT GGTCATTTCA AACACAGTTT TCACCACTTT GAACAAGCTT TAAGTCCTTT 120

TTGGTTGCTT GTAAATGATT TTTGGAGTTT TCTAACCCCT TTTGAGCCAT TTTTTCTGTT 180

TCTAACCCAT TTTTTCAACA GTTCTAGCTC GGTTTAAGTA GTTTTTGCTT TTTTCTAACC 240

CATTGATCCC ATGACTAATC AGTTTTAAGC CGTTTTTGTC CATTTCTGAC CCATCTTTGC 300

CCAGCTCTAG CTTTGTTTAA GCCGTTTTTC TCCATTTCTA ACCCGTTTCT AGCCCATTCC 360

TGACCTGTTT TAAGCCTATT CCTAACCCAT TTCCAACCCA TTTTTGGTCT TGTCAAATGC 420

ATTTTTCACC TCTTCTGACT CGCTTGAAGA CCTTTTTGCC G 461

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 208 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CGCTTGAAGA CCTTTTTGCC GGTTTCTAAA GCATTTTTGG CGTTTTCTAA CCCGGTTTAA 60

TCCTTTTCTT GGCTCTTTCT CACCCATTTC TCACATTTCT CGTCCATTTT AATCCATTTT 120

TGGTCATTTC TAAACCATTT CTGCCCATGT GCAACCTGTT TCAAGCAGGT TTTGGGGATT 180

TCTAACCGGC TTTCGTCCAT TCCTAACC 208

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GTTTTCGTCC 10

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATTCCTAACC AC 12

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATTAAAGCCT 10

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATTTTTCACC C 11

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

ATTTCCAACC A 11

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ATTTTAAGCA 10

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

ATTTGTGGTC 10

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ATTTCAAACA CA 12

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GTTTTCACCA 10

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CTTTGAACAA G 11

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CTTTAAGTC 9

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CTTTTTGGTT G 11

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CTTGTAAATG 10

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

ATTTTTGGA 9

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GTTTTCTAAC CC 12

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CTTTTGAGCC 10

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

ATTTTTTCT 9

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GTTTCTAACC C 11

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

ATTTTTTCAA CA 12

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GTTCTAGCTC G 11

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GTTTAAGTA 9

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GTTTTTG 7

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CTTTTTTCTA ACCC 14

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

ATTGATCCCA TGACTAATCA 20

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GTTTTAAGCC 10

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GTTTTTGTCC 10

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

ATTTCTGACC CAT 13

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

CTTTGGCCAG CTCTAG 16

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

CTTT 4

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GTTTAAGCC 9

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GTTTTTCTCC 10

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

ATTTCTAACC C 11

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GTTTCTAGCC C 11

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

ATTCCTGACC T 11

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

GTTTTAAGCC T 11

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

ATTCCTAACC C 11

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

ATTTCCAACC C 11

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

ATTTTTGGT 9

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

CTTGTCAAAT GC 12

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

ATTTTTCACC T 11

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

CTTCTGACTC G 11

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

CTTGAAGAC 9

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

CTTTTTGCCG 10

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

GTTTCTAAAG C 11

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

ATTTTTGGC 9

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

CTTTTCTAAC CCG 13

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

GTTTAATC 8

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

CTTTTCTTGG CT 12

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

CTTTCTCACC C 11

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

ATTTCTCAC 9

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

ATTTCTCGTC C 11

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

ATTTTAATCC 10

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

ATTTTTGGTC 10

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

ATTTCTAAAC C 11

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

ATTTCTGCCC ATGTGCAACC T 21

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

GTTTCAAGCA G 11

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

GTTTTGGGG 9

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

ATTTCTAACC G 11

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

GGTTAGGAAT GGACRAAARC G 21

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

GCTTGAAGAC CTTTTTGCC 19

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

TTAAGCCGTT TTGTCCATTT CTGACCCATC TTTGCC 36

CLAIMS:

Having described my invention, I claim:

(1) An oligonucleotide probe having an effective number of nucleotides for hybridizing specifically with a DNA segment which is characteristic for female parrots of the Psittacidae genus.

(2) An oligonucleotide probe as recited in claim 1, the DNA segment being located on the W chromosome of the female parrot of the Psittacidae genus.

(3) An oligonucleotide probe as recited in claim 2, said probe further including a labeled moiety so that, when said labeled probe is hybridized to the DNA segment, the DNA segment on the W chromosome can be detected.

(4) An oligonucleotide probe as recited in claim 3, said labeled moiety being a fluorophore.

(5) An oligonucleotide probe as recited in claim 4, said fluorophore being selected from a group consisting of a fluorescein, a rhodamine or any other fluorophore.

(6) An oligonucleotide probe as recited in claim 3, said labeled moiety being selected from a group consisting of biotin and a radioisotope.

(7) An oligonucleotide probe as recited in claim 2, said probe having a periodicity of groups of about 2 to about 6 thymidine or adenine residues, said groups being repeated on average of about 10.5 nucleotides.

(8) An oligonucleotide probe as recited in claim 2, said oligonucleotide probe further having a curved DNA element.

(9) An oligonucleotide probe as recited in claim 2, said oligonucleotide probe having a nucleotide sequence selected from a group having the following 5'-3' sequence or 3'-5' sequence or an equivalent sequence or an effective segment thereof:

```
5'-GTTTTCGTCC ATTCCTAACC ACATTAAAGC CTATTTTTCA CCCATTTCCA
3'-CAAAAGCAGG TAAGGATTGG TGTAATTTCG GATAAAAAGT GGGTAAAGGT

   ACCAATTTTA AGCAATTTGT GGTCATTTCA AACACAGTTT TCACCACTTT
   TGGTTAAAAT TCGTTAAACA CCAGTAAAGT TTGTGTCAAA AGTGGTGAAA

   GAACAAGCTT TAAGTCCTTT TTGGTTGCTT GTAAATGATT TTTGGAGTTT
   CTTGTTCGAA ATTCAGGAAA AACCAACGAA CATTTACTAA AAACCTCAAA

   TCTAACCCCT TTTGAGCCAT TTTTTCTGTT TCTAACCCAT TTTTTCAACA
   AGATTGGGGA AAACTCGGTA AAAAAGACAA AGATTGGGTA AAAAAGTTGT

   GTTCTAGCTC GGTTTAAGTA GTTTTTGCTT TTTTCTAACC CATTGATCCC
   CAAGATCGAG CCAAATTCAT CAAAAACGAA AAAAGATTGG GTAACTAGGG

   ATGACTAATC AGTTTTAAGC CGTTTTTGTC CATTTCTGAC CCATCTTTGC
   TACTGATTAG TCAAAATTCG GCAAAAACAG GTAAAGACTG GGTAGAAACG

   CCAGCTCTAG CTTTGTTTAA GCCGTTTTTC TCCATTTCTA ACCCGTTTCT
   GGTCGAGATC GAAACAAATT CGGCAAAAAG AGGTAAAGAT TGGGCAAAGA

   AGCCCATTCC TGACCTGTTT TAAGCCTATT CCTAACCCAT TTCCAACCCA
   TCGGGTAAGG ACTGGACAAA ATTCGGATAA GGATTGGGTA AAGGTTGGGT

   TTTTTGGTCT TGTCAAATGC ATTTTTCACC ACTTCTGACT CGCTTGAAGA
   AAAAACCAGA ACAGTTTACG TAAAAAGTGG TGAAGACTGA GCGAACTTCT

   CCTTTTTGCC G-3'
   GGAAAAACGG C-5'
```

(10) An oligonucleotide probe as recited in claim 1, said probe being a DNA probe.

(11) An oligonucleotide probe as recited in claim 1, said probe being a RNA probe.

(12) A method of determining the sex of a parrot of the Psittacidae genus, said method comprising:

hybridizing the labeled probe of claim 3 to the female chromosome DNA of the animal; and

detecting the labeled probe hybridized to the DNA segment on the female chromosome of the parrot to identify the female chromosome of the parrot.

(13) A method as recited in claim 12, said detecting step comprising:

visualizing the labeled probe hybridized to the DNA segment.

(14) A method as recited in claim 12, the labeled probe being a labeled DNA probe.

(15) A method as recited in claim 12, the labeled probe being a labeled RNA probe.

(16) A kit for determining the sex of a parrot of the Psittacidae genus, said kit comprising:

an oligonucleotide probe of claim 1; and

components for carrying out an assay to determine the sex of the parrot.

(17) A kit as recited in claim 16, said oligonucleotide probe being a DNA probe.

(18) A kit as recited in claim 16, said oligonucleotide probe being a RNA probe.

(19) An oligonucleotide probe as recited in claim 9, said probe being PARsex 1.

(20) An oligonucleotide probe as recited in claim 2, said oligonucleotide probe having a nucleotide sequence selected from a group having the following 5'-3' sequence or 3'-5' sequence or an equivalent sequence or an effective segment thereof:

```
5'-GCTTTTGTCC ATTCCTAACC CCATTACAGC CTATTTTTAA CCCATTTCCA
3'-CGAAAACAGG TAAGGATTGG GGTAATGTCG GATAAAAATT GGGTAAAGGT

   ACCAATTTTA AGCAATTTGT GGTCATTTCA AACACAGTTT TCACCACTTT
   TGGTTAAAAT TCGTTAAACA CCAGTAAAGT TTGTGTCAAA AGTGGTGAAA

   GAACAAGCTT TAAGTCCTTT TTGGTTGCTT GTAAATGATT TTTGGAGTTT
   CTTGTTCGAA ATTCAGGAAA AACCAACGAA CATTTACTAA AAACCTCAAA

   TCTAACCCCT TTTGAGCCAT TTTTTCTGTT TCTAACCCAT TTTTTCAACA
   AGATTGGGGA AAACTCGGTA AAAAAGACAA AGATTGGGTA AAAAAGTTGT

   GTTCTAGCTC GGTTTAAGTA GTTTTTGCTT TTTTCTAACC CATTGATCCC
   CAAGATCGAG CCAAATTCAT CAAAAACGAA AAAAGATTGG GTAACTAGGG

   ATGACTAATC AGTTTTAAGC TGTTTTTGTC CATTTCTGAC CCATCTTTGC
   TACTGATTAG TCAAAATTCG ACAAAAACAG GTAAAGACTG GGTAGAAACG

   CCAGCTCTAG CTTTGTTTAA GCCGTTTTTC TCCATTTCTA ACCCGTTTCT
   GGTCGAGATC GAAACAAATT CGGCAAAAAG AGGTAAAGAT TGGGCAAAGA

   AGCCCATTCC TAACCTGTTT TAAGCCTATT TCTAACCCAT TTTCAAGCAA
   TCGGGTAAGG ATTGGACAAA ATTCGGATAA AGATTGGGTA AAAGTTCGTT

   TTTTTCGTCA TGTCAAACGC ATTTTTCACC ACTTCTGACT CGCTTGAAGT
   AAAAAGCAGT ACAGTTTGCG TAAAAAGTGG TGAAGACTGA GCGAACTTCA

   CCTTTTTGCC G-3'
   GGAAAAACGG C-5'
```

(21) An oligonucleotide probe as recited in claim 20, said probe being PARsex 2.

(22) An oligonucleotide probe as recited in claim 2, said oligonucleotide probe having a nucleotide sequence selected from a group having the following 5'-3' sequence or 3'-5' sequence or an equivalent sequence or an effective segment thereof:

```
5'-CGCTTGAAGA CCTTTTTGCC GGTTTCTAAA GCATTTTTGG CGTTTTCTAA
3'-GCGAACTTCT GGAAAAACGG CCAAAGATTT CGTAAAAACC GCAAAAGATT

   CCCGGTTTAA TCCTTTTCTT GGCTCTTTCT CACCCATTTC TCACATTTCT
   GGGCCAAATT AGGAAAAGAA CCGAGAAAGA GTGGGTAAAG AGTGTAAAGA

   CGTCCATTTT AATCCATTTT TGGTCATTTC TAAACCATTT CTGCCCATGT
   GCAGGTAAAA TTAGGTAAAA ACCAGTAAAG ATTTGGTAAA GACGGGTACA

   GCAACCTGTT TCAAGCAGGT TTTGGGGATT TCTAACCGGC TTTCGTCCAT
   CGTTGGACAA AGTTCGTCCA AAACCCCTAA AGATTGGCCG AAAGCAGGTA

   AGGATTGG-3'
   TCCTAACC-5'
```

(23) An oligonucleotide probe as recited in claim 22, said probe being PARsex 11.

(24) An oligonucleotide probe as recited in claim 2, said oligonucleotide probe having a nucleotide sequence selected from a group having the following 5'-3' sequence or 3'-5' sequence or an equivalent sequence or an effective segment thereof:

```
5'-CGCTTGAAGT CCTTTTTGCC GGTTTCTAAA GCATTTTTGG CGTTTTCCAA
3'-GCGAACTTCA GGAAAAACGG CCAAAGATTT CGTAAAAACC GCAAAAGGTT

   CCCAGTTTAA TCCTTTTCTT GACTGTTTCT CACCCATTTC TCACATATCT
   GGGTCAAATT AGGAAAAGAA CTGACAAAGA GTGGGTAAAG AGTGTATAGA

   CGTCCTTTTT AATCCATTTT TGGTCATTTC TAAACCATTT CTGCCCATGT
   GCAGGAAAAA TTAGGTAAAA ACCAGTAAAG ATTTGGTAAA GACGGGTACA

   GCAACCTGTT TCAAGCAGGT TTTGGGGATT TCTAACCGGT TTTCGTCCAT
   CGTTGGACAA AGTTCGTCCA AAACCCCTAA AGATTGGCCA AAAGCAGGTA

   AGGATTGG-3'
   TCCTAACC-5'
```

(25) An oligonucleotide probe as recited in claim 24, said probe being PARsex 12.

(26) An oligonucleotide probe comprising a MspI-Sau3A digest fragment consisting of nucleotides 1-245 of the nucleotide sequence recited in claim 9 or an equivalent sequence.

(27) An oligonucleotide probe comprising a MspI-Sau3A digest fragment consisting of nucleotides 1-245 of the nucleotide sequence recited in claim 20 or an equivalent sequence.

(28) An oligonucleotide probe comprising a MspI-Sau3A digest fragment consisting of nucleotides 245-461 of the nucleotide sequence recited in claim 9 or an equivalent sequence.

(29) An oligonucleotide probe comprising a MspI-Sau3A digest fragment consisting of nucleotides 245-461 of the nucleotide sequence recited in claim 20 or an equivalent sequence.

(30) An oligonucleotide probe comprising a PCR amplified fragment consisting of nucleotides 462-628 of the nucleotide sequence or an equivalent sequence.

(31) An oligonucleotide probe having at least one sequence equivalent to the following concensus sequence:

$$R_{0.74}T_{1.00}T_{1.00}T_{0.84}T_{0.55}T_{0.51}R_{0.82}R_{0.58}C_{0.65}C_{0.61}Y_{0.65}$$

(32) An oligonucleotide probe as recited in claim 31, said probe having an internal T_{2-6} repeat length of about 10.8 nucleotides.

(33) An oligonucleotide probe having the following 5'-3' sequence or an equivalent sequence or an effective segment thereof:

5'-TTAAGC CGTTTTTGTC CATTTCTGAC CCATCTTTGC C-3'

(34) An oligonucleotide probe as recited in claim 3, said labeled moiety being alkaline phosphatase.

(35) A method as recited in claim 12, said hybridizing step using a labled probe, wherein said probe is a probe having the following 5'-3' sequence or an equivalent sequence or an effective segment thereof:

5'-TTAAGC CGTTTTTGTC CATTTCTGAC CCATCTTTGC C-3'

(36) A kit as recited in claim 16, wherein said oligonucleotde probe is a probe having the following 5'-3' sequence or an equivalent sequence or an effective segment thereof:

5'-TTAAGC CGTTTTTGTC CATTTCTGAC CCATCTTTGC C-3'

Figure 1

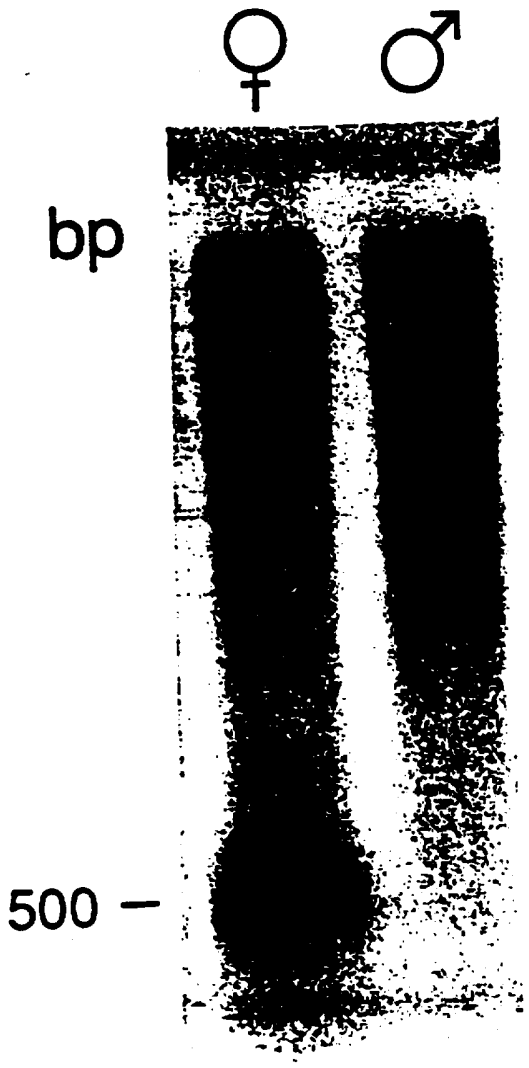


Figure 2

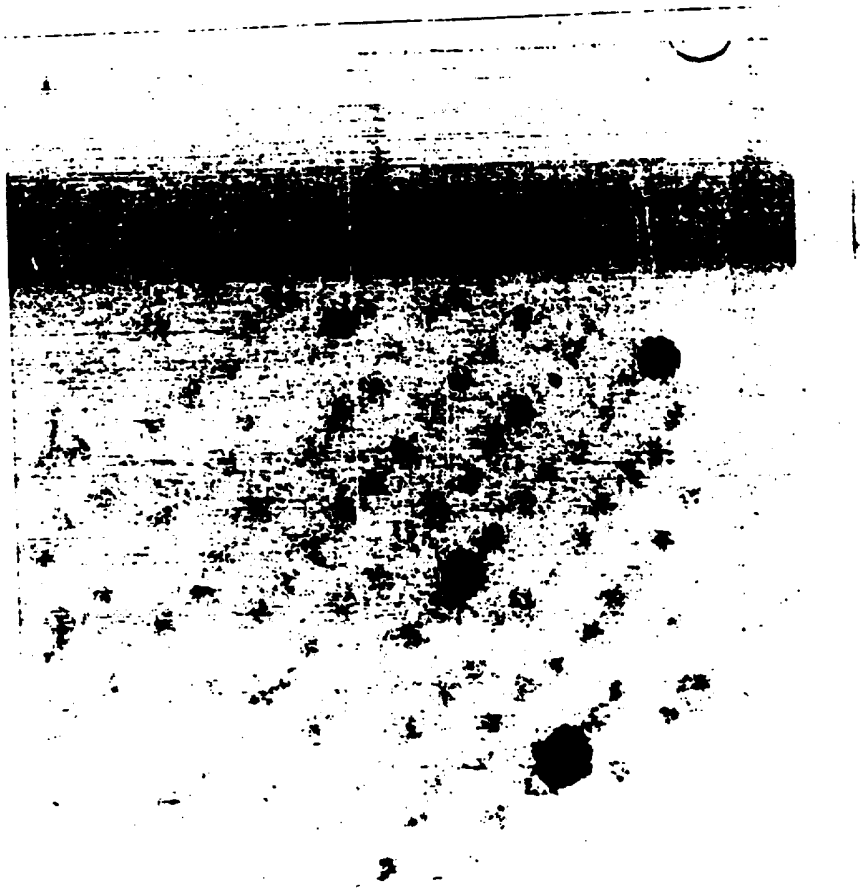


Figure 3

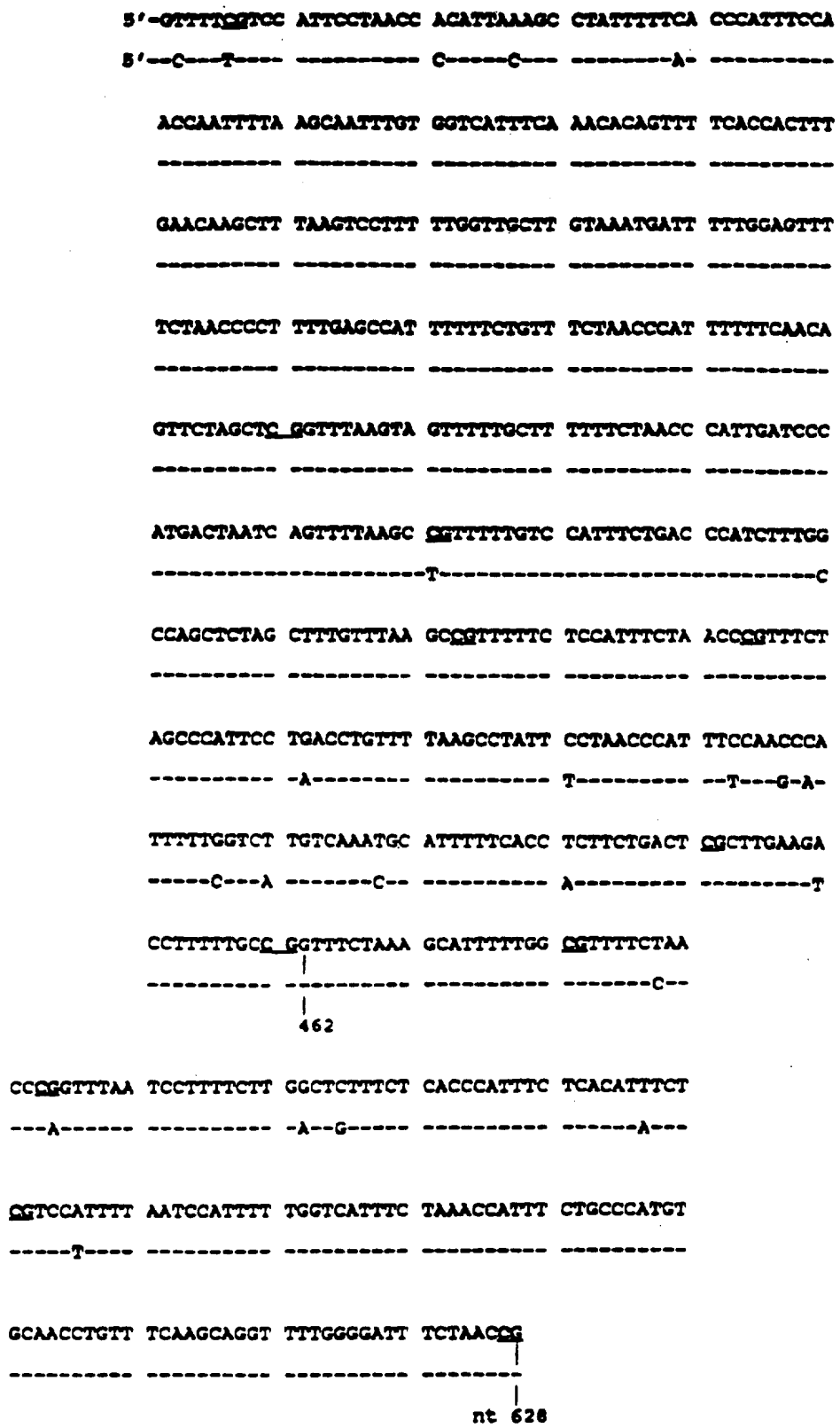

5'-GTTTTCGTCC ATTCCTAACC ACATTAAAGC CTATTTTTCA CCCATTTCCA
5'--C---T---- ---------- C-----C--- --------A- ----------
ACGAATTTTA AGCAATTTGT GGTCATTTCA AACACAGTTT TCACCACTTT
GAACAAGCTT TAAGTCCTTT TTGGTTGCTT GTAAATGATT TTTGGAGTTT
TCTAACCCCT TTTGAGCCAT TTTTTCTGTT TCTAACCCAT TTTTTCAACA
GTTCTAGCTC GGTTTAAGTA GTTTTTGCTT TTTTCTAACC CATTGATCCC
ATGACTAATC AGTTTTAAGC CGTTTTTGTC CATTTCTGAC CCATCTTTGG
----------T----------C
CCAGCTCTAG CTTTGTTTAA GCCGTTTTTC TCCATTTCTA ACCCGTTTCT
AGCCCATTCC TGACCTGTTT TAAGCCTATT CCTAACCCAT TTCCAACCCA
---------- -A-------- ---------- T---------- --T---G-A-
TTTTTGGTCT TGTCAAATGC ATTTTTCACC TCTTCTGACT CGCTTGAAGA
-----C---A -------C-- ---------- A--------- ---------T
CCTTTTTGCC GGTTTCTAAA GCATTTTTGG CGTTTTCTAA
---------- ---------- ---------- -------C--
462
CCCGGTTTAA TCCTTTTCTT GGCTCTTTCT CACCCATTTC TCACATTTCT
---A------ ---------- -A--G----- ---------- ------A---
CGTCCATTTT AATCCATTTT TGGTCATTTC TAAACCATTT CTGCCCATGT
-----T---- ---------- ---------- ---------- ----------
GCAACCTGTT TCAAGCAGGT TTTGGGGATT TCTAACCG
---------- ---------- ---------- --------
nt 628

Figure 3a

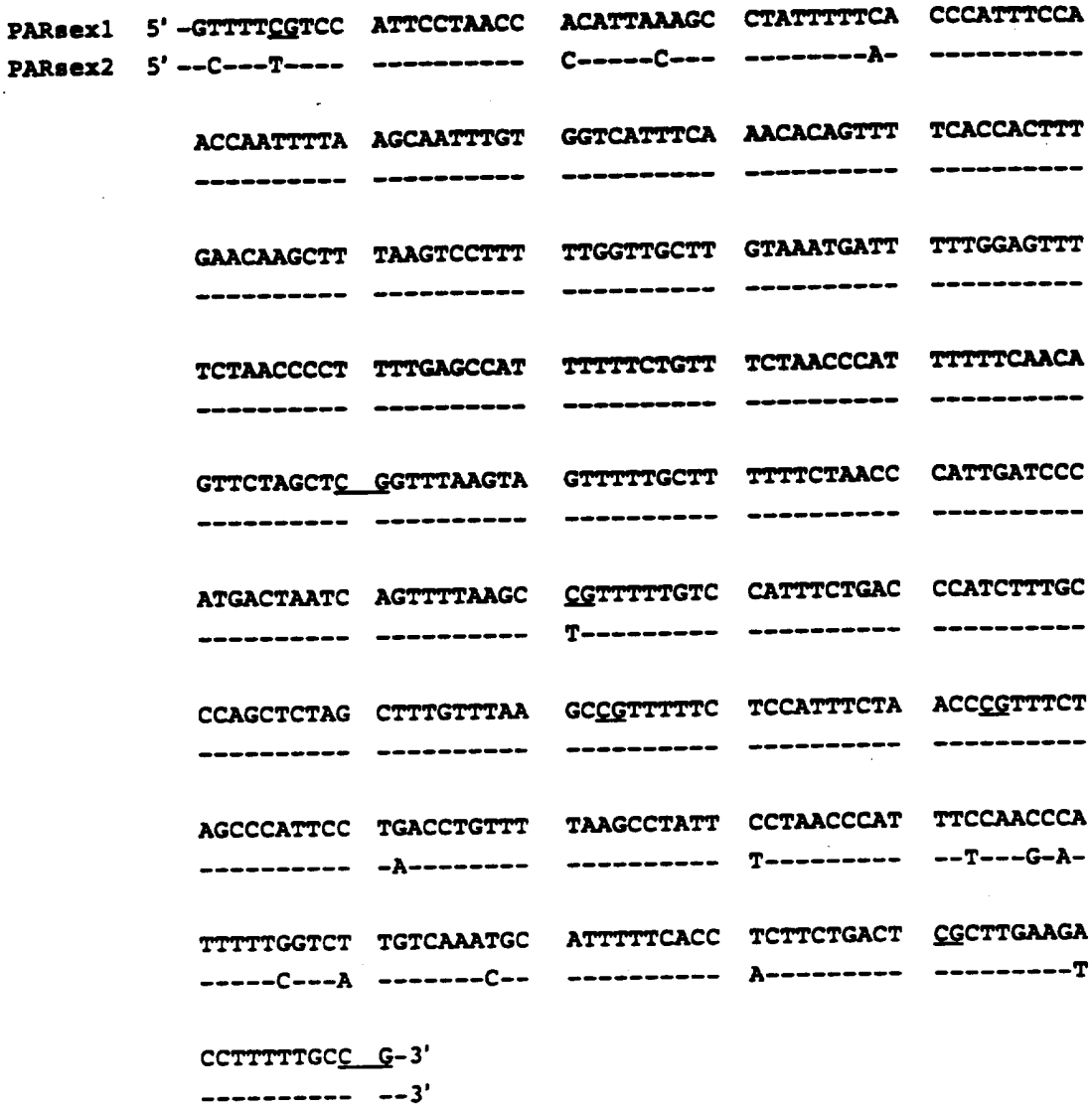

PARsex1 5' -GTTTTCGTCC ATTCCTAACC ACATTAAAGC CTATTTTTCA CCCATTTCCA
PARsex2 5' --C---T---- ---------- C-----C--- --------A- ----------
ACCAATTTTA AGCAATTTGT GGTCATTTCA AACACAGTTT TCACCACTTT
---------- ---------- ---------- ---------- ----------
GAACAAGCTT TAAGTCCTTT TTGGTTGCTT GTAAATGATT TTTGGAGTTT
---------- ---------- ---------- ---------- ----------
TCTAACCCCT TTTGAGCCAT TTTTTCTGTT TCTAACCCAT TTTTTCAACA
---------- ---------- ---------- ---------- ----------
GTTCTAGCTC GGTTTAAGTA GTTTTTGCTT TTTTCTAACC CATTGATCCC
---------- ---------- ---------- ---------- ----------
ATGACTAATC AGTTTTAAGC CGTTTTTGTC CATTTCTGAC CCATCTTTGC
---------- ---------- T--------- ---------- ----------
CCAGCTCTAG CTTTGTTTAA GCCGTTTTTC TCCATTTCTA ACCCGTTTCT
---------- ---------- ---------- ---------- ----------
AGCCCATTCC TGACCTGTTT TAAGCCTATT CCTAACCCAT TTCCAACCCA
---------- -A-------- ---------- T--------- --T---G-A-
TTTTTGGTCT TGTCAAATGC ATTTTTCACC TCTTCTGACT CGCTTGAAGA
-----C---A -------C-- ---------- A--------- ---------T
CCTTTTTGCC G-3'
---------- --3'

Figure 3b

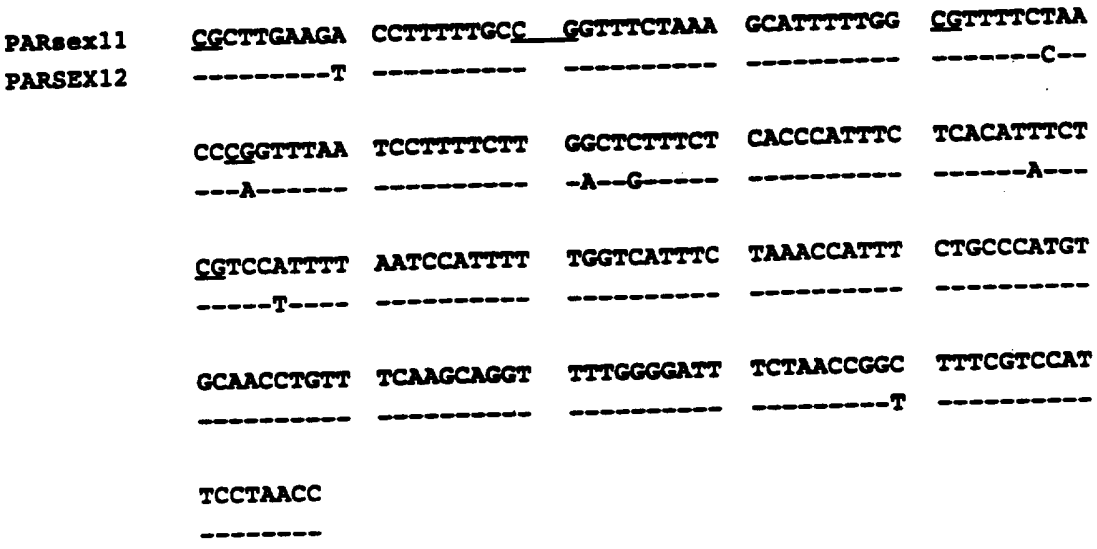

PARsex11 CGCTTGAAGA CCTTTTTGCC GGTTTCTAAA GCATTTTTGG CGTTTTCTAA
PARSEX12 ----------T ---------- ---------- ---------- --------C--
CCCGGTTTAA TCCTTTTCTT GGCTCTTTCT CACCCATTTC TCACATTTCT
---A------ ---------- -A--G----- ---------- ------A---
CGTCCATTTT AATCCATTTT TGGTCATTTC TAAACCATTT CTGCCCATGT
-----T---- ---------- ---------- ---------- ----------
GCAACCTGTT TCAAGCAGGT TTTGGGGATT TCTAACCGGC TTTCGTCCAT
---------- ---------- ---------- ---------T ----------
TCCTAACC

Figure 4. Internal repeat structure of the sex specific repeat of the African grey parrot. The subfragments begin with the nucleotide preceeding the individual oligo dT_{2-6} segments. Initial nt indicates the position of the initial nt in Fig. 3a and 3B.

Fragment length	Starting position	Fragment Sequence	Fragment length (nts)	oligo dT (nts)
1	1	5'-GTTTTCGTCC	10	4
2	11	ATTCCTAACCAC	12	2
3	23	ATTAAAGCCT	10	2
4	33	ATTTTTCACCC	11	5
5	44	ATTTCCAACCA	11	3
6	55	ATTTTAAGCA	10	4
7	65	ATTTGTGGTC	10	3
8	75	ATTTCAAACACA	12	3
9	87	GTTTTCACCA	10	4
10	97	CTTTGAACAAG	11	3
11	108	CTTTAAGTC	9	3
12	117	CTTTTTGGTTG	11	5
13	128	CTTGTAAATG	10	2
14	138	ATTTTTGGA	9	5
15	147	GTTTTCTAACCC	12	4
16	159	CTTTTGAGCC	10	4
17	169	ATTTTTTCT	9	6
18	178	GTTTCTAACCC	11	3
19	189	ATTTTTTCAACA	12	6
20	201	GTTCTAGCTCG	11	2
21	212	GTTTAAGTA	9	3
22	221	GTTTTTG	7	5
23	228	CTTTTTTCTAACCC	14	6
24	242	ATTGATCCCATGACTAATCA	20	2
25	262	GTTTTAAGCC	10	4
26	272	GTTTTTGTCC	10	5
27	282	ATTTCTGACCCAT	13	3
28	295	CTTTGGCCAGCTCTAG	16	3
29	311	CTTT	4	3
30	315	GTTTAAGCC	9	3
31	324	GTTTTTCTCC	10	5
32	334	ATTTCTAACCC	11	3
33	345	GTTTCTAGCCC	11	3
34	356	ATTCCTGACCT	11	2
35	367	GTTTTAAGCCT	11	4
36	378	ATTCCTAACCC	11	2
37	389	ATTTCCAACCC	11	3
38	400	ATTTTTGGT	9	5
39	409	CTTGTCAAATGC	12	2
40	421	ATTTTTCACCT	11	5
41	432	CTTCTGACTCG	11	2
42	443	CTTGAAGAC	9	2
43	452	CTTTTTGCCG	10	5
44	462	GTTTCTAAAGC	11	3
45	473	ATTTTTGGC	9	5
46	482	CTTTTCTAACCCG	13	4
47	495	GTTTAATC	8	3
48	503	CTTTTCTTGGCT	12	4
49	515	CTTTCTCACCC	11	3
50	526	ATTTCTCAC	9	3
51	535	ATTTCTCGTCC	11	3
52	546	ATTTTAATCC	10	4
53	556	ATTTTTGGTC	10	5
54	566	ATTTCTAAACC	11	3
55	577	ATTTCTGCCCATGTGCAACCT	21	3
56	598	GTTTCAAGCAG	11	3
57	609	GTTTTGGGG	9	4
58	618	ATTTCTAACCG-3'	11	3

Concensus: $R_{0.74}T_{1.00}T_{1.00}T_{0.84}T_{0.55}T_{0.51}R_{0.82}R_{0.58}C_{0.65}C_{0.61}Y_{0.65}$

Figure 5

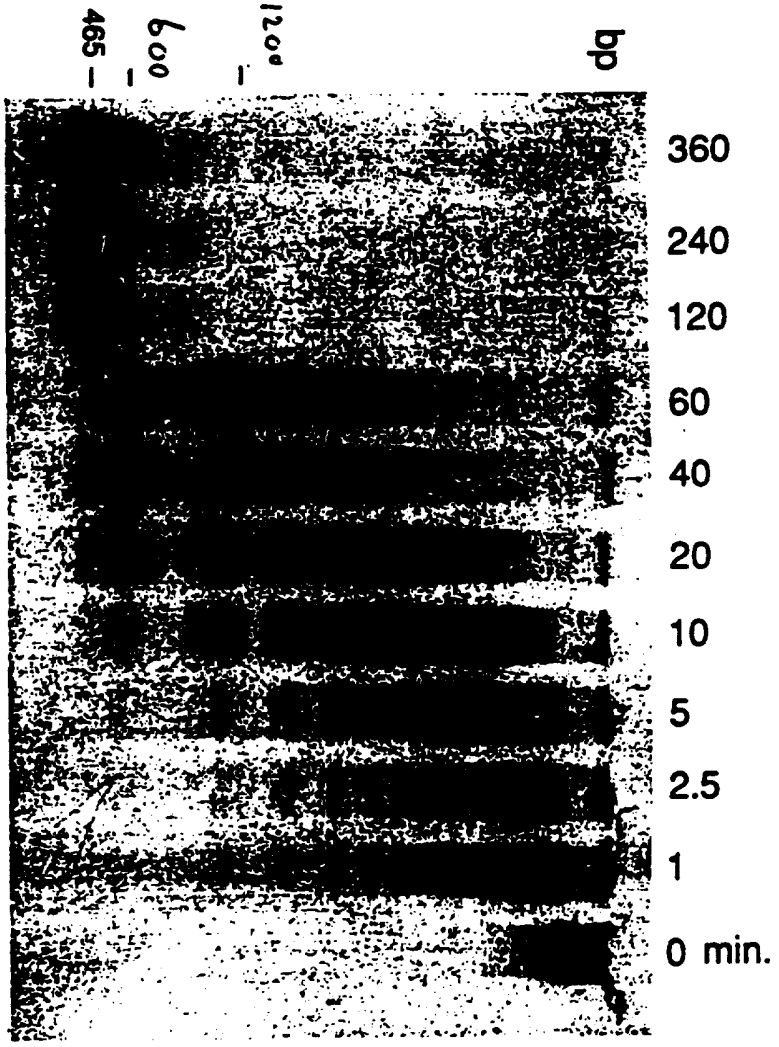


Figure 6

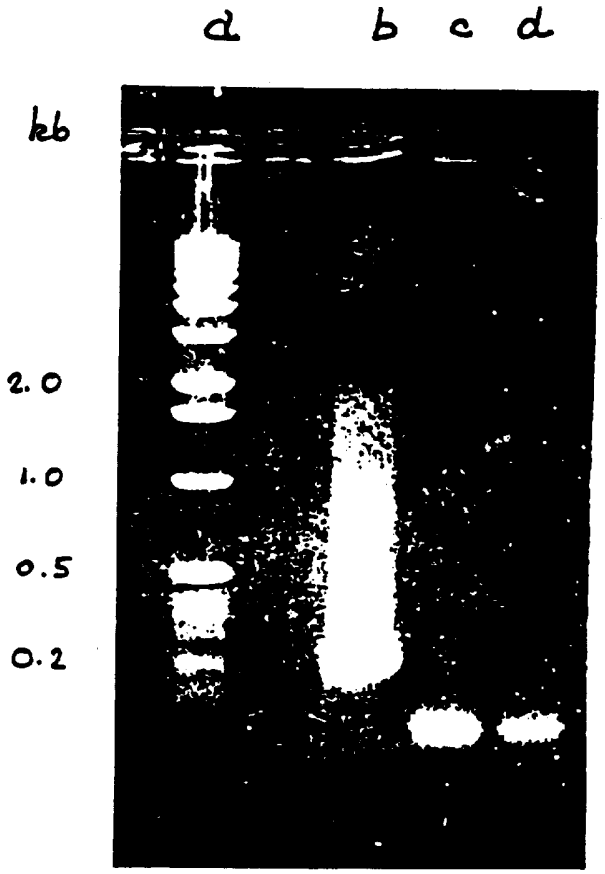


Figure 7

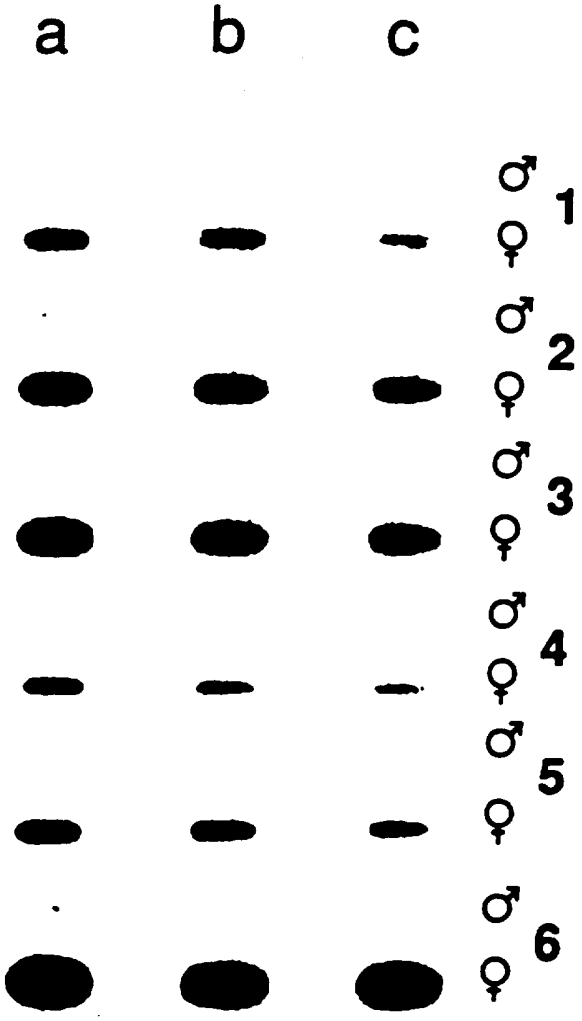


Figure 8

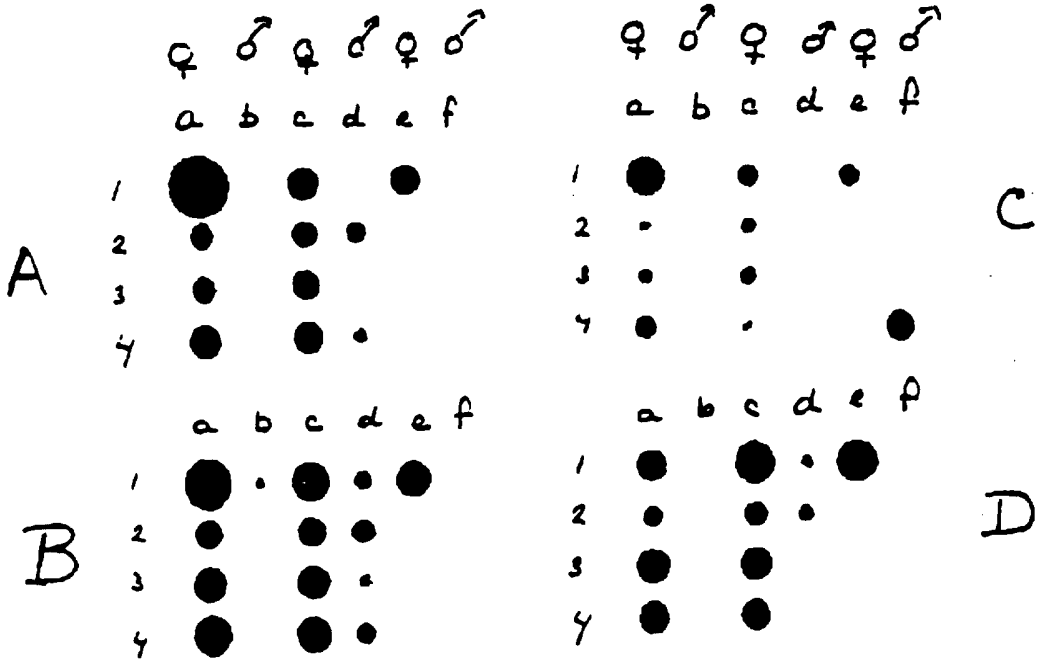
Figure 9

Hybridization of subfragments of *PARsex* with DNA of female and male Psittacidae. 1 µg of DNA of males and females of the species indicated was spotted on a Nylon membrane with a dot blot apparatus (GIBCO BRL) and hybridized with probes A - D. Probes (25 - 50 ng per ml were labelled with P^{32} by random primer labelling and α-$dCTP^{32}$ (A, B, C) or at the 5' terminus with polynucleotide kinase and γ-ATP^{32} (D).

A = 245 bp MspI - Sau3A fragment 1 - 245
B = 220 bp Sau3A - MspI fragment 241 - 461
C = PCR fragment 461 - 628 (+ primers)
D = 37 nt fragment at nt

a,c,e = females
b,d,f = males

1 ab = African grey parrot
2 ab = blue-fronted amazon
3 ab = budgerigar
4 ab = moluccan cockatoo
1 cd = cherry-headed conure
2 cd = eclectus parrot
3 cd = chattering lory
4 cd = blue and gold macaw
1 ef = Indian ringneck parrakeet
4 f = isolated PCR fragment 461 - 1



INTERNATIONAL SEARCH REPORT

Int. ..ational application No.
PCT/US96/00768

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07H 21/04; C12Q 1/68
US CL :435/6; 536/24.31
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6; 536/24.31; 935/77, 78

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P	Griffiths et al. Sex of the Last Wild Spix's Macaw. Nature. 08 June 1995, Vol. 375, page 454, entire document.	12-18
Y	Quinn et al. Molecular Sexing of Geese Using a Cloned Z Chromosomal Sequence with Homology to the W Chromosome. Auk. January 1990, Vol. 107, pages 199-202, especially pages 200-201.	1-8, 10-19, 30-32
Y	Madsen et al. Highly Repeated DNA Sequences in Birds: The Structure and Evolution of an Abundant, Tandemly Repeated 190-bp DNA Fragment in Parrots. Genomics. 1992, Vol. 14, pages 462-469, entire document.	1-32

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 04 MAY 1996	Date of mailing of the international search report 22 MAY 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer STEPHANIE W. ZITOMER, Ph.D. Telephone No. 703-308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/00768

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Griffiths et al. A Novel Avian W Chromosome DNA Repeat Sequence in the Lesser Black-backed Gull (Larus fuscus). 1990, Vol. 99, pages 243-250, entire document.	1-8, 12-19, 30-32

INTERNATIONAL SEARCH REPORT

In..national application No.
PCT/US96/00768

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, DIALOG ONESEARCH: EMBASE, MEDLINE, BIOSIS, BIOTECH. ABSTRACTS, CHEMICAL ABSTRACTS, LIFE SCIENCES, WPI, IMPADOC, DISSERTATION ABSTRACTS.
SEARCH TERMS: w chromosome, sex?, parrot?, repetitive sequence?