Paternity Testing

Hongbao Ma *, Huaijie Zhu **, Fangxia Guan ***, Shen Cherng ****

* Michigan State University, East Lansing, Michigan 48824, USA, hongbao@msu.edu, 517-303-3990

** Columbia University, New York, New York 10027, USA, hz42@columbia.edu; Jacksun Easy Biotech Inc., New York, New York 10027, USA, hjz689@yahoo.com, 718-513-0385

*** Bioengineering Department, Zhenzhou University, 100 Science Road, Zhengzhou, Henan 450001, China, fxguan78@yahoo.com

**** Department of Electrical Engineering, Chengshiu University, Niaosong, Taiwan 833, Republic of China; cherngs@csu.edu.tw, 011886-7731-0606 ext 3423

Abstract: Paternity testing can be done by older methods including ABO blood group typing, analysis of various other proteins and enzymes, or using human leukocyte antigen (HLA). However, DNA testing became the only formal and exact method for the paternity testing now. For DNA testing of paternity determination, the DNA collection using an easily obtained cheek swab saliva sample. Fast, proprietary and confidential genetic profiling of the child and alleged father with 99.999% probability/accuracy of results. In the common DNA paternity case, the alleged father and child are tested using polymerase chain reaction (PCR), Short tandem repeat (STR) and restriction fragment length polymorphism (RFLP) methodologies. Results for a DNA paternity case are available in an average of 3-4 days from receipt by the most paternity testing laboratory. Cheek swab saliva testing can be done immediately after birth, and typically the child and alleged father are tested. Results exclude or confirm the biological father with an accuracy rate exceeding 99.9%. [The Journal of American Science. 2006;2(4):76-92].

Keywords: deoxyribonucleic acid (DNA); maternity; paternity; polymerase chain reaction (PCR); restriction fragment length polymorphism (RFLP); testing

Abbreviations: DNA, deoxyribonucleic acid; GVHD, graft-versus-host disease; HLA, human leukocyte antigen; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; Rh factor, rhesus factor; RNA, ribonucleic acid; STR, short tandem repeat

Introduction

The report of paternity testing in scientific journal can be traced to 1956 (Henningsen, 1956).

A paternity testing is to determine paternity, i.e., whether a man is the biological father of another person. Similarly, a maternity testing can be done to prove whether a woman is the biological mother of another person. The maternity testing is less common, because at least during childbirth, except in the case of a pregnancy involving embryo transfer or egg donation, it is obvious who the mother is. But, the maternity testing can be useful for the condition that the mother is long time separated from her children and the provement of biological relationship requires. However, the techinque of paternity and maternity is same. All the paternity testing techniques described in this artilce are applied to maternity testing.

Paternity testing can be done by older methods including ABO blood group typing, analysis of various other proteins and enzymes, or using human leukocyte antigen (HLA). However, DNA testing has become the only formal and exact method for the paternity testing now (Jolly, 2000).

A blood type is a description an individual's characteristics of red blood cells due to substances (carbohydrates and proteins) on the cell membrane. The two most important classifications to describe blood types in humans are ABO and Rhesus factor (Rh factor) (Apostolopoulos, 2002).

The HLA test is a test that detects antigens on white blood cells. There are four types of HLAs: HLA-A, HLA-B, HLA-C, and HLA-D. The HLA test provides evidence of tissue compatibility typing of tissue recipients and donors. It is also an aid in genetic counseling and in paternity testing. HLA is a substance that is located on the surface of white blood cells. This substance plays an important role in the body's immune response. Because the HLAs are essential to immunity, identification aids in determination of the degree of tissue compatibility between transplant recipients and donors. Testing is done to diminish the likelihood of rejection after transplant, and to avoid graft-versus-host disease (GVHD) following major organ or bone

marrow transplantation. It should be noted that risk of GVHD exists even when the donor and recipient share major antigens. It was recently discovered that a mismatch of HA-1 (a minor antigen) was a cause of GVHD in bone marrow grafts from otherwise HLA-identical donors. HLA can aid in paternity exclusion testing. To resolve cases of disputed paternity, a man who demonstrates a phenotype (two haplotypes: one from the father and one from the mother) with no haplotype or antigen pair identical to one of the child's is excluded as the father. Conversely, a man who has one haplotype identical to one of the child's may be the father (the probability varies with the appearance of that particular haplotype in the population). Certain HLA types have been linked to diseases, such as rheumatoid arthritis, multiple sclerosis, serum lupus erythematosus, and other autoimmune disorders. By themselves, however, none of the HLA types are considered definitive. Because the clinical significance of many of the marker antigens has not yet been well defined, definitive diagnosis of disease is obtained by the use of more specific tests. The HLA test requires a blood sample. However, there is no need for the patient to be fasting before the test. Risks for this test are minimal, but may include slight bleeding from the blood-drawing site, fainting or feeling lightheaded after venipuncture, or hematoma. The HLA testing in paternity determination is to identify specific leukocyte antigens, HLA-A, HLA-B, HLA-C and HLA-D (Schonemann, 1998).

The DNA of a person is almost exactly same in each somatic cell of this person. Sexual reproduction brings the DNA of both parents together randomly to create a unique combination of genetic material in a new cell, so the genetic material of an individual is derived from the genetic material of both their parents. The genetic material is located in the nucleus. Except some genes located in mitochondrion that come from mother's egg, half genes of a person come from his/her father that locate in 23 chromosomes and the other half genes of the person come from his/her father that locate in the other 23 chormosomes. Comparing the DNA sequence of a person to that of another person can show if one of them is derived from the other or not. Specific sequences are usually looked at to see if they are copied verbatim from one of the individual's genome to the other (Henry, 1993).

Besides the nuclear DNA in the nucleus, the mitochondria in the cells also have their own genetic material termed the mitochondrial genome. Mitochondrial is not in neuclear and mitochondrial DNA comes only from the mother. Proving a relationship based on comparison of the mitochondrial genome is much easier than that based on the nuclear genome. However, testing the mitochondrial genome can only prove if two individuals are related by common descent through maternal lines and it could not be used to test for paternity (Wenk, 2004; Eyre-Walker, 2001).

Meta-analysis of a wide variety of genetic studies appears to show that the rates of non-paternity in the general population are on the order of 4% to over 30%, depending on the social group involved.

Beside the important valuation in the scientific researches, paternity testing also has significant comercial value in by its application.

Legal Issues in Paternity Testing

Many countries have restrictions/regulations on the human genetics testing. As an example, in Britain there were no restictions on paternity tests until the Human Tissue Act came into force in September 2006. Section 45 of Britain states that it is an offence to possess without appropriate consent any human bodily material with the intent of analyzing its DNA. In the Britain, legally declared fathers have access to paternity testing services under the new regulations, provided the putative parental DNA being tested is their own. Tests are sometimes ordered by courts when proof of paternity is required. In the Britain, the Department for Constitutional Affairs accredits organizations which can conduct this testing. The Department of Health is also in the process of updating its voluntary code of practice on genetic paternity testing. In the United States, there is less regulation for the paternity testing and there are a lot of companies doing this service with the cost of about US$100/testing and about three days one testing can be done. There are paternity testing service in China and it normally offer by the legal system, such as police deparmtents of govermnment. In the whole woprld, it is a big requirement for the parnity testing and it is a big market on this testing. The profit could be more than 60%.

DNA

1. DNA is the abbreviation for deoxyribonucleic acid.
2. It is the basic genetic material contained in all living most cells of the body.
3. DNA governs the structure and function of every component in the body.
4. Everyone has a unique DNA pattern except genetically identical twins.
5. As individuals, half of our DNA "characteristic" is inherited from the biological mother and half from the biological father.

Genes

The human body consists of trillions of cells. Most of these cells have a nucleus (some cells have no nucleus, such as platelet). The nucleus contains genes

that are the functional units of heredity. The genes exist in the chromosomes. There are 46 chromosomes inside every cell in a normal human body, which compose 23 pairs and each pair has 2 chromosomes. Twenty-three of these chromosomes come from the biological father and the other 23 come from the biological mother. Chromosomes are named by a letter "D" followed by a number (e.g. chromosome #1 is named D1 and chromosome #2 is named D2). Genes are named by the chromosome number, and by the location that the genes occupy on the chromosome. A DNA parentage test report identifies the genes that are analyzed during the process of testing. Fro example, when the results refer to D2S44, the gene analyzed is on chromosome #2 at locus 44. The whole genes of human have been determined by the world Genome Project and the gene structure and DNA sequence can be checked in GenBank and other resources (GenBank, 2006).

Twenty-six letters of the alphabet are the basic units of the English language. All English words and sentences are formed from these 26 letters. Genes on the other hand are formed from only 4 chemicals, or bases. These bases are named as: adenine (A), cytosine (C), guanine (G) and thymine (T). Like the letters of the alphabet that form words, sentences and paragraphs, these 4 bases form long gene fragments called deoxyribonucleic acid that is abbreviated DNA. The length of the gene fragments can be determined by the number of bases these gene fragments contain. Fro example, when the gene fragment on a DNA paternity test report is 2 kb that means the gene fragment consists of 2000 bases. All children have a biological mother and a biological father. For each gene in the child, he/she has a pair of the gene (allele) and one comes from the biological father and the other one comes from the biological mother. A DNA paternity test determines and examines the allele found in the mother, the child and the alleged father. The accuracy of the DNA test results depends on the extent of the DNA testing process. With sufficient testing, DNA technology provides an extremely powerful method of discriminating between fathers and non-fathers.

Paternity testing simply means establishing fatherhood. Everyone is born with a unique genetic blueprint known as DNA. Because the DNA molecular structure and genetic characteristics of a child are inherited from or determined by the DNA structure of the biological mother and father, DNA identification provides a conclusive and definitive way to establish biological relationships. Consequently, DNA testing has become the most accepted method to determine identity within the legal and scientific communities.

The standard methods for paternity testing are polymerase chain reaction (PCR), short tandem repeat (STR) and restriction fragment length polymorphism (RFLP), with which exclusion or inclusion of a parent's DNA to that of a child results in a probability factor of 99.999999% or 1 in 10 billion of the population.

What is DNA Testing?

DNA testing is the most advanced form of proving or disproving biological relationships. The testing is based on the analysis of genetic material between two people (for example: a child and alleged father). DNA is every person's genetic "blueprint". Half of their DNA is inherited from their mother and half from their father. When the child's DNA is compared to an alleged parent and no match exists, that person is excluded 100% as the biological parent. If there is a match in the DNA patterns, a probability of 99% or greater is calculated thus establishing a biological relationship (Klein, 2005).

DNA parentage testing is the most reliable and powerful method of proving or disproving parentage - for legal, personal, or medical reasons. It conclusively answers difficult questions, resolves disputes, helps streamline court proceedings and facilitates pretrial settlements.

Testing is based on a highly accurate analysis of the genetic profiles of the mother, child and alleged father. DNA, the unique genetic blueprint within each nucleated cell of a person's body, determines the genetic pattern and individual characteristics. A child inherits half of this DNA pattern from the mother and half from the father. If the mother's and child's patterns are known, the father's can be deduced with virtual certainty.

Purpose of Parental Testing

1. Main Aim:

1) To establish an accurate medical history for the child.
2) To prevent disputes in adoption.
3) To create a record for immigration.
4) To foster peace of mind for all involved parties.
5) To obtain child support.
6) To determine the biological father or the biological mother.

2. Normally the DNA testing is for:

1) A woman seeking child support from a man who denies he is the child's father.
2) A man attempting to win custody or visitation.
3) Adoptive children seeking their biological families.
4) People seeking to identify one parent when the other parent is absent or deceased, or desiring to identify other lost relatives.
5) People wanting to determine grandparentage, inheritance rights or whether twins are identical or fraternal.

6) People seeking entry into a country on the grounds that he/she is a blood relative of a citizen, or someone seeking to establish born rights.
7) Those who have received inconclusive results from other methods or who want a second opinion.
8) Criminal cases related, such as a rape, homicides and incest.

How do DNA Testing usually Work?

1. The test is based upon the principals of inheritance. A child gets one half of his/her genetic makeup from the mother and the other half from the biological father.
2. The test reveals a control batch of genetic markers from all parties. The genetic markers that the child shares with the mother are first located. Then to determine paternity, the child's remaining markers are compared to the alleged father. If the man is indeed the father, all of the markers that did not match the mother's should match his. If all of the child's remaining markers match the alleged father, evidence is provided that he is the biological father of the child. If they do not match, he is excluded as the biological father of the child.
3. When the mother is not tested, a larger batch of control markers is revealed from the alleged father and child. If these markers show that the man is contributing half of the genetic makeup of the child, he is given evidence that he is the biological father of the child. If markers are found not to match between the alleged father and child, the man is excluded from being the biological father of the child.
4. A self collection kit can be sent to your home if the customers do not need a legal notarized result.

Test Purpose/Samples Needed From

1. **Routine paternity:** mother, child and alleged father.
2. **Motherless paternity:** Child and alleged father.
3. **Prenatal paternity:** Amniocentesis CVS from mother, fetus and alleged father.
4. **Absent alleged father:** Mother, child, both paternal grandparents.
5. **Siblingship studies:** Two siblings, mother (if available).
6. **Identical twin studies:** One set of twins.
7. **Sperm detection:** One garment sample.
8. **Marital fidelity studies:** Control samples from two people.
9. Forensic studies: One evidence sample, 1 suspect sample and 1 victim sample (if any).

Principle of Methods

The DNA test is accomplished by taking buccal saliva samples (or drops of blood) from the child and alleged father. Along with the required forms, the samples are sent to the laboratory for DNA extraction and processing. Results can generally be available within 3 days after receipt by a laboratory, and it can be finished in one day with an additional cost.

The following is the normal steps for the paternity testing:

1. **Collect samples:** Cheek swab samples (or drops of blood) are obtained from the child and father using the sterile swabs provided.
2. **Send to laboratory:** Samples and completed forms are sent to the DNA testing laboratory.
3. **Lab check:** Samples are checked in, identified with tracking numbers and unique test codes and assigned a confidential case file.
4. **DNA extractions:** DNA is extracted from the cheek swab samples (or blood) for the child and parent. The phenol-chloroform is used in the DNA extraction.
5. **DNA profiles:** Comparison DNA profiles are obtained using the PCR or RFLP processes.
6. **DNA correlations:** Identification of similar and dissimilar genetic markers and test result.

DNA paternity testing could come with the following guarantee from the laboratory: The paternity by DNA technique is the most extensive and accurate DNA test available, with typical power of exclusion of 99.999999%. In all cases to test a child and an alleged parent, it guarantees one of the following results:

1. The paternity testing proves conclusively that the tested person is not the biological parent of the child. The probability of paternity is 0%, and it can be 100% certain that one is not the child's biological father.
2. The probability of paternity is greater than 99.9% and it documents that more than 99.9% of the male population cannot be the biological father of the tested child.

In an article of 2000, Professor Garrison (Brooklyn Law School, USA) showed that most commentators on technological conception had employed a "top-down" methodology, deriving rules for specific cases from an abstract global principle such as reproductive autonomy or freedom of contract. Garrison criticized

these and several other approaches, showing that they offered little concrete guidance in many cases, risked the introduction of discordant values into the law of parentage, and failed to capture all of the values that had traditionally guided parentage determination. In their place, she proposed an interpretive methodology which, by relying heavily on current rules governing parentage determination in other contexts, would assimilate technological conception within the broader law of parental obligation (Garrison, 2000).

Brief Description of Methods

DNA is extracted from cheek cells, a few drops of blood or cultured cells. DNA restriction endonuclease is used to cut the DNA sample into fragments, which are then placed into a gel matrix. Electrophoresis is applied for the electric current to drive the fragments across the gel - the smaller fragments move faster, while the bigger fragments move slower. The separated DNA fragments are transferred to a nylon membrane, which is exposed to a labeled DNA probe, a short piece of customized DNA that recognizes and binds to a unique segment of the test person's DNA. This nylon membrane is placed against a film, which when developed, reveals black bands where the probes bound to the DNA. The visible band pattern of the child is unique - half matches the mother and half matches the father. This process is repeated several times, with each probe identifying a different area in the DNA and producing a distinct pattern. Using several probes greater than 99.9% certainty about parentage can be achieved. The current techniques for paternity testing are using PCR and RFLP.

1. **DNA testing with PCR:** In the PCR method of testing, DNA is first isolated from the sample. Individual gene fragments are then synthetically produced in the laboratory and they are labeled with special fluorescent tags. These fluorescent tags enable detection of the genes.
2. **DNA testing with STR:** STR markers are polymorphic DNA loci that contain a repeated nucleotide sequence. The STR repeat unit can be from two to seven nucleotides in length. The number of nucleotides per repeat unit is the same for a majority of repeats within an STR locus. The number of repeat units at an STR locus may differ, so alleles of many different lengths are possible. Polymorphic STR loci are therefore very useful for human identification purposes. STR loci can be amplified using PCR process and the PCR products are then analyzed by electrophoresis to separate the alleles according to size. PCR-amplified STR alleles can be detected using various methods, such as fluorescent dye labeling, silver staining, or fluorescent dye staining.
2. **DNA testing with RFLP:** In the RFLP method of testing, the DNA isolated from the sample is cut into fragments by DNA restriction endonucleases. Then, an electric current is used to separate DNA fragments by size. The separated DNA fragments are identified with DNA probes. These probes are pieces of custom made DNA that bind and identify a specific gene locus.

Briefly, DNA test method can be described as the following:

1. **Extraction and purification of DNA.** The sample is treated with chemicals to break open the blood cells. DNA is separated from the cells and later purified.
2. **Cut DNA into fragments.** Enzymes that recognize certain sequences in the chemical base patterns are added to the DNA. These enzymes act like molecular scissors and cut the DNA molecule at specific points, leaving fragments of various lengths.
3. **Sort fragments by length.** The DNA fragments are placed on a gel, and an electric current is applied. The DNA, which is negatively charged, moves toward the positive end, the smaller fragments moving faster than large ones. Hours later the fragments have become arranged by length.
4. **Attach probes.** The nylon sheet is immersed in bath, and a probe - a DNA segment of known sequence - is added. The probe targets specific base sequences and bonds to them.
5. **Make a print and analyze it.** X-ray film is exposed to the nylon sheet containing the luminescent labeled probe. Dark bands develop at the probe sites.

DNA Paternity Testing

1. **Convenience:** The person who wants to take the paternity testing can collect the samples anywhere (such as in home) when it is convenient and send the sample through post office or any other delivery ways (such as Fedex or UPS in USA).
2. **Privacy:** The person who wants to take the paternity testing can remain anonymous if he/she wants. A test number is assigned that corresponds with a reference number on the kit.
3. **Easy:** The person who wants to take the paternity testing can use just a buccal (mouth) swab that means no needles. No bruising from

the blood draw or needle marks if use buccal swab sample. A buccal swab is as easy as brushing teeth.

4. **Reliability:** The report of the paternity testing results can be easy to understand by a non-scientist.
5. **In-home collection for the testing:** The DNA testing company could offer an in-home collection kit for privacy and convenience. Other DNA testing laboratories stipulate that a strict identification process must be followed. This chain of custody ensures test results are admissible in a court of law, but may be inconvenient for patients not interested in using the results for legal reasons.

DNA Laboratory Testing Process

1. Ordering and sample collections

1) Customer contacts the paternity testing company and orders a DNA test (e.g., paternity, siblingship, grand-parentage).
2) DNA collection kit is mailed to customer. Kits should be bar-coded and linked to the customer's name.
3) Each participant should fill out the order form including signing their informed consent.
4) Personal tests have the specimens collected by the participants and there is no validation of authenticity of collection by the laboratory.
5) Legal testing requires independent third party collection and identification of each participant. Legal tests maintain full legal chain of custody of specimens and identification of participants from collection through reporting.
6) Customer (or collector) sends the DNA specimens to the DNA testing company.
7) The paternity testing company receives the specimens and submits them into the accessioning process.
8) The DNA testing is made by the DNA testing company.
9) The testing result report is completed.
10) The results are offered to the customers.

For the blood collection, collect several drops or 5–10 ml of whole blood in EDTA tubes (lavender top), ACD tubes (yellow top, acid citrate dextrose) or heparinized tubes (green top). Whole blood specimens received by the lab may be stored in the refrigerator up to one month. Whole blood may be spotted on untreated, autoclaved cotton cloth, dried at room temperature, and stored frozen at –15 to –25 °C.

2. Basic laboratory procedures and contamination control

It needs four distinct and physically separated laboratory rooms for the testing**:**

1) Accessioning and DNA extraction.
2) PCR setup.
3) DNA amplification.
4) DNA detection.

Workflow is always unidirectional from extraction through detection to ensure that amplified DNA never moves to upstream processes**.** This includes specimens, reagents, and paperwork. Every laboratory work surface is disinfected with 10% bleach in water followed by a 100% ethanol rinse at least once per day. This removes any traces of contamination from the surface. General protocols require that all surfaces and equipment are cleaned at least once per day as well as before and after any operation or process setup.

3. Accessioning

1) **Mail is received** and sorted by type. Orders and specimens are delivered to the accessioning work station.
2) **Envelopes are opened** and processed one at a time. Each specimen envelope or container is labeled with a bar code and entered into the laboratory tracking system linking the container to the name on the container.
3) **The order form is used to setup the order in the laboratory tracking system and into the financial processing system**. The identity, ethnicity, and relationship of each participant are entered at this step in the process. An order number is assigned to the case. This order number becomes the primary identifier for the case as it progresses through the system and into reporting.
4) **Each specimen for the case is linked to the order**.
5) **The specimens are queued for the applicable extraction process** depending on the type of test ordered and the type of specimens.

4. DNA extraction

1) The extractions (i.e., swab specimens) can be performed on a Qiagen Biorobot 8000 Liquid Handling System, which can extract 4 plates of 96 samples and controls (a total of 384 samples) at one time, simultaneously (Lab Centraal B.V., Postbus 312, 2000AH Haarlem, The Netherlands). Each extraction batch includes quality controls to monitor for, and detect possibilities of contamination. This ensures reagent quality and the specificity of the test. The phenol-chloroform and the Chelex method can be used

for DNA extraction. The phenol-chloroform method removes proteins and other cellular components from nucleic acids, resulting in relatively purified DNA preparations. This is popular in the modern molecular biology research and application. The patented reagent TRIzol reagent for DNA and RNA isolation is the application of phenol-chloroform method. DNA extracted by the phenol-chloroform method is also suitable for RFLP analysis provided it is not significantly degraded. This method is also recommended when extracting DNA from relatively large samples (*i.e.*, when the amount of DNA in a sample is expected to be greater than 100 ng). The Chelex method of DNA extraction is more rapid than the phenol-chloroform method. It involves fewer steps, resulting in fewer opportunities for sample-to-sample contamination.

2) Many efficient reagents can be used to extract DNA from the samples, such as the Ten-minute DNA Release Kits can extract DNA within 10 min (Zhu, et al, 2006).

5. Manual DNA extraction

1) Each participant typically submits 3 separate swab specimens. The normal process will automatically queue two of the swabs for extraction. These two swabs are extracted in different batches by different laboratory technicians. In the normal DNA extraction method, the simultaneous extraction of two swabs enables the laboratory to greatly improve turn around times for those situations where one of the swabs may not yield significant DNA. The duplicate testing also allows for direct verification of results and provides superior confidence in preventing sample switching during the extraction setup process.
2) Once the specimen has been extracted into a plate the position of this sample is maintained throughout the remaining processes with all liquid transfers being handled by computer driven robotics.

6. Storage

1) Each extracted DNA plate is bar coded so that the information system knows exactly which plate a specified DNA is in as well as its physical location within the plate.
2) The extracted DNA plate is then sent to PCR setup for further processing. After this processing the plate is stored in our 4ºC refrigerator or -20ºC freezer for a period determined by the type of test offered. All paternity related extractions are required to be destroyed after a period of 6 months. The regular storage time for all other test types can be 5 years unless the customer has ordered the guaranteed banking service which is for 25 years.
3) All biologic materials that are to be destroyed are incinerated to ensure the complete destruction of the material.

7. PCR Setup

1) All reagents for PCR are prepared in a dedicated dead air box, under low light conditions, and on ice to ensure contamination free master mix and optimum sensitivity for all fluorescent labels.
2) Extracted DNA, master mix, and controls are automatically combined and loaded onto 384 well plates.

8. Amplification

1) After PCR setup, the plates are transferred into the amplification laboratory through a dedicated one way pass-through box on the wall of the laboratory. This pass through box helps to minimize contamination and maintain unidirectional work flow into the amplification area.
2) The thermo cycler (PCR equipment for DNA amplification) is used for DNA amplification.

9. Detection

1) After amplification, the amplified DNA is combined with formamide and size standard on a Biomek FX robot. Formamide is a chemical that denatures the DNA amplicon and facilitates detection and electrophoresis. The size standard is called the ladder which ensures that each allele is correctly located in the associated bin for its specific fragment size.
2) The plate is quickly heated to 96ºC for 3 min to denature DNA and then placed in the freezer or on the ice for 5 min for cool.
3) The plate is then loaded onto a detection machine. ABI 3100, ABI 3700 and ABI 3730 can be used for the fragment analysis.
4) Once the plate has been run through analysis it is placed in the detection refrigerator for short term storage to ensure that the run passes all quality control checks.

10. Analysis

1) The laboratory tracking system automatically uploads the data files from the detection instruments and launches GeneScan and GenoTyper software programs. These programs are used to assess the quality of

each run and sample as well as to assign the specific alleles to each fragment analyzed.

2) Each sample in each batch run through detection is analyzed and called by two independent analysts. Their calls are compared by the laboratory information system and any discrepancies are brought out for resolution by a lead analyst.
3) Since each swab specimen is extracted in duplicate, there are two distinct batches for each sample that comes off the detection instruments. If any discrepancy results then the sample may be recollected or the third swab extracted.

11. Quality control (QC)

The following quality control(s) (QC) are used on every batch and plate processed, and is reviewed at the analysis step:

1) **Negative extraction:** This is basically a check to ensure that the extraction reagents are clean and that no amplification occurs. If any peaks come up in this control the whole batch fails and is re-extracted.
2) **PCR negative control:** Like the extraction negative control we ensure that the reagents and master mix for PCR are clean and do not give rise to spurious peaks.
3) **PCR positive control(s):** One or more controls are added to each plate to ensure that the correct calls are being made for each plate. Positive controls use a combination of NIST SRM, CEPH DNA, and employee DNA samples. These controls allow ensuring that each plate runs correctly, that it gives the correct results without shifting data, and allows for an assessment of contamination control.
4) **DNA ladders:** Each sample's alleles line up within specified bins defined by the size standard or ladder each was processed with. A QC check is performed to ensure that each allele is within the expected bin location.
5) **Data matching between analysts and batches:** The analysts ensure consistency between the two batches and the two independent reviews. This makes a total of 4 unique readings and reviews of each person's genotype.

12. Reporting

1) The detecting report is given by the analyst individual sample.
2) The final report is then reviewed by the laboratory supervisor, manager or director.
3) Once all reviews are conducted the report is signed by the final reviewer(s) and given to client.

13. Release of results

1) Client support representatives review the case file and determine how the customer chooses to have results. In many cases results are simply released to each customer's secure personal web page where they can access and print their results.
2) In some cases if customers prefer hard copy or email results, the hard copy or email will be sent to the customers.

To do a testing, the person can simply call the service coordinators of the testing company to order a collection kit. Once the company receives samples and payment, the customer will be assigned a permanent test number. Using strict lab guidelines and quality control procedures, testing is completed within 1-10 days normally, and 1 day possibly. To protect the customers' confidentiality, results will be sent in a discreet envelope to only the addresses the customers provide if it is through the mail system.

Normally a company can offer the following services:

1. The process is relatively easy and reasonable.
2. Costs very but figure around US$80-US$500 in the United States for a trio with Buccal swabs or blood samples, and US$100 is normally.
3. Additional cost would be to a private clinic for specimen collection, which may be around $20 per person.
4. If the customer prefers not to go to a medical site and do not need a legal notarized result, a self-collection kit can be sent to his/her home for around US$50-US$300 in the United States. One kit will collect up to 3-10 people. Cost of testing is usually separate and additional cost.
5. Results will take around 3-20 days, and it can offer 1 day service with additional speedy cost.
6. The customer can often set-up the process over the Internet. However, to have legal clout, a lab visit is necessary for the legal document confidence.
7. Usual prepay for testing by credit card, personal check, bank check or money order.

Improving analyses of DNA microarray data

DNA microarrays are increasingly being used for analyzing gene expression in life science studies and

clinical medicine. However, converting the thousands of data points from these microarrays into clear interpretable information has been challenging (Simon, 2006).

What is required for the test?

1. Buccal swabs or blood samples are normally used for testing.
2. Samples from the child (children), mother and alleged father(s) are required.
3. They must be properly collected, packed and preserved and submitted with all the relevant documents so that they will meet the legal and scientific requirements for admissibility in a court of law, if necessary
4. Paternity can also be accurately determined in the absence of the mother, by testing the child and alleged father only.
5. A DNA paternity test can be performed on individuals of any age.
6. Results are usually available within 3-20 days.

Other related DNA tests

1. Maternity testing.
2. DNA testing can conclusively answer questions relating to maternity.
3. Grandparentage testing.
4. Determines whether a person is the true grandparent of a child.
5. Sibling relationship testing.
6. Determines whether or not brothers/sisters are sibling.
7. Body identification.
8. Confirms the sex and identity of an unidentified body.

As the analysis is only carried out in a DNA analysis laboratory, the blood samples must be properly collected, packed and preserved and submitted with all the relevant documents so that they will meet the legal and scientific requirements for admissibility in a court of law, if necessary.

For a testing, normally it needs buccal swabs or blood (about 2 ml) taken from each of the parties concerned (mother, child/children and alleged father(s)). The blood samples should be collected in EDTA specimen tubes only which should then be sealed and clearly labeled with the identification name/number and the date of collection. All samples collected should be kept at low temperature (4^oC). If samples are not to be examined for several weeks, they should be frozen. Repeated freezing and thawing should be avoided since this can reduce the amount of DNA which can be recovered. The samples should be sent via one of the courier agencies or hand carried so that chain of custody can be traced.

DNA Quantitation

The efficiency of a PCR amplification is influenced by the quality (degree of degradation), purity, and total quantity of DNA in a sample. Lack of amplification is usually due to highly degraded DNA, the presence of PCR inhibitors, insufficient DNA quantity, or any combination of these factors.

The QuantiBlot Human DNA Quantitation Kit (P/N N808-0114) from Applied Biosystems Company (Foster City, CA, USA) is one of an ideal method for accurate quantitation of human DNA. One kit of the reagent can make 480 tests with a cost of US$300.

1. QuantiBlot Kit

If the QuantiBlot Kit determines that sufficient DNA is present in the extracted sample (greater than approximately 0.05 ng/µl concentration), then lack of amplification is most likely due to PCR inhibitors or severe degradation of the DNA.

Quantitation of samples determines if there is a sufficient amount of DNA present for amplification. Also, PCR inhibition can be minimized by adding the smallest volume of DNA extract necessary for successful amplification (volume containing approximately 1.0 ng). Lastly, by using the minimal volume of extracted DNA for PCR, the number of different genetic marker tests or repeat analyses that can be performed is maximized. Adding greater than 2.5 ng of DNA can result in too much PCR product, such that the dynamic range of the instrument used to detect and analyze the PCR product is exceeded.

2. Analysis by the QuantiBlot Kit method

The QuantiBlot Human DNA Quantitation Kit method of DNA quantitation is based on probe hybridization to the human alpha satellite locus, D17Z1. A biotinylated probe specific for the D17Z1 sequence is hybridized to sample DNA that has been immobilized via slot blot onto a nylon membrane. The subsequent binding of horseradish peroxidase/streptavidin enzyme conjugate (HRP-SA) to the bound probe allows for either colorimetric or chemiluminescent detection. In the case of colorimetric detection, the oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB) catalyzed by HRP-SA results in the formation of a blue precipitate directly on the nylon membrane. For chemiluminescent detection, the oxidation of a luminol-based reagent catalyzed by HRP-SA results in the emission of photons that are detected on standard autoradiography. This process is called enhanced chemiluminescence (ECL). In both cases, the quantity of sample DNA is determined by comparison of the sample signal intensity to human

DNA standards that have been calibrated against two DNA controls of known quantity.

The colorimetric method allows for detection and quantitation down to 150 pg. The chemiluminescent method can detect 150 pg with a 15-minute exposure to film and can detect as little as 20 pg with longer film exposures (three hours to overnight).

3. Specificity for primate DNA

One significant advantage of the QuantiBlot Human DNA Quantitation Kit method is that the probe is highly specific for human/primate DNA. When tested, 300 ng quantities of several non-primate DNA samples (*E. coli*, yeast, dog, cat, mouse, rat, pig, cow, chicken, fish, and turkey) are found to give either no signals or signals that are less than or equal to that obtained for 0.15 ng of human DNA. This high degree of specificity for human/primate DNA allows for the accurate quantitation of target human DNA in samples that also contain significant amounts of microbial or other non-primate DNA (Applied Biosystems, 2006)

DNA and RNA extraction using TRIzol reagent

DNA extraction is the first step of paternity testing and it is important for the testing. TRIzol reagent is a commercial reagent that is convenient for the RNA extraction, and with TRIzol reagent it can also obtain the extraction of DNA and protein. The following is the decription of the RNA, protein and DNA isolation using TRIzol reagent.

1. DNA Extraction by TRIzol Reagent

TRIzol reagent can be used to RNA, DNA and protein from tissue and cells. The protocol of DNA isolation by TRIzol is the following:

1) Homogenization

(1) *Tissues*

Homogenize tissue samples in 1 ml of TRIzol Reagent per 50-100 mg of tissue.

(2) *Cells Grown in Monolayer*

Lyse cells directly in a culture dish by adding 1 ml of TRIzol reagent to a 3.5 cm diameter dish, and passing the cell lysate several times through a pipette.

(3) *Cells Grown in Suspension*

Pellet cells by centrifugation. Lyse cells in TRIzol reagent by repetitive pipetting. Use 1 ml of the reagent per 5-10 × 10^6 of animal, plant or yeast cells, or per 1 × 10^7 bacterial cells. Washing cells before addition of TRIzol reagent should be avoided as this increases the possibility of mRNA degradation (this should be paid attention to if mRNA extract will be done for the sample). Disruption of some yeast and bacterial cells may require the use of a homogenizer. An additional isolation step may be required for samples with high content of proteins, fat, polysaccharides or extracellular material such as muscles, fat tissue, and tuberous parts of plants. Following homogenization, remove insoluble material from the homogenate by centrifugation at 12,000 × *g* for 10 min at 2 to 8°C. The resulting pellet contains extracellular membranes, polysaccharides, and high molecular weight DNA, while the supernatant contains RNA. In samples from fat tissue, an excess of fat collects as a top layer which should be removed. In each case, transfer the cleared homogenate solution to a fresh tube and proceed with chloroform addition and phase separation as described.

2) Phase Separation

Incubate the homogenized samples for 5 min at 15 to 30°C to permit the complete dissociation of nucleoprotein complexes. Add 0.2 ml of chloroform per 1 ml of TRIzol reagent. Cap sample tubes securely. Shake tubes vigorously by hand for 15 seconds and incubate them at 15 to 30°C for 2 to 3 min. Centrifuge the samples at no more than 12,000 × *g* for 15 min at 2 to 8°C. Following centrifugation, the mixture separates into a lower red, phenol-chloroform phase, an interphase, and a colorless upper aqueous phase. RNA remains exclusively in the aqueous phase. The volume of the aqueous phase is about 60% of the volume of TRIzol reagent used for homogenization.

3) DNA precipitation

Remove the remaining aqueous phase overlying the interphase, and precipitate the DNA from the interphase and organic phase with ethanol. Add 0.3 ml of 100% ethanol per 1 ml of TRIzol reagent used for the initial homogenization, and mix samples by inversion. Next, store the samples at 15°C to 30°C for 2-3 min and sediment DNA by centrifugation at no more than 2,000 × *g* for 5 min at 2 to 8°C.

4) DNA wash

Remove the phenol-ethanol supernatant, and if desired, save it for protein isolation. Wash the DNA pellet twice in a solution containing 0.1 M sodium citrate in 10% ethanol. Use 1 ml of the solution per 1 ml of TRIzol reagent used for the initial homogenization. At each wash, store the DNA pellet in the washing solution for 30 min at 15 to 30°C (with periodic mixing) and centrifuge at 2,000×*g* for 5 min at 2 to 8°C. Following these two washes, suspend the DNA pellet in 75% ethanol (1.5-2 ml of 75% ethanol per 1 ml TRIzol reagent), store for 10-20 min at 15°C to 30°C (with periodic mixing) and centrifuge at 2,000×*g* for 5 min at 2 to 8°C.

5) Redissolving the DNA

Air dry the DNA 5 to 15 min in an open tube. Dissolve DNA in 8 mM NaOH such that the concentration of DNA is 0.2–0.3 µg/µl. Typically add 300–600 µl of 8 mM NaOH to DNA isolated from 1×10^7 cells or 50–70 mg of tissue. Resuspending in weak base is recommended since isolated DNA does not resuspend well in water or in Tris buffers. The pH of the 8 mM NaOH is only about 9 and should be easily adjusted with TE or HEPES once the DNA is in solution. At this stage, the DNA preparations may contain insoluble gel-like material. Remove the insoluble material by centrifugation at >12,000×*g* for 10 min. Transfer the supernatant containing the DNA to a new tube. DNA solubilized in 8 mM NaOH can be stored overnight at 4°C; for prolonged storage, samples should be adjusted with HEPES to pH 7-8 and supplemented with 1 mM EDTA. Once the pH is adjusted, DNA can be stored at 4°C or –20°C.

6) Quantitation and Expected Yields of DNA

Take an aliquot of the DNA preparation solubilized in 8 mM NaOH, mix it with water and measure the A260 of the resulting solution. Calculate the DNA content using the A260 value for double-stranded DNA. One A260 unit equals 50 µg of double-stranded DNA/ml. For calculation of cell number in analyzed samples, assume that the amount of DNA per 1×10^6 diploid cells of human, rat, and mouse origin equals: 7.1 µg, 6.5 µg, and 5.8 µg, respectively.

2. RNA extraction by TRIzol Reagent

1) Homogenization

(1) *Tissues*

Homogenize tissue samples in 1 ml of TRIzol reagent per 50-100 mg of tissue using a glass-Teflon ® or power homogenizer (Polytron, or Tekmar's TISSUMIZER ® or equivalent). The sample volume should not exceed 10% of the volume of TRIzol reagent used for homogenization.

(2) Cells grown in monolayer

Lyse cells directly in a culture dish by adding 1 ml of TRIzol Reagent to a 3.5 cm diameter dish, and passing the cell lysate several times through a pipette. The amount of TRIzol Reagent added is based on the area of the culture dish (1 ml per 10 cm 2) and not on the number of cells present. An insufficient amount of TRIzol Reagent may result in contamination of the isolated RNA with DNA.

(3) Cells grown in suspension

Pellet cells by centrifugation. Lyse cells in TRIzol reagent by repetitive pipetting. Use 1 ml of the reagent per $5\text{-}10\times10^6$ of animal, plant or yeast cells, or per 1×10^7 bacterial cells. Washing cells before addition of TRIzol Reagent should be avoided as this increases the possibility of mRNA degradation. Disruption of some yeast and bacterial cells may require the use of a homogenizer.

Optional: An additional isolation step may be required for samples with high content of proteins, fat, polysaccharides or extracellular material such as muscles, fat tissue, and tuberous parts of plants. Following homogenization, remove insoluble material from the homogenate by centrifugation at 12,000×*g* for 10 min at 2 to 8°C. The resulting pellet contains extracellular membranes, polysaccharides, and high molecular weight DNA, while the supernatant contains RNA. In samples from fat tissue, an excess of fat collects as a top layer which should be removed. In each case, transfer the cleared homogenate solution to a fresh tube and proceed with chloroform addition and phase separation as described.

2) Phase separation

Incubate the homogenized samples for 5 min at 15 to 30°C to permit the complete dissociation of nucleoprotein complexes. Add 0.2 ml of chloroform per 1 ml of TRIzol reagent. Cap sample tubes securely. Shake tubes vigorously by hand for 15 seconds and incubate them at 15 to 30°C for 2 to 3 min. Centrifuge the samples at no more than 12,000×*g* for 15 min at 2 to 8°C. Following centrifugation, the mixture separates into a lower red, phenol-chloroform phase, an interphase, and a colorless upper aqueous phase. RNA remains exclusively in the aqueous phase. The volume of the aqueous phase is about 60% of the volume of TRIzol reagent used for homogenization.

3) RNA precipitation

Transfer the aqueous phase to a fresh tube, and save the organic phase if isolation of DNA or protein is desired. Precipitate the RNA from the aqueous phase by mixing with isopropyl alcohol. Use 0.5 ml of isopropyl alcohol per 1 ml of TRIzol Reagent used for the initial homogenization. Incubate samples at 15 to 30°C for 10 min and centrifuge at no more than 12,000×*g* for 10 min at 2 to 8°C. The RNA precipitate, often invisible before centrifugation, forms a gel-like pellet on the side and bottom of the tube.

4) RNA wash

Remove the supernatant. Wash the RNA pellet once with 75% ethanol, adding at least 1 ml of 75% ethanol per 1 ml of TRIzol Reagent used for the initial homogenization. Mix the sample by vortexing and centrifuge at no more than 7,500×*g* for 5 min at 2 to 8°C.

5) RNA redissolving

At the end of the procedure, briefly dry the RNA pellet (air-dry or vacuum-dry for 5-10 min). Do not dry the RNA by centrifugation under vacuum. It is important not to let the RNA pellet dry completely as this will greatly decrease its solubility. Partially dissolved RNA samples have an A260/280 ratio <1.6. Dissolve RNA in RNase-free water or 0.5% SDS solution by passing the solution a few times through a pipette tip, and incubating for 10 min at 55 to 60°C. (Avoid SDS when RNA will be used in subsequent enzymatic reactions.) RNA can also be redissolved in 100% formamide (deionized) and stored at -70°C (DNA Solutions, 2006).

6) Quantitation of RNA

Total RNA concentration was determined spectrophotometrically by absorption of O.D.260 nm: RNA concentration (μg/ml) = $OD_{260\ nm}$ × 40 μg/ml × dilution factor. Consider ratio of optional density $OD_{260\ nm/280\ nm}$ > 1.8 as pure.

Brief steps for RNA isolation using TRIzol reagent:

1) Add TRIzol into sample (5-10×10^6 cells/1 ml).
2) Incubate at room temperature for 5 min.
3) Add 0.2 ml chloroform/1 ml TRIzol.
4) Shake tubes vigorously by hand for 15 seconds.
5) Incubate at room tempearture for 3 min.
6) Centrifuge 15 min at 4°C at less than 12,000×g (10000 rpm, r=65 mm).
7) RNA is in top (aqueous phase, about 60% volume).
8) Transfer RNA aqueous phase (top) to a fresh tube.
9) Save organic phase for DNA and protein isolation.
10) Pricipitate RNA from aqueous phase with isopropanol (isopropyl alcohol, C_3H_8O).
11) Add 0.5 ml isopropanol/ml TRIzol reagent.
12) Incubate 10 min at room temperature.
13) Centrifuge 10 minutes at 4°C by less than 12000×g (10000 rom, r=65 mm).
14) RNA is in bottom as gel-like pellet.
15) Remove and dispose supernatant.
16) Wash RNA pellet once with 75% ethanol: Add 75% ethanol 1 ml/ml TRIzol reagent.
17) Vortex and centrifuge 5 min at 4°C by less that 7500 g (8000 rpm, r=65 mm).
18) Save RNA pellet (air-dry 5-10 min).
19) Dissolve RNA in 0.05 ml diethyl-pyrocarbonate (DEPC) treated water.
20) Mix with pipett.
21) Incubate 10 min at 55-60°C.
22) Total RNA is determined spectrophotometrically by absorption of A260 nm: RNA concentration (μg/ml) = $A_{260\ nm}$ × 40 μg/ml × dilution factor.
23) Consider ratio of optional density $OD_{260\ nm/280\ nm}$ > 1.8 as pure.
24) Keep at -70°C unitil used, or redissolved in 100% formamide (deionized) at -70°C for longer storage.
25) De-frozen and for PCR usage.

Figure 1 gives a brief steps for the TRIzol usage on RNA isolation (Figure 1). RNA can be reverse transcripted into DNA and used for paternity testing.

3. Protein Extraction by TRIzol reagent

1) Protein precipitation

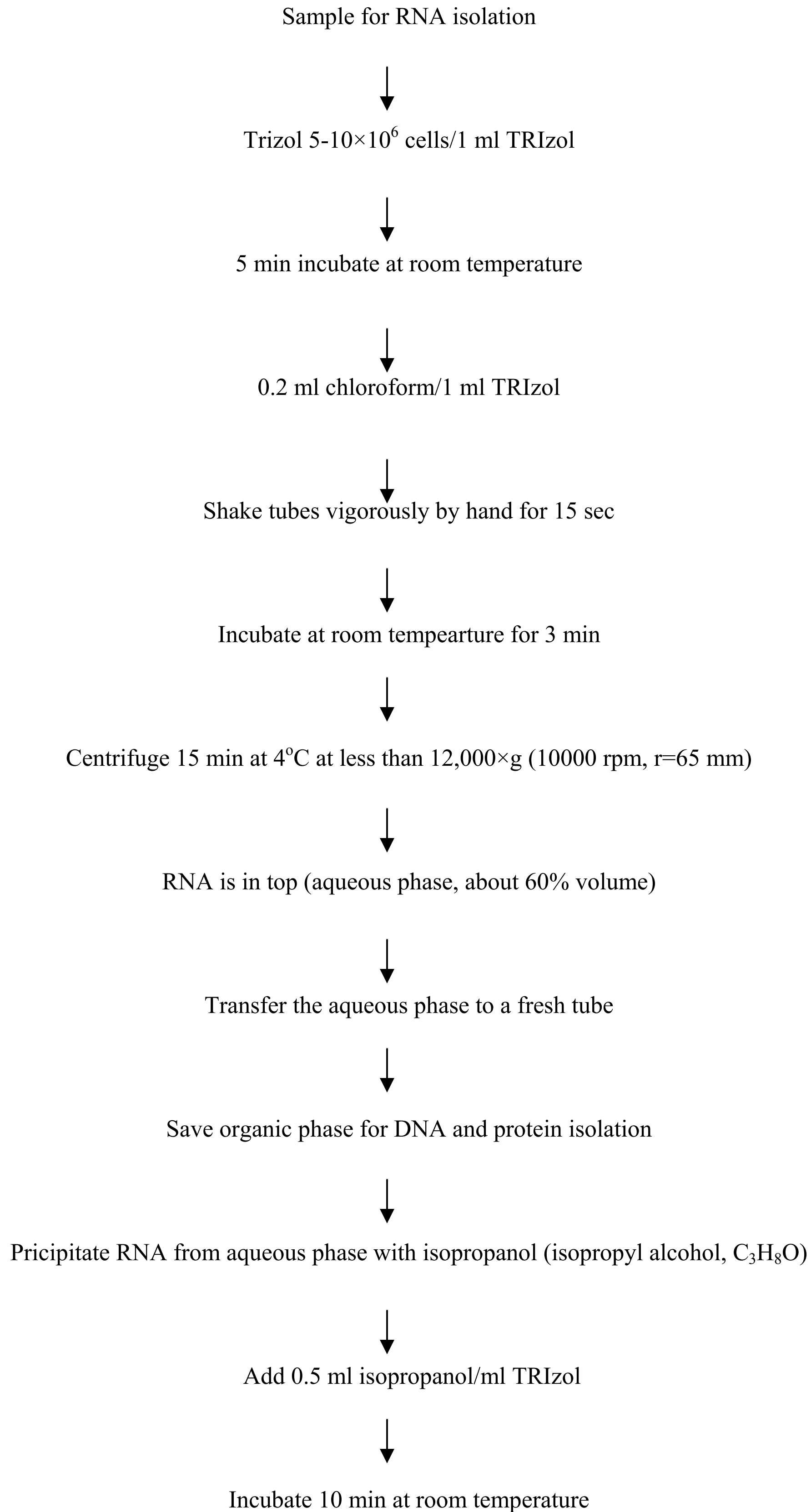
Precipitate proteins from the phenol-ethanol supernatant (approximate volume 0.8 ml per 1 ml of TRIzol reagent) with isopropyl alcohol. Add 1.5 ml of isopropanol per 1 ml of TRIzol reagent used for the initial homogenization. Store samples for 10 min at 15 to 30°C, and sediment the protein precipitate at 12,000×*g* for 10 min at 2 to 8°C. The purified proteins can be used as the result references.

2) Protein wash

Remove the supernatant and wash the protein pellet 3 times in a solution containing 0.3 M guanidine hydrochloride in 95% ethanol. Add 2 ml of wash solution per1 ml of TRIzol reagent used for the initial homogenization. During each wash cycle, store the protein pellet in the wash solution for 20 min at 15 to 30°C and centrifuge at 7,500×*g* for 5 min at 2 to 8°C. After the final wash, vortex the protein pellet in 2 ml of ethanol. Store the protein *g* for 5 min at 2 to 8°C. Vacuum dry the protein pellet for 5-10 min. Dissolve it in 1% SDS by pipetting. Complete dissolution of the protein pellet may require incubating the sample at 50°C. Sediment any insoluble material by centrifugation at 10,000×*g* for 10 min at 2 to 8°C, and transfer the supernatant to a fresh tube. The sample is ready for use in Western blotting or may be stored at -5 to -20°C for future use.

Facilities needed and budget estimated for a paternity testing laboratory setting up (Table 1)

To set up a paternity testing laboratory, it needs a munimum of 1000-2000 ft^2 space, US$100,000 and 3-10 people in the beginning. Table 1 gives the facilities needed and budget estimated for a paternity testing laboratory setting up, and the budget could be very various depending on the scale of the laboratory. The profit of the testing could be 50-80%. This laboratory can also be used for other clinical purposes and reserches, such as genetic diagnosis, etc.


Sample for RNA isolation
Trizol 5-10×10^6 cells/1 ml TRIzol
5 min incubate at room temperature
0.2 ml chloroform/1 ml TRIzol
Shake tubes vigorously by hand for 15 sec
Incubate at room tempearture for 3 min
Centrifuge 15 min at 4°C at less than 12,000×g (10000 rpm, r=65 mm)
RNA is in top (aqueous phase, about 60% volume)
Transfer the aqueous phase to a fresh tube
Save organic phase for DNA and protein isolation
Pricipitate RNA from aqueous phase with isopropanol (isopropyl alcohol, C_3H_8O)
Add 0.5 ml isopropanol/ml TRIzol
Incubate 10 min at room temperature

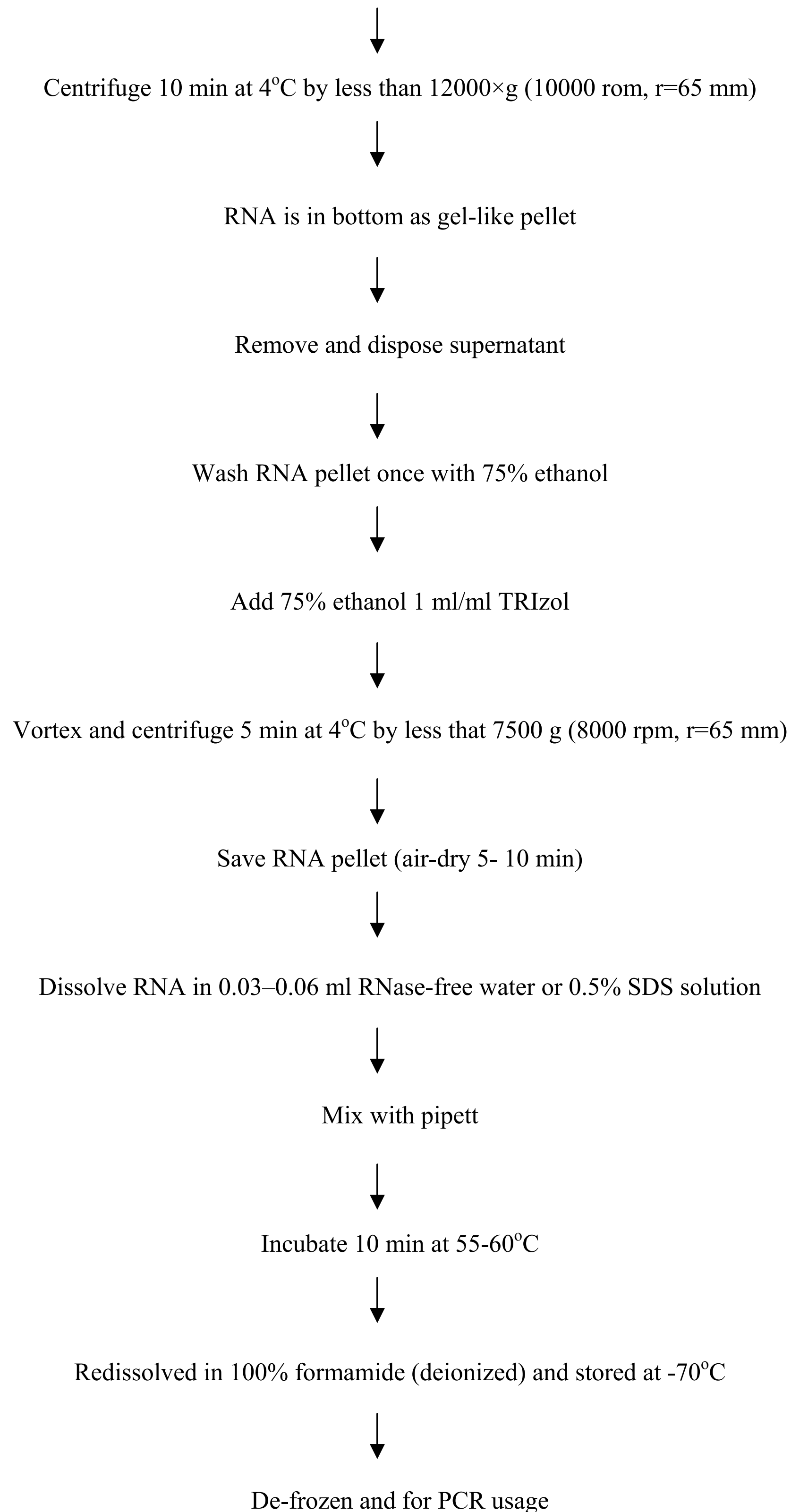


Figure 1. TRIzol steps for RNA isolation

Table 1. Facilities needed and budget estimated for a paternity testing laboratory setting up

Facilities	Cost (US$)
Thermal cycler (PCR equipment)	30000
Balance	5000
Centrifuge	10000
Electrophoresis system	2000
Hood	6000
pH meter	1000
Spectrophotometer	2000
Refrigerator 4°C	2000
Freezer -70°C	10000
Water bath	1000
Magnetic stir	1000
Liquid nitrogen tank	2000
Ultraviolet transilluminator	2000
Pipetters	3000
Distill water system	2000
Film developer	2000
Computer	2000
Camera	1000
Initial reagents	8000
Others	8000
Sum	100000

Discussion

A person has thousands of genes. Half from each parent. The randomness of this combination makes each person's genetic make-up unique, except for identical twins. It is this unique combination within each cell that makes DNA testing so powerful in human identification. The results could either 100% exclude someone as a biological parent of a child or confirm a 99% or higher probability of parentage. Legally, a 99% or greater probability of a biological relationship is considered proof of paternity.

When the biological father of a child is in doubt, a DNA test is the most accurate form of paternity testing available. With the exception of identical twins, every person's DNA is unique, just like a fingerprint. DNA contains all the information needed to produce a living person and is found in all nucleated cells in the body in structures known as chromosomes.

All children receive half of their chromosomes from their biological mother and the other half from their biological father. Each person typically has two different sized fragments of DNA at each chromosome location which can be detected by specific DNA probes. Therefore for each region of the DNA tested, a child will have one sized fragment of DNA that he/she inherited from his/her mother and another one from his/her father. A DNA paternity test directly examines specific fragments of DNA at predetermined regions on the chromosomes found in the mother, child and putative father, with a high degree of accuracy in identifying biological fathers.

Initially, the fragments of DNA found in the mother and child are compared. The child will have a fragment of DNA that is exactly the same size as a fragment of DNA found in the mother. Knowing which one of a pair of DNA fragments that the child inherited from the mother identifies the other sized fragment that the child must have received from the biological father. If the DNA fragments of the putative father do not match at two or more of the DNA probes tested, then this man is 100% excluded from being the biological father of the child. Alternatively, if the putative father matches the child at every DNA probe tested, then this individual is not excluded as a potential biological father.

Next, using a computer program, it can calculate the probability of paternity that this man is the biological father of the child when compared to the general population of men of the same race. In cases of inclusion, a probability of usually 99% or greater is achieved by DNA testing, indicating that the man being tested is for "practical purposes proven" to be the biological father of the child.

The results of DNA paternity testing are valid in court and may be used to settle child support and custody cases, questions in estate litigations and for identification of identical twins and other family members. This procedure can also be used to establish other relationships within families.

Traditional blood tests involve the study of genetic traits or "markers" such as blood types (A, B, AB or O) and human leukocyre antigen (HLA). A battery of tests is necessary, requiring a relatively large blood sample. This is often a problem with newborns and young infants. DNA-based testing, which studies the genetic material directly, is 10 to 100 times more precise than traditional testing and requires only a few drops of blood or a swab of the mouth.

Recent genetic analyses show that, in social mammals, loss of paternity by breeding males varies with strategies of mate guarding rather than with the degree of polygyny (Clutton-Brock, 2006). The last few years, many paternity tests on buccal swabs have been sold over the Internet as "test kits". The commercial providers of these DNA testing services facilitate controversial practices, including clandestine sampling at home, anonymous sending off for analysis, motherless testing and using "stolen" personal objects containing biological material (combs, cigarette butts). In reaction, legal initiatives are appearing throughout

the world. The Britain Human Genetics Commission has advised that the non-consensual obtaining and analysis of personal genetic information should be a new criminal offence. The German Federal Court of Justice has ruled that paternity tests performed without the mother's knowledge are inadmissible as evidence in lawsuits. French law strictly forbids the application of DNA testing without the involvement of the court system (Mertens, 2006). The United States has its own control. DNA testing is recognized and accepted by the U.S. Court System and the American Association of Blood Banks (AABB).

There are no age restrictions, because DNA makeup is set at conception. Testing can be performed before a child is born and newborns can be safely tested at delivery using umbilical cord blood. In fact, samples can be taken from persons of any age, even post mortem. Only one half of a teaspoon of blood or a cheek swab is required.

According to Cifuentes's report on the March of this year, the average exclusion probability is a measure of efficiency in paternity testing. This parameter measures the capacity of the system to detect a false accusation of paternity. Traditionally, this average exclusion probability has been estimated as the probability of excluding a man who is not the father by an inconsistency in at least one of the studied loci. They suggested that this criterion should be corrected, as currently the presumed father is excluded when at least three genetic inconsistencies were found with the child being tested, not just one. This change of criterion had occurred because of the use of microsatellite loci, whose mutation rates were much greater than those of the coding genes used previously in paternity studies. They proposed the use of the average probability of exclusion for at least three loci (not only one), as an honest measure of the combined probability of exclusion of several loci, and they also proposed an algebraic expression to calculate it (Cifuentes, 2006). According to Marjanovic's report, the standard molecular techniques, with only a slight modification, are very useful in obtaining and interpreting the final results in paternity testing. Data obtained through this analysis are highly reliable. However, success and swiftness of DNA typing of biological evidence either that found at a crime scene or used in disputed paternity testing, depends on the optimization of numerous factors (Marjanovic, 2006).

Paternity testing can distinguish the pure father and the father plus uncle relationship. In the application, the patenity testing also can be used in the case of incest between brother and sister. In Macan colleagues' study in 2003, DNA from blood samples of the alleged parents and their two children was extracted using Chelex DNA extraction method and quantified with Applied Biosystems QuantiBlot quantitation kit. PCR was performed with AmpFlSTR SGM Plus PCR amplification kit and GenePrint PowerPlex PCR amplification kit. PCR products were separated and detected using the Perkin Elmer's ABI PRISM trade mark 310 Genetic Analyser. DNA and data analysis of 17 loci and Amelogenin confirmed the suspicion of brother-sister incest. Since both children had inherited all of the obligate alleles from the alleged father, they confirmed with certainty of 99.999999% that the oldest brother in the family was the biological father of both children. Calculated data showed that even in a case of brother-sister incest, paternity could be proved by the analysis of Amelogenin and 17 DNA loci (Macan, 2003).

Without DNA testing, women can lie to children about who their father is and be rewarded with eighteen or more years of child support. In Kirk Kerkorian's case, that's worth more than $300,000 a month. Several US states have already passed laws that encourage honesty about paternity, which is a better public policy (Bentham, 2007).

The application of paternity testing will be increase greatly in the world and it is a big market in the globe.

Correspondence to:
Hongbao Ma, Ph.D.
Michigan State University
East Lansing, Michigan 48824, USA
Telephone: 517-303-3990
Email: hongbao@msu.edu

Receipted: June 5, 2006

References

1. Apostolopoulos K, Labropoulou E, Konstantinos B, Rhageed S, Ferekidis E. Blood group in otitis media with effusion. ORL J Otorhinolaryngol Relat Spec. 2002;64(6):433-5.
2. Applied Biosystems. AmpFlSTR® Profiler® PCR Amplification Kit User's Manual. 2006:1-2.
3. Cifuentes LO, Martinez EH, Acuna MP, Jonquera HG. Probability of exclusion in paternity testing: time to reassess. J Forensic Sci. 2006;51(2):349-50.
4. Clutton-Brock TH, Isvaran K. Biol Lett. 2006;2(4):513-6.
5. Bentham M, Fraser L. Move to outlaw secret DNA testing by fathers. **http://www.telegraph.co.uk/news/main.jhtml;$sessionid$3UKQVPAAACSV3QFIQMGCFF4AVCBQUIV0?xml=/news/2002/05/19/ndna19.xml&sSheet=/news/2002/05/19/ixhome.html**. 2007.
6. Dalya Rosner. Naked Science Articles: How does genetic fingerprinting work?

http://www.thenakedscientists.com/html/columnists/dalyacolumn8.htm. 2006.
7. DNA Solutions. Legal DNA Paternity Testing from DNA Solutions. http://www.dnaaction.com. 2006.
8. Eyre-Walker A, Awadalla P. Does human mtDNA recombine? J Mol Evol. 2001;53(4-5):430-5.
9. Garrison M. Law making for baby making: an interpretive approach to the determination of legal parentage. Harv Law Rev. 2000;113(4):837-923.
10. GenBank. American NIH official website. http://www.ncbi.nlm.nih.gov/Genbank/index.html. 2006.
11. Henningsen K. On the application of blood group testing to cases of disputed paternity in Denmark. Acta Med Leg Soc (Liege). 1956;9:95-104.
12. Henry I, Hoovers J, Barichard F, Bertheas MF, Puech A, Prieur F, Gessler M, Bruns G, Mannens M, Junien C. Pericentric intrachromosomal insertion responsible for recurrence of del(11)(p13p14) in a family. Genes Chromosomes Cancer. 1993;7(1):57-62.
13. Jolly JG. Medicolegal significance of human blood groups. J Indian Med Assoc. 2000;98(6):340-1.
14. Klein RD, Dykas DJ, Bale AE. Clinical testing for the nevoid basal cell carcinoma syndrome in a DNA diagnostic laboratory. Genet Med. 2005;7(9):611-9.
15. Macan M, Uvodic P, Botica V. Paternity testing in case of brother-sister incest. Croat Med J. 2003;44(3):347-9.
16. Marjanovic D, Bakal N, Kovacevic L, Hodzic M, Haveric A, Haveric S, Ibrulj S, Durmic A. Optimisation of forensic genetics procedures used in disputed paternity testing: adjustment of the PCR reaction volume. Bosn J Basic Med Sci. 2006;6(2):76-81.
17. MarketTools, Inc. Cybertory™ Virtual Laboratory Exercises. **http://www.cybertory.org/exercises/#paternity**. 2007a.
18. MarketTools, Inc. Paternity Determination using the Cybertory™ Virtual Molecular Biology Laboratory. **http://www.cybertory.org/exercises/forensics/paternity.html**. 2007b.
19. Mertens G. Acta Clin Belg. 2006;61(2):74-8.
20. Schonemann C, Groth J, Leverenz S, May G. HLA class I and class II antibodies: monitoring before and after kidney transplantation and their clinical relevance. Transplantation. 1998;65(11):1519-23.
21. Simon R. Journal of the National Cancer Institute. www.intelihealth.com/IH/ihtIH/EMIHC000/333/333/359682.html. 2006.
22. Wenk RE. Testing for parentage and kinship. Curr Opin Hematol. 2004;11(5):357-61.
23. Zhu H, Zhu Y, Ma H, Lu J. Ten-minute DNA Release Kits. Nature and Science. 2006;4(2):58-70.