AN INTRODUCTION TO THE DNA IQ™ SYSTEM: THE SMART WAY TO PURIFY DNA

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Abstract

A new DNA purification system, DNA IQ™, specifically designed for the special needs of the forensic and paternity community is described. This system uses a limiting amount of a DNA binding paramagnetic resin to isolate a set amount of DNA from database and paternity samples such as blood, bloodstains and buccal swabs. The DNA IQ™ System becomes very efficient at purifying DNA from small casework samples, providing DNA free of PCR inhibitors. Sample types from which DNA has been successfully isolated include bloodstains on clothing ,including denim and leather, blood in soil, sperm and epithelial fractions from differentially extracted rape kits, tissue, hair, bone, cigarette butts, toothbrush bristles and paraffin embedded formalin fixed tissue. The system uses a standard extraction protocol for stains on solid supports or a protease K digestion of tissue, hair and bone. A standard 45 minute protocol then is used to purify the DNA. This purification procedure has been automated on a Beckman Biomek® 2000 robotic system.

Introduction and theory

DNA analysis is a multi-step process whose outcome is dependent on the quality of each step. Increased casework loads have spurred the development of megaplex STR amplification systems but it is important to examine other stages of the process to maximize efficiency and quality. Current DNA purification systems were designed to purify bacterial and plasmid DNA and frequently have problems with the diverse sample types encountered in forensic work.

To obtain consistent results, we have developed a nearly universal purification system that is designed specifically around the needs of the forensic community. This system isolates DNA free of PCR inhibitors from a wide variety of difficult samples such as blood on denim, soil and leather using a common protocol for purification following a standardized extraction protocol for stains on solid supports or a Protease K digestion for tissue, bone and hair. The system is based on a paramagnetic resin that binds DNA and can be easily manipulated.

This system employs two approaches depending on the type of sample. For database, paternity and reference samples where DNA is abundant, the limiting amount of resin is saturated and thus captures a set amount of DNA (see Fig. 1, upper box). This amount has been set at 100ng but can be easily scaled up or down. The practical result is automatic quantitation of DNA during purification, eliminating the need for an extra quantitation step and ensuring that the right amount of DNA is placed in amplification reactions to give optimal STR results.

For casework samples the same protocol is used with smaller sample sizes. The DNA IQ™ resin becomes very efficient at recovering DNA from these smaller samples (Fig. 1, lower box). The extraction protocol takes about 45 minutes which includes a 30 minute heat step. A protease K digestion replaces the heat step for tissue, hair and bone samples. Differentially extracted sperm and epithelial fractions do not need any further extraction and are placed directly into the purification protocol by adding at least 2 volumes of Lysis Buffer. Liquid blood, either fresh or frozen, can be placed directly into the purification protocol.

The DNA purification system has been thoroughly tested with other Promega products such as the AluQuant™ System for human specific quantitation and PowerPlex® STR amplification systems including PowerPlex® 16. This package of reagents increases the likelihood of obtaining reliable and reproducible results.

Results

Database Applications

Database, reference and paternity samples are routinely composed of buccal swabs, blood on solid supports (FTA® or S&S 903 paper) or liquid blood. These samples are single source and contain abundant amounts of DNA. While not presenting significant problems in obtaining DNA free of PCR inhibitors, the amount and variability between samples, especially with buccal swabs, requires a quantitation step following purification. The DNA IQ™ system eliminates this quantitation need by isolating a set 100ng of total DNA.

FTA® paper was designed to provide a quick and safe way to purify and analyze DNA from blood. After washing and drying, small punches can be placed directly into the amplification reaction. Unfortunately, the paper has a very high capacity requiring manipulation of the cycle number and even then frequently results in significant imbalance of peak heights. In addition, variations in white cell counts and drying dynamics result in significant differences in DNA content within different areas of a bloodstain.

The DNA IQ™ system eliminates this variation by removing and quantitating the DNA from FTA® paper. Taking about the same time as the traditional FTA® process, DNA was isolated from blood-stained FTA® paper. One µl of purified DNA was placed in a standard PowerPlex® 1.1 amplification reaction and then analyzed on an FMBIO® II fluorescent scanner. Fig. 2 shows both a picture of the gel and tracings of the peak heights. The well balanced peak height make genotyping simple. DNA also is available for additional amplification reactions while the entire purification process would have to be repeated using the standard FTA® protocol for placing punches directly into PCR reactions.

Buccal swabs are routinely used because of the non-invasive collection procedure. However, significant variations in DNA content occur because of variations in collection procedure and cell shedding between individuals. Variations of from 0.2 to 3 µg of DNA are frequently encountered. This necessitates a post purification quantitation step to avoid re-amplifying a significant percentage of samples. To show the utility of the DNA IQ™ system, swabs collected from 12 individuals were extracted and purified using the standard protocol. One µl of isolated DNA was amplified using PowerPlex® 1.1 and analyzed on an FMBIO® II fluorescent scanner. Fig. 3 shows the gel image demonstrating relatively uniform bands that were easily used to genotype each individual.

Casework Applications

Ideally a DNA purification system for forensic samples should be able to use a standard protocol for a wide variety of sample types and be able to isolate small amounts of DNA. Stains on a wide variety of solid supports including FTA® and S&S 903 papers, swabs, cotton clothing, denim, leather, cigarette butts and soil are heated in the presence of Lysis Buffer to liberate the biological material. The solution and support are then centrifuged to liberate the DNA containing liquid. This results in a solution that can be treated just like liquid blood. Heat sensitive material can be treated without heating or the biological material removed with a swab. Tissue including hair and bone are treated with a

Protease K solution in place of the heat extraction. The purification protocol is the same for all resulting samples, only adjusting the initial and elution volumes depending on sample amount. The DNA IQ™ resin is capable of purifying both single stranded and double stranded DNA including mitochondrial DNA. However, the resin does not purify small fragments of DNA (below 80 bases) which are too small to amplify with forensic primer sets but tie up polymerase and primers and thus reduce amplification.

To demonstrate the sensitivity of the DNA IQ™ System, blood was diluted 10 fold and 1 µl stains were placed on denim. After storage at room temperature for 3 weeks, the stains were cut out, extracted, the DNA purified and eluted into 25µl of Elution Buffer. Five µl of the DNA solution was amplified with PowerPlex® 16 and analyzed on an ABI PRISM® 310 Genetic Analyzer. Fig. 4 shows the uniform pattern which was easily genotyped.

Paraffin embedded formalin fixed tissue is occasionally used for forensic or paternity samples and is one of the most difficult sample types to obtain usable DNA. To show the versatility of the DNA IQ™ System, paraffin embedded formalin fixed thin sections were treated with a solution of Protease K overnight and then 100µl of Lysis Buffer was added and the DNA purified. The resulting DNA was amplified with PowerPlex® 16 and analyzed on an ABI PRISM® 310 Genetic Analyzer. Fig. 5 shows the relatively uniform peak heights even out to 400 bases.

Resin compatible with capillary electrophoresis

Some paramagnetic resins have created problems when the DNA isolated using these particles is analyzed by capillary electrophoresis. To demonstrate that the DNA IQ™ resin has no effect on capillaries, 16 samples were isolated using the DNA IQ™ System and amplified with PowerPlex® 16. These 16 amplified samples were then analyzed on an ABI PRISM® 310 Genetic Analyzer, cycling for 100 injections. Fig. 6 shows sample number 4 patterns on injection 4, 36, 52, 68 and 84. All patterns are identical demonstrating that the DNA IQ™ resin has no apparent effect on the capillaries.

Automation of the DNA IQ™ System

Using paramagnetic particles, the DNA IQ™ System is relatively easy to automate. Most of the automation was performed on the Beckman Biomek® 2000 platform. This platform uses an eight channel liquid dispensing tool and a gripper arm for moving 96-well plates. An integrated shaker and heating system provides efficient washing and elution capabilities. This system has been installed in four state forensic laboratories (see manuscript by Susan Greenspoon in these proceedings) and has been used to purify DNA from a number of different sample types including, blood, sperm and epithelial fractions from differentially extracted rape kit material, tissue, bloodstains on cards and toothbrush bristles. It should be emphasized that at present, pre-processing such as protease K digestion for tissue and extraction from solid supports must be done prior to placing the samples on the robot. The robotic processing time takes between 90 and 130 minutes for 88 samples depending on the sample type and is completely walk-away. Protocols are also being developed for the Tecan Genesis 150 robotic system.

Conclusions

We have developed a DNA purification system that was designed specifically for the forensic and paternity community. A nearly universal extraction process removes DNA from solid supports while a standard Protease K digestion is used on tissue, hair and bone. This system then uses a limiting

amount of resin so that with database and paternity samples, where DNA is plentiful, a constant 100ng of DNA are isolated independent of sample size. For casework samples, the resin is very efficient at capturing small amounts of DNA free of PCR inhibitors. In either case, the system is fast, does not use hazardous reagents, works on most sample types and has been automated.

Acknowledgments
We wish to acknowledge the contributions of Rex Bitner and Susan Koller for their support in the early phases of this work and Jeff Bacher for his support with tissue extraction protocols.

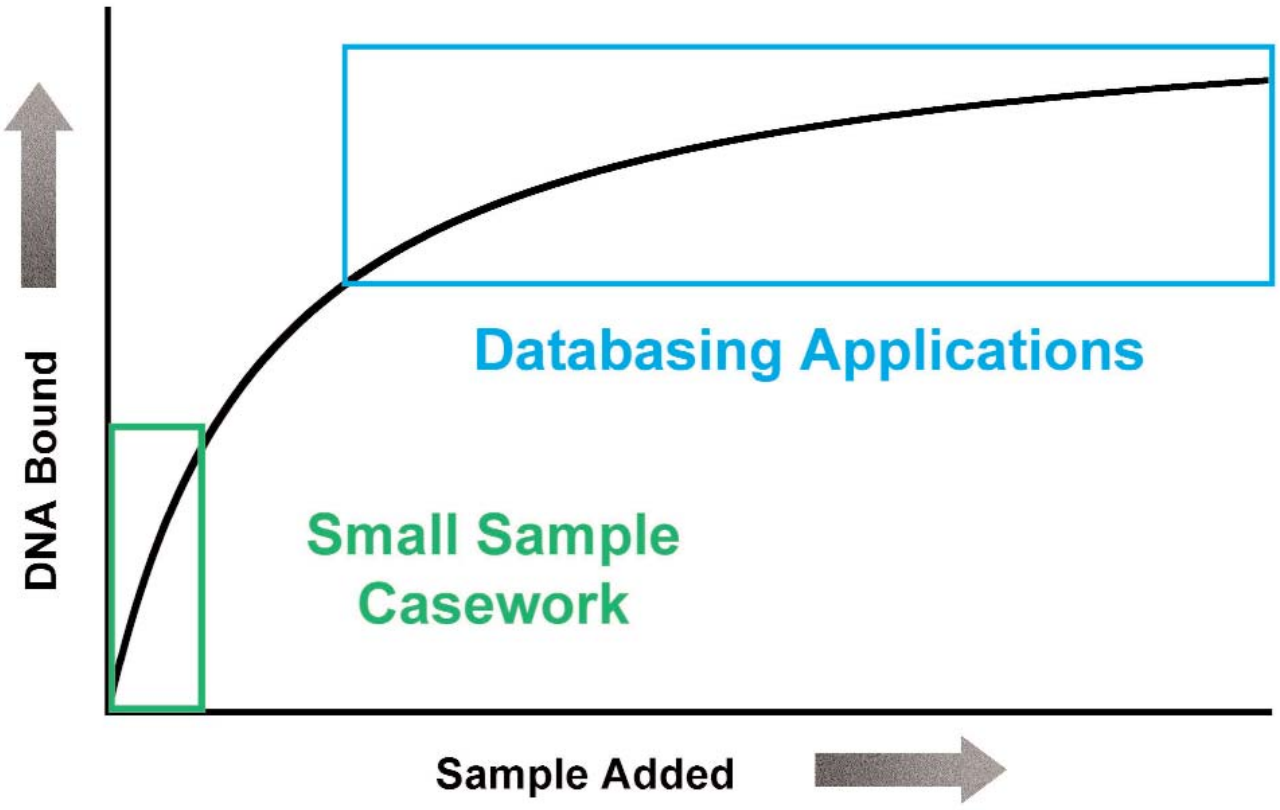

Figure 1. Theory behind the DNA IQ™ System. All DNA-binding resin will saturate in a manner similar to the curve shown. The DNA IQ™ resin has been selected to saturate in a DNA concentration relevant to the forensic and paternity community. When the sample contains an excess amount of DNA, upper box, a relatively uniform amount of DNA is recovered regardless of sample size above the saturation point. With smaller amounts of DNA, lower box, such as those commonly found at crime scenes, the resin becomes very efficient at capturing the available DNA.

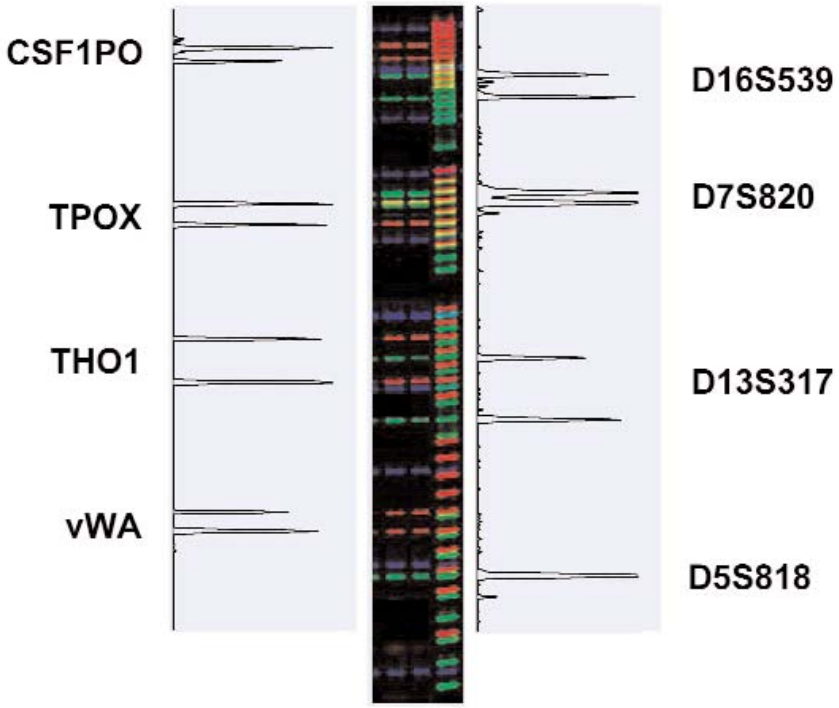

Figure 2. Analysis of DNA purified from bloodstains on FTA® paper. Bloodstains on FTA® paper were processed by the standard DNA IQ™ method, 1µl of DNA solution from each sample was amplified with PowerPlex® 1.1 and then analyzed on an FMBIO® II fluorescent scanner. Lane 1 of the gel image was scanned for peak heights in the two sample colors and indicate the uniform peak heights obtained.

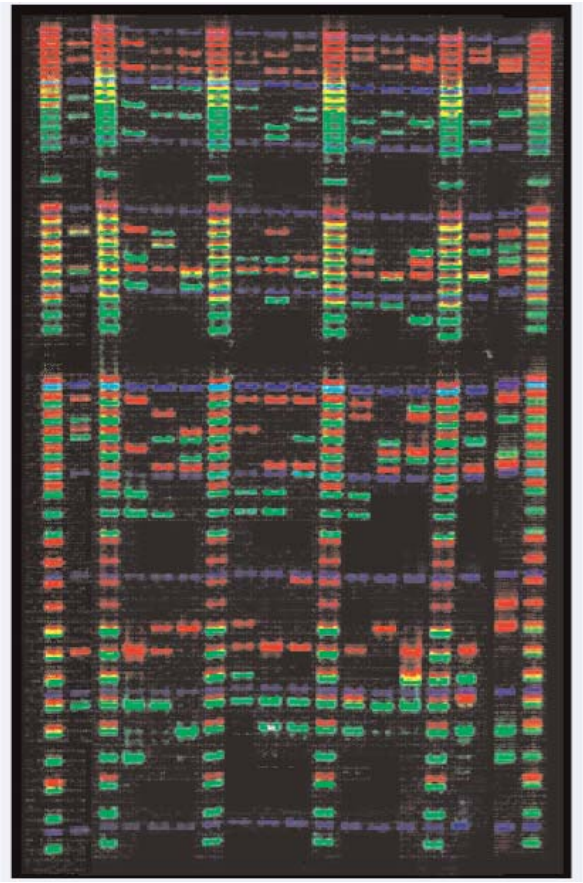

Figure 3. Analysis of DNA purified from buccal swabs. Cotton buccal swabs were obtained from 12 individuals, processed by the standard DNA IQ™ method and 1µl of each sample was amplified with PowerPlex® 1.1 and then analyzed on an FMBIO® II fluorescent scanner. The gel image demonstrates the relatively uniform banding of each individual which were easily genotyped.

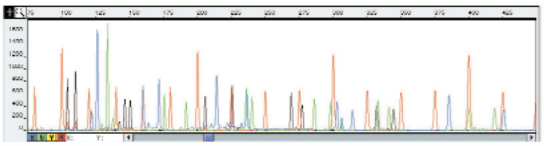

Figure 4. Sensitivity of the DNA IQ™ System. Blood was diluted 10 fold with phosphate buffered saline and 1µl spots were made on denim. After storage at room temperature for 3 weeks, the spots were processed using the standard DNA IQ™ method and eluted in 25µl of Elution Buffer. Five µl samples were amplified with PowerPlex® 16 and analyzed on an ABI PRISM® 310 Genetic Analyzer. The scan shows uniform peak heights with RFU values well above acceptable lower limits.

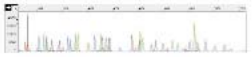

Figure 5. Isolation of DNA from Paraffin embedded formalin fixed tissue. Thin sections of paraffin embedded formalin fixed tissue was treated with protease K overnight followed by the standard DNA IQ™ purification method. The DNA was amplified with PowerPlex® 16 and analyzed on an ABI PRISM® 310 Genetic Analyzer. The relatively uniform peak heights even at 400 bases demonstrates the robustness of this system.

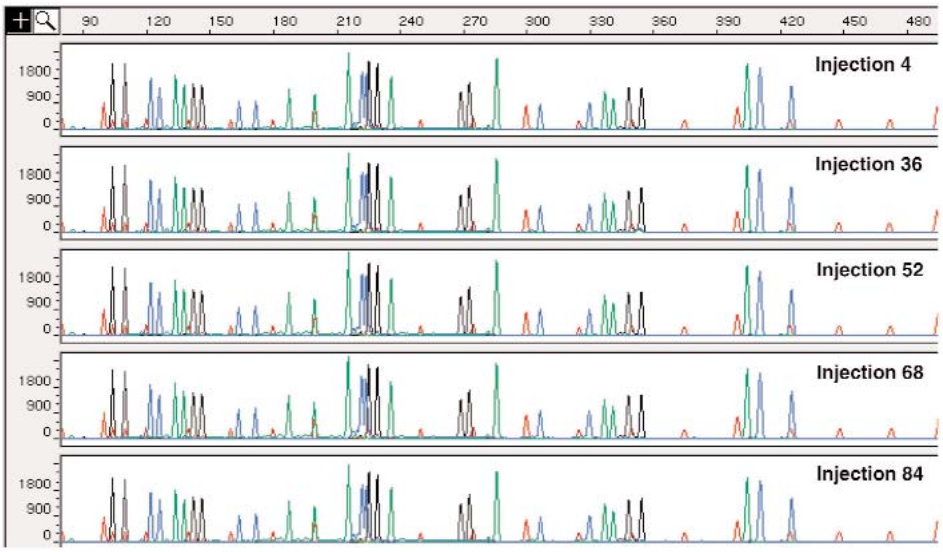

Figure 6. No detrimental effect observed on capillaries. To demonstrate that DNA purified by the DNA IQ™ System could be used on capillary electrophoresis systems, 16 samples were purified with the DNA IQ™ System, amplified with PowerPlex® 16 and then run on an ABI PRISM® 310 Genetic Analyzer. The samples were cycled for a total of 100 injections. The image shows the scan of sample number 4 at injections 4, 36, 52, 68 and 84. All scans are identical.