Comprehensive Gene Sequence Analysis from Bloodspot and Saliva DNA

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Abstract

Ambry Genetics’ proprietary full gene sequence detection methods have been validated for DNA isolated from blood spots and saliva, in addition to DNA from whole blood and cultured amniocytes used to date. Blood can be collected on Schleicher & Schuell (S&S) 903 specimen collection paper commonly used in newborn screening programs. Alternatively, saliva can be collected into an Oragene™ DNA Self-Collection container (DNA Genotek Inc.). High quality DNA can be obtained from either starting material, with a half of a blood spot (0.5 inch diameter) or 250 ul saliva giving yields of approximately 1.5-4.5 ug and 8-12 ug DNA, respectively. The isolated DNA has been successfully analyzed for sequence variations in the entire Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) coding sequence using the Ambry Test™: CF, utilizing modified temporal temperature gradient electrophoresis (mTTGE) and dye terminator sequencing methods. We observed a PCR amplification template size limit of approximately 800 bp for DNA isolated from bloodspots. Analytical sensitivities and specificities between the specimen types for these two genes were shown to be similar. Furthermore, the specimens are intact for years in their collected media and sensitivities and specificities between the specimen types for these two genes were shown using a dye terminator sequencing method. We observed a PCR amplification template length was kept under 800 bp. Dye terminator sequencing was set up using Beckman DTCS reagents and templates were analyzed on a CEG8000.

Introduction

Comprehensive gene sequence detection methods typically rely on ample amounts of DNA isolated from whole blood for analysis. Our assays, in particular for cystic fibrosis (CF) and beta globin (HBB) are often requested for newborns, babies and small children. In addition, specimens are sent from locations worldwide. We investigated if our methods could be optimized for use with bloodspot DNA and saliva DNA in order to accommodate a broader range of sample collection and shipping conditions. Advantages of providing these options are time and cost savings, and expedited diagnoses.

Methods

DNA extraction from blood spots: Blood was collected on FDA approved Schleicher & Schuell (S&S) 903 specimen collection paper (1), and DNA was extracted based on a method published by Walsh et al. (2). Briefly, half of a dried blood spot was incubated and rinsed three times in deionized water prior to centrifugation. A small volume of freshly made 5% Chelex 100 resin was then added. The suspension was incubated at 55°C for 2 hours, then boiled for 8 minutes, and centrifuged at high speed. The DNA supernatant was removed from the resin pellet and used for PCR.

DNA extraction from saliva: Two milliliters (4-5 spots) of saliva were collected into a DNA Genotek’s Oragene™ DNA Self-Collection container, which contains ~2 ml of cell culture media and cultured amniocytes used to date. Blood spots were collected on Schleicher & Schuell (S&S) 903 specimen collection paper commonly used in newborn screening programs. Alternatively, saliva can be collected into a DNA Genotek’s Oragene™ DNA Self-Collection container (DNA Genotek Inc.). High quality DNA can be obtained from either starting material, with a half of a blood spot (0.5 inch diameter) or 250 ul saliva giving yields of approximately 1.5-4.5 ug and 8-12 ug DNA, respectively. The isolated DNA has been successfully analyzed for sequence variations in the entire Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) coding sequence using the Ambry Test™: CF, utilizing modified temporal temperature gradient electrophoresis (mTTGE) and dye terminator sequencing methods. We observed a PCR amplification template size limit of approximately 800 bp for DNA isolated from bloodspots. Analytical sensitivities and specificities between the specimen types for these two genes were shown to be similar. Furthermore, the specimens are intact for years in their collected media and sensitivities and specificities between the specimen types for these two genes were shown using a dye terminator sequencing method. We observed a PCR amplification template length was kept under 800 bp. Dye terminator sequencing was set up using Beckman DTCS reagents and templates were analyzed on a CEG8000.

Conclusion

• Ambry Genetics’ proprietary full gene sequence detection method utilizing a combination of TTGE scanning and sequencing has been validated for DNA extracted from blood spots and saliva specimens.
• Analytical sensitivities and specificities for blood spot and saliva DNA are similar to those of whole blood DNA in The Ambry Test™: CF and in The Ambry Test™: HBB.
• Blood spots collected as part of newborn screening programs now can be used in Ambry Genetics’ CFTR and HBB full gene sequence analysis when follow-up molecular studies are needed, thus avoiding multiple blood collection and office visits, while expediting diagnosis. Potentially, DNA from stored bloodspots of deceased patients could be evaluated to help establish diagnoses and complete pedigrees.
• Clinics or individuals without customary access to phlebotomists can utilize the Oragene saliva kits to submit their DNA to Ambry Genetics for CFTR and HBB full gene sequence analysis.

References

1. www.schleicher-schuell.com