

GenVault: Genotyping Recovered DNA

I. Abstract

Shortcomings in current methods for biosample storage and preparation can lead to poor genotyping data quality, sample mix-ups, and processing delays. GenVault has developed a complete biosample management system which addresses these deficiencies and is simple to integrate with existing sample workflows. The following data show that DNA stored in the GenVault system generates high-quality results from the Illumina Golden Gate Assay while providing researchers with an inexpensive, space-saving system for sample management.

II. Introduction

Remarkable progress has been made in the development of analytical tools for high-throughput genotyping, with Illumina leading the way. Unfortunately, the full potential of this technology may not always be obtained using current methods for sample storage and preparation. Current methods of storing biosamples are primarily limited to cryostorage, where samples containing DNA are placed into a freezer and/or liquid nitrogen tanks. These storage methods are accessed manually and are often part of a scattered network of freezers or tanks. The contents of such an archive, even when tracked in a central database, can make retrieval a tedious and labor-intensive process. This prevents researchers from taking full advantage of high-throughput genotyping assays on statistically significant population sizes.

GenVault recently developed an integrated system to address these shortcomings. GenPlates, which are the core consumable of this technology, stabilize DNA at room temperature in a format that is compatible with laboratory automation. The plates contain an integrated biological barcode, referred to as the GenCode, which provide a permanent tracking mechanism for both the plate and the individual samples within. The GenPlate is paired with a storage module that maximizes storage efficiency and requires minimal electricity to operate. For example, GenVault's Desktop Archive can hold up to 38,400 aliquots in only two cubic feet. GenConnect, GenVault's sample management software, enables researchers to track their samples throughout the process and pair sample status with clinical data for the creation of meaningful study cohorts.

III. Materials and Methods

Sample Application and DNA Recovery

Ten microliters of anti-coagulated blood were applied overnight in a 40% humidity chamber, and then the samples were stored for six months at room temperature. After that, the DNA was recovered from the blood-spotted elements using the method described in the *GenSolve DNA Recovery User Guide*. The DNA was concentrated using QiaAmp Midi columns to achieve a final concentration of 50 ng/μl [2.5 μg] DNA. A total of 38 unique samples were recovered. An additional 2.5 μg of DNA was recovered from five of the unique samples to be used as replicates, and four DNA samples from Coriell Cell Repositories were included as controls.

DNA Quantitation

The PicoGreen dsDNA Quantitation Assay was used to determine DNA concentration according to the Pico Green protocol for 1:20 dilution of sample into prepared dye solution on a Tecan Genios plate reader. Only one sample was below the 50 ng/μl requirement at 47.7 ng/μl. The highest concentration recorded was 93.2 ng/μl. The mean concentration was 62.8 ng/μl.

SNP Detection

DNA was submitted directly to Illumina FastTrack Genotyping Services for genotyping on the BeadLab platform using the GoldenGate Assay. These samples were analyzed using a single OPA (Oligo Pool All) Linkage Array. This OPA is the first oligo pool in the four-OPA Linkage Panel, and includes 1,516 loci.

IV. Results

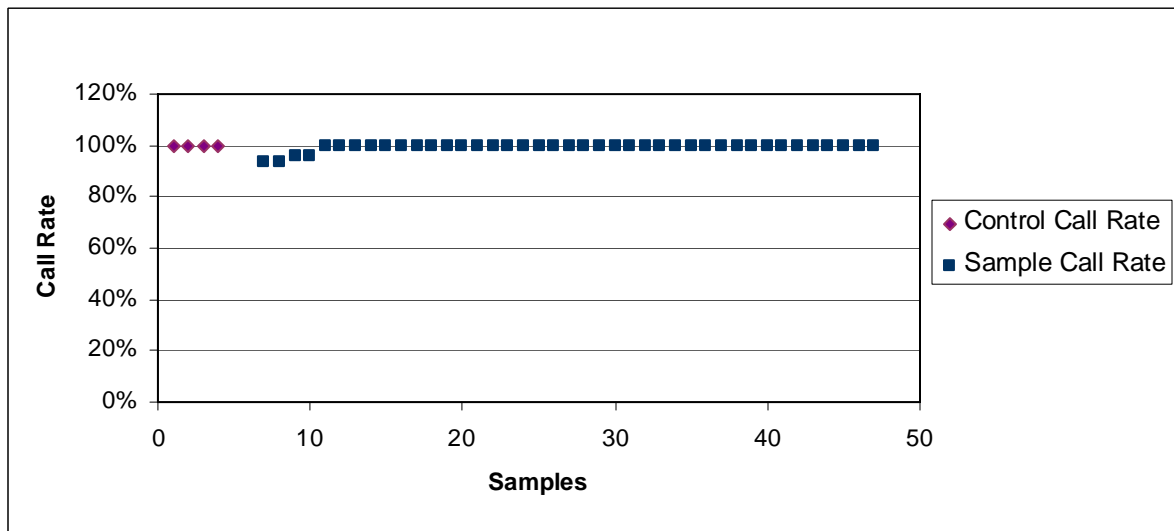
A total of 38 unique samples were submitted for analysis on Illumina’s BeadLab platform. Five of these samples were submitted in duplicate to examine reproducibility. A single Sentrix Array Matrix from the Linkage IV Panel was run using Illumina’s GoldenGate chemistry.

Two samples were dropped from the overall analysis due to low call frequencies. From the 41 successful samples, a total of 62,156 calls were attempted. Of those, 61,818 were successful, resulting in an overall call rate of 99.46% (*Table 1*). Additionally, 100% of calls between replicates were identical. All four of the Coriell control samples were successfully genotyped with a call rate of 99.9%. Call rates for successful samples ranged between 93% and 100%. The median call rate was 100% with 29 of the 41 samples all sharing this call frequency. *Figure 1* shows the call rates for all successfully genotyped samples relative to the call rates of the four control samples.

Table 1: Data Quality

Parameter	Number Successful	Number Possible	Success Rate
Sample Success Rate	41	43	95.35%
Genotypes (Call Rate)	61,818	62,156	99.46%
Reproducibility	13,296	13,296	100%

Figure 1: Call Rate



These call rates are based on Illumina’s GenCall scoring algorithm, which is based on the position of a particular genotype cluster. Genotypes with high GenCall scores are located in the center of a cluster and the lower GenCall scores are located outside or on the edge of the clusters. The GenCall score cutoff considered reliable with the GoldenGate Assay is 0.25.

V. Concluding Remarks

DNA stored and recovered from GenPlates can be successfully applied to SNP detection. The samples submitted to Illumina for genotyping show a high genotyping success rate and excellent reproducibility. The data shown here demonstrate that the GenSolve method of recovering DNA from dry-state storage provides a high-quality DNA sample that is effective in one of the most sophisticated methods of high-throughput genetic analysis.

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