Using Whole-Genome Amplified (WGA) DNA Samples in the GoldenGate® Genotyping Assay

Introduction

Illumina recognizes the need of some investigators to use whole-genome amplified (WGA) DNA because of limited sources of genomic DNA (gDNA) for their genetic studies. An increasing number of publications are being generated on the viability of WGA products. This document provides information from publications and customer feedback that will assist researchers considering the use of WGA DNA samples for their genetic studies using the GoldenGate genotyping assay.

Publications using the Illumina platform and WGA products

The use of WGA products on the Illumina platform was first described by Barker et. al[1]. In this study, high quality, intact DNA was used to assess the reliability of two methods of WGA using Illumina's GoldenGate assay. Multiple displacement amplification (REPLI-g®, Qiagen®, Inc.) and OmniPlex® (Rubicon Genomics®) technologies were tested in five Centre d'Etudes du Polymorphisme Humain (CEPH) DNA samples from Coriell Cell Repositories. Both methods yielded >99.8% concordance relative to gDNA samples and >98% genotype call rates.

A later study by Pask et. al[2] examined a larger cohort of 86 gDNA samples and their corresponding WGA products. In this study, WGA products were generated using F29 polymerase Multiple Displacement Amplification. A concordance rate of 98.8% between the WGA and gDNA samples was observed, as well as a genotype call rate of 99.8%.

While both of these studies provide positive indicators for using WGA products on the Illumina platform, it must be noted that both studies used high quality, intact DNA within the recommended ranges of the manufacturer's specifications.

A more recent study also examined the use of WGA samples on the Illumina platform. Sawcer et. al[3] used 508 WGA samples as part of their study. While only 104 of the 508 samples generated genotypes, the study was clear to note that the average starting concentration of the genomic DNA input into the WGA reaction was clearly lower in the failed samples (5.9 ng/ml) than in the 104 samples which were successful (17.4 ng/ml). No mention was made as to the quality or age of the samples used for the WGA reactions. While concordance rates and genotype call rates were not directly addressed, the authors did demonstrate a crude error rate of 0.025% using the WGA-derived samples.

Important suggestions for using WGA samples on the Illumina GoldenGate platform

Based on the publications described above as well as customer feedback, Illumina has compiled the following list of important considerations when deciding to use WGA samples on the Illumina GoldenGate platform:

1. **Know your DNA.** The quality of DNA used in the WGA reaction appears to be one of the most important factors for the success of samples used with the GoldenGate chemistry. High-quality, intact DNA has been shown to generate the best results. Sample preparation, storage conditions, length of storage, source of DNA, as well as a host of other factors can affect quality of DNA.
2. **Use sufficient amounts of input DNA.** Although some WGA methods describe successful whole genome amplification with smaller amounts of starting DNA, Illumina suggests using a minimum of 50 ng of intact, high quality DNA for input into the WGA reaction. DNA suspected or known to have undergone degradation should be used in minimum quantities of 100-200 ng. These suggestions are not only supported by the above publications, but also by Illumina customer feedback.

3. **Accurately quantify your DNA prior to addition to the WGA reaction.** Since the quality and quantity of DNA used in WGA reactions is an important contributing factor to sample success on the Illumina platform, accurate quantification of input DNA is essential. Samples should be quantified using a DNA-specific method or reagent such as PicoGreen®. Products from the WGA reaction should also be quantified using the same method prior to entry into the GoldenGate procedures, as recommended in current BeadLab and BeadStation protocols. For accurate quantitation of highly degraded DNA, particularly formalin-fixed, paraffin-embedded (FFPE) samples, the use of real-time PCR with the ALU Yb8 subfamily may be useful[4].
4. **Test your samples.** Regardless of the WGA method used, Illumina strongly suggests testing a small number of samples prior to initiating a study that partially or completely utilizes WGA samples. This is not only important for WGA kits that are new to the market and for which prior information is not available, but also warranted for the methods described in the publications above. Formulary changes in kits can adversely affect the successful generation of results. Testing 12-16 samples side-by-side on an array with gDNA samples using a well-performing oligo pool (OPA) is highly recommended before undertaking any projects that include WGA samples. When possible, include family DNAs in these tests to increase the confidence level of the test.

Frequently asked questions

1. **Where can I get more information and order the kits described in the publications summarized above?** The REPLI-g kit is no longer available from Molecular Staging, Inc. It is now sold under the same name by Qiagen (www.qiagen.com), catalog #59043. The OmniPlex method is available as a service from Rubicon Genomics (www.rubicongenomics.com). The kit is available for purchase from Sigma-Aldrich® (www.sigmaaldrich.com/genomeplex) under the name GenomePlex®. Illumina strongly suggests testing samples with these kits prior to performing a complete study, as formulary changes may have occurred when these products were transferred to new companies or as part of the product's evolution.
2. **Do I need to clean WGA products prior to using them in GoldenGate assays?** Most importantly, Illumina suggests that customers refer to the manufacturer's protocols for the kit they are using. If a purification step is not included in the protocol, customers can then decide whether it is a cost-efficient step to add one or not. Illumina customer feedback has suggested that cleaning WGA products will not cause WGA samples that would not normally work to now perform well. Anecdotally, however, customers have stated they have seen slightly improved genotyping call rates. Of note, the Barker et. al1 implemented a purification step using QIAquick® (Qiagen) columns.
3. **How can I tell if my DNA samples are intact or degraded?** Prior to WGA, molecular weight and sample degradation can be assessed by electrophoresis through agarose. For the purposes of WGA, samples with high molecular weights (20 kb and above) can be considered relatively intact. Samples with low molecular weights (lower than 20 kb) should be considered degraded.
4. **Can I use optical density (OD_{260}) to quantify DNAs that will be added to the WGA reaction?** Use of OD_{260} is strongly discouraged as it is not a direct measurement of DNA quantity, and is subject to a variety of influences that can falsely elevate calculated DNA concentration. Samples should be quantified using a DNA-specific method (i.e., RediPlateTM 96 PicoGreen® dsDNA Quantitation Kit, Molecular ProbesTM, catalog #R-21495, www.invitrogen.com). Products of the WGA reaction should also be quantified using the same method prior to entry into the GoldenGate procedures, as recommended in current BeadLab and BeadStation protocols.
5. **Can results from WGA samples be analyzed with gDNA results?** For many loci, WGA samples will cluster well with gDNA samples in GenCall. However, for some loci a slight shift in intensity or theta values may be observed. Therefore, it is recommended that samples from WGA not be analyzed concurrently with non-WGA derived DNA samples. Including family DNA samples when possible can also increase confidence in the clustering and calling of genotypes by estimated Mendelian error rates.
6. **Can GenTrain files be used for analyzing the results for WGA samples?** GenTrain files that were created with WGA samples can certainly be used to increase efficient data processing. However, GenTrain files generated using gDNA samples should not be used for WGA projects. GenTrain files for standard products, such as the Linkage IVb panels, have been generated from gDNA samples and therefore are not recommended for use with WGA samples.
7. **Is there allelic bias observed for WGA samples on the Illumina platform?** Amplification and allelic bias is a concern with in any WGA product. Pask et. al[2] found that potential allelic bias, as demonstrated by inheritance errors in their family DNAs, was nearly identical and occurred for the same loci between the Illumina and TaqMan® (Applied Biosystems®, Inc.) platforms. This demonstrates that any allelic bias most likely stems from the WGA reaction and is not platform-specific. In addition, insufficient DNA added to WGA reactions can be a major source of allelic bias. Starting with sufficient, high-quality DNA will reduce these effects.
8. **Can WGA samples be used with the Infinium® Assay?** The GoldenGate and Infinium assays use different chemistries and could potentially perform differently with WGA DNA samples. Additionally, the Infinium Assay includes a WGA step as part of the protocol.As such, any potential amplification bias that may be present in WGA samples could theoretically be enhanced in the WGA reaction of the Infinium protocol. The use of WGA samples with the Infinium product is currently being explored by the Illumina Research and Development team.

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