Optimizing for DNA sample success at Invitae

The success of any molecular assay depends on the quality, integrity, and quantity of the analyzed DNA. Tissue age and storage, as well as DNA extraction methods, storage, and quantification can affect these properties.

This guide is intended to provide suggestions on how to prepare and store DNA samples so they will have the greatest likelihood of yielding complete and reliable results.

Background

Invitae specimen requirements can be found at www.invitae.com/specimen-requirements. Whenever possible, we highly recommend supplying fresh blood for testing, with saliva as an alternate choice. We accept DNA isolated in other labs,* and derived from most tissue types (as detailed at www.invitae.com/specimen-requirements), with the following caveats.

Copy number analyses are especially sensitive to biases introduced during sample preparation and storage. Compromised DNA quality (degradation, etc.), differences in DNA composition due to alternate extraction methods, or interfering contaminants introduced during extraction can all impact copy number analysis. In our experience, the majority of received DNA samples can support full sequencing and copy number variation (CNV) analysis, although some can support only sequencing analysis. In comparison, for DNA extracted from fresh blood and saliva at Invitae, >99% of samples support full sequencing and CNV analysis.

Unfortunately, there is no upfront QC method that can flag which DNA samples will support CNV analysis and which will not. We re-run DNA samples multiple times in an attempt to get robust CNV data. If valid CNV data is not possible, we will still provide a comprehensive report on SNVs and indels. Billing is the same regardless of whether CNV analysis is included.

Detection of copy number variation at Invitae

Invitae uses in-solution hybridization capture to enrich target regions of the genome for sequencing. The validity of the copy number analysis is evaluated based on a comparison of the proband sample's global coverage profile with the global coverage profile of other concurrently processed samples. If the proband sample performs as expected globally, specific deviations from the baseline form the basis for our deletion/duplication calling algorithm. Using a baseline from within the batch allows us to understand and account for small variations in our laboratory processes.

Specific causes of variability in DNA extractions

DNA quality or profiles may differ based on source tissue, DNA extraction chemistry, lysis and resuspension incubation temperatures, elution buffers, pre- and post-extraction storage conditions, and freeze-thaw cycles. DNA isolated using different extraction chemistries and collected using different protocols are more likely to yield deviant results.

An outline follows describing our recommended best practices for ensuring the most reliable results when providing isolated DNA to Invitae. In addition, we continue to work to improve the yield for non-standard DNA isolates.

**DNA must be extracted in a CLIA or other suitably certified laboratory.*

DNA best practices for reducing sample failures

Sample amount	We require 5 micrograms (ug) of gDNA with a concentration of 50ng/uL. It is commonly recognized that when the same sample is measured in different labs (or even by different users/instruments within the same lab), there will be variability in the measurement (ref: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2765451). We cannot accept samples that do not meet our quantity requirements based on our own PicoGreen measurement; therefore it is important to accurately quantitate your sample to prevent it from being rejected due to insufficient quantity. ▪ Use a PicoGreen-based method to measure amount and concentration, as this method is specific to double-stranded DNA. Be aware that quantifying using UV absorbance can result in over-estimation of the quantity since the measurement is non-specific and can include single-stranded DNA, RNA, free nucleotides, and contaminants (ref: https://www.ncbi.nlm.nih.gov/pubmed/24495734). ▪ If UV absorbance is used, A260/A280 should be in the range expected for pure DNA (1.8-2.0). Deviations from this indicate contaminants that can affect our process.
Sample integrity	Our process assumes that DNA starts intact, double-stranded, and with a high molecular weight (> 20 kb). If you are concerned about sample integrity and have sufficient starting material, we suggest running the sample in an agarose gel to check the integrity.
DNA storage buffer	We exclusively use 10 mM Tris-HCl, pH 8.5, which is Qiagen's EB buffer. We can support TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) or LowTE (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA). We actively recommend against DNA that has been stored in water.
DNA storage temperature	We recommend DNA be stored frozen for periods longer than one week after extraction, and submitted prior to freeze-thaw cycles. DNA undergoes non-enzymatic depurination rapidly at low pH, with a strong dependence on temperature (ref: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4278771), so DNA in unbuffered solutions stored at 4C or room temperature will be more susceptible to failures.
Volume	We strongly recommend screw-cap tubes with an integrated rubber O-ring to prevent evaporation or leakage in transit. We also strongly recommend against small volumes of highly concentrated DNA. We recommend ≥ 75 µl.
Concentration	If samples are too concentrated to meet our minimum volume requirements, they can be diluted by adding a small amount of one of the above-mentioned buffers. In practice, concentrations over 250 ng/ul often produce non-linear quantitation results, so it is preferable to dilute to a working concentration and then requantitate prior to submission. If samples are too dilute and do not meet our concentration requirements: ▪ Concentrate the DNA using traditional ethanol precipitation, avoiding carrier nucleic acids or carbohydrates if possible. Do not over dry the pellet or resuspend a pellet with heated incubations. ▪ Concentrate the sample using a column or bead-based approach. ▪ As a last resort, use a vacuum concentrator for samples only if it is essential that there be no DNA lost, as this approach risks cross-contamination and increased salt in the sample.
Extraction kits	Invitae uses two commercial bead-based extraction kits, the Qiagen Qiasymphony DSP chemistry, and Mag-Bind kits from Omega Bio-tek. We strongly recommend the use of a commercial kit, specifically products from the Qiagen DNeasy line.
Shipping	Ship samples in screw-cap tube overnight with a cold pack if possible.

HEADQUARTERS | 1400 16th Street, San Francisco, CA 94103 | **ONLINE** | www.invitae.com | **CONTACT** | www.invitae.com/contact