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Maximizing DNA Yield from
Whole Blood Extraction

White Paper

Executive Summary

When you perform genomic DNA extraction from whole blood, low yield or low quality DNA can result in many issues. No matter your intended downstream application—qPCR, next generation sequencing, Sanger sequencing, and so on—you need high quality DNA. We've made this step-by-step guide to assist you in getting the highest possible DNA yield and quality, and set you on course to achieving great results.

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Choose (or Know) the Best Sample Storage Conditions

Anticoagulant

There are a number of choices for an anti-coagulant. From a DNA yield and quality perspective, EDTA is the best choice, followed by sodium citrate.

Heparin should be avoided if at all possible as it interferes with the purity of your DNA end product. That's because it is very difficult to remove from the DNA sample during the purification process, and it inhibits downstream PCR analyses. EDTA on the other hand, is easily removed during standard purification.

Evidence that EDTA has a slight edge over sodium citrate as the better anticoagulant choice comes from an interesting paper that tracked the impact of DNA extraction yield after samples were stored in EDTA, sodium citrate, and heparin at room temperature (RT).[1]

The results showed a clear difference in DNA yield after the samples were stored at RT for 12 hours. The DNA yields from EDTA-stored samples were unaffected, while samples stored with sodium citrate or heparin had greatly decreased DNA yield.

While we don't recommend that you store your samples at RT, this study shows that EDTA is adding an extra layer of protection to the DNA sample. This extra protection puts EDTA above sodium citrate in the order of preference.

Light Exposure and Temperature

Exposing your sample to sunlight and/or temperatures above 4ºC should be minimized to preserve the DNA in the sample. UV light harms DNA by causing formation of thymine dimers in DNA. As temperatures increase, so do chemical reactions that damage DNA like oxidation and acid hydrolysis.

Therefore, the faster that you can refrigerate or freeze your sample, the better.

If a sample will be used within three days, refrigerate it at 4ºC as quickly as possible following sample draw. One study reported significant increases in DNA yield when the time from blood collection to refrigeration was decreased.[2]

On the other hand, if the sample will not be processed within 3 days, freeze and store it at -80ºC. Ideally this should happen within two hours of sample draw to avoid any impact on yield from your genomic DNA extraction, according to Caboux et al 2012.[2]

Hemolysis

Finally, once a sample is safely in your hands and stored properly, avoid lysing the red blood cells in your sample (hemolysis). You can avoid hemolysis by only mixing your blood sample if necessary and only then by gentle inversion. One particular study that looked at over 50,000 DNA samples found an inverse correlation between the degree of hemolysis and DNA yield.[2]

Therefore, treat your samples gently!

Cell Count

It is best practice to check the cell count of the sample being processed, either by hand using a hemocytometer or with an automated cell counter.

Of course, starting with too few cells will result in a low yield, but using more cells only works up to a certain point. Starting with too many cells than the chemistry being used can handle will result in poor yield and increased likelihood of incomplete lysis as well as carryover of proteins and other contaminants, which will interfere with downstream applications.

Check the manual for your chosen DNA isolation method to determine the optimal cell count, or determine the ideal concentration empirically with a dilution series.

If you find that your cell count is too high, split the sample into two aliquots and perform two separate extractions.

Choose the Best Whole Blood Genomic DNA Extraction Method

With the proper storage and pertinent sample information in hand, the next step is to determine which extraction protocol to use to get the best DNA yield.

The most common options are phenol-chloroform extraction, magnetic bead separation, and precipitation chemistry (or "salting out"). The method that results in highest DNA yield and purity is precipitation chemistry.

- Phenol and chloroform is the oldest of these approaches, but uses harsh chemicals that need to be handled extremely carefully. Aside from being a safety concern, any phenol or chloroform that is carried through to the end of the extraction can negatively affect downstream applications.[2] So, much like heparin, this approach to DNA isolation should be avoided if at all possible.
- DNA extraction using magnetic beads is a less toxic approach, but beads have the possibility to be carried over to cause contamination and inhibition of downstream processes. Again, like the phenol-chloroform method, you want to avoid this if possible because the carry-over of beads affects your ability to use it for PCR and other downstream applications.
- Precipitation chemistry or "salting out" is a popular DNA extraction method because it results in little-to-no contamination and consistent yields. There are a variety of kits available for this method. The Puregene method sequentially lyses the red and white blood cells, treats the lysed samples with RNase, and then precipitates protein and other contaminants. In contrast, the newer Flexigene method lyses the whole blood sample in one step and immediately removes RNA contamination by collecting the white blood cell nuclei pellet, then subsequent incubation with a buffer containing proteases and a chaotropic salt removes the remaining contaminants. In both methods the DNA is recovered by alcohol precipitation.

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Additional Top Tips for Improving Your DNA Yield from Blood Samples

The biggest opportunities to increase your DNA yield from blood samples lie within sample storage and handling. Still, here are a few additional top tips that could help increase your yield:

- Consider automating. Not only is automated DNA extraction from whole blood more consistent between each sample, it also removes human error.[2–4] Automated DNA extraction will also save you hands-on time, especially if you have a large number of samples to process. You can set up the automation system and come back later to high quality, pure DNA.
- Look after your enzymes. This might be a newbie tip, but sometimes those are the best. To prevent protease degradation caused by excessive freeze-thaw cycles, use aliquots of enzyme-containing buffers. And if the procedure is not working, consider trying again with a new aliquot of enzymes as a first step.
- Careful with your DNA pellet. Most DNA extraction procedures end with a pellet drying step after the alcohol precipitation. This step removes any excess alcohol, but be careful to not overdo it. If you have difficulties resuspending the DNA, try heating the DNA pellet with the respective rehydration buffer at 55ºC for about 5 minutes.

All in all, DNA extraction from whole blood isn't very complicated but can be intimidating with lots of room for errors that negatively impact the yield and purity of your end product. These tips will help you navigate the process to avoid most of the pitfalls and give you high yields of contamination-free DNA.

Sources:
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About AutoGen

AutoGen is a leading provider of automated nucleic acid extraction workflows that allows lab professionals to produce premier quality and value-added extraction results. Our workflows provide solutions that are the best fit for our customers' laboratory needs and budget, and our customers include biorepositories, contract research organizations, academic research laboratories, pharmaceutical companies, clinical diagnostic laboratories, and government institutions all over the world. We strive to provide quality instrumentation and chemistries, as well as dedicated technical support – all with a level of post-sale service that is truly unmatched. Visit www.AutoGen.com to learn more.

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