SBP Recommendation

Preanalytical conditions to prepare human DNA from whole blood

Introduction

Pre-analytical factors include all factors that can influence the results of a sample analysis, particularly during the processes of sample collection, transport, processing, and storage. Their impact, often underestimated, can be critical. Identifying and controlling these factors at each step, and standardizing processes to obtain reliable, reproducible, and accurate results, will help ensure sample quality and suitability for advanced research applications, particularly for omics studies. Indeed, the integration of multi-omics data, facilitated by technological advancements, is crucial for accelerating both research progress and advancements in precision medicine. In this context, ensuring the reliability and integrity of biological samples is essential and biobanks and researchers play a key role in this.

The aim of this document is to support and guide researchers and biobanks to prepare high-quality DNA for genomic projects.

Before considering each process in detail and focusing on sample quality, you need to establish standardized procedures for tracking samples effectively (incl. naming, coding) and mitigate the risk of sample swapping.

For each pre-analytical process, from collection to storage, a set of recommendations including DNA quality criteria is given, highlighting which steps are important when handling samples to preserve the integrity of the DNA, minimize the possibility of any chemical modifications, and avoid any agents that could contaminate nucleic acid samples or inhibit downstream enzymatic manipulations (e.g., residual proteins, chemical reagents). These recommendations are aligned with the ISO 20186-2: Molecular in vitro diagnostic examinations - Specifications for pre-examination processes for venous whole blood - Part 2: Isolated genomic DNA[1].

To further support researchers and biobanks, a list of key pre-analytical variables to be documented is provided at the end of the document.

FACTORS INFLUENCING DNA QUALITY & RECOMMENDATIONS [i]

SAMPLE COLLECTION

Collection tube is a critical factor in the blood collection process. The **type of tube material** as well as the **additives** used to preserve the blood are factors to be considered to ensure DNA preservation.

We recommend:

1) Use a **commercially available collection tube** containing an anticoagulant/genomic DNA stabilizer (e.g. EDTA tube, PAXgene Blood DNA tube).
2) **Do not underfill or overfill the tube** to maintain the correct ratio of additives. Care must be taken when filling the tube to respect the recommended volume.
3) Homogenize blood with additives. To avoid DNA degradation, **homogenization must be carried out gently. Do not shake or spin the tube.** The mixing of blood and additives is often achieved by inverting the tube repeatedly.

SAMPLE TRANSPORT / RECEPTION

Time and temperature are major factors of variability of these two pre-analytical steps.

We recommend:

1) Document the storage duration of biological material prior to processing. If collection and processing do not take place on the same site, the overall storage duration includes, if applicable, the duration of storage at the collection site, the duration of transport and the duration of storage prior to extraction at the processing site.
2) Follow the manufacturer's instructions regarding the maximum duration before treatment permitted. In all cases, **maximum storage duration must be controlled and kept as short as possible.**
3) Respect and document temperature conditions.
    a. In the absence of recommendations, and for immediate extraction, keep the blood sample at 4°C (or on ice) for periods up to 3 days and maintain the cold chain until processing.
    b. For non-immediate DNA isolation, freeze the blood sample in ultra-low freezer (range between -70°C and -80°C). Avoid freeze/thaw cycles to prevent DNA degradation, which can be achieved by optimizing sample preservation through aliquoting in smaller volumes. If freezing and thawing are necessary, do not exceed one cycle to minimize potential DNA damage.
        › Note that it is preferable to store DNA in extracted form over the long term rather than in blood, where it is more subject to degradation over time and more sensitive to a possible freeze/thaw cycle (e.g. in the event of a freezer failure).

SAMPLE PROCESSING

During processing, DNA is extracted from the blood sample. Precautions must be taken to avoid any contamination that could affect the quality of the DNA. The method used is also key in this process.

We recommend:

1) Carry out nucleic acid isolation according to a precise, validated and verified protocol. In this case, refer to SBP SOP on method validation (available here). We however strongly recommend using **commercially available isolation kits** and follow the manufacturers' instructions.

[i] In all cases, and where applicable, the manufacturer's instructions must be followed precisely.

   a. If the manufacturer of the blood collection tube specifies a kit to be used, follow its recommendations.
   b. If DNA Isolation is carried out by an automated method, using a robot, follow the manufacturer's instructions concerning the specific kits to be used.
   c. If a manufacturer's protocol needs to be adapted, validation is required.

2) Perform DNA isolation in a **separate area** from the amplification step, to avoid cross-contamination.
3) Use **DNase-free reagents and consumables during processing** to avoid DNase contamination and thus DNA degradation.
4) Include **an RNase step** to remove any remaining RNA.
5) Elute nucleic acid in an **appropriate buffer included in the kit or recommended** by the manufacturer.
6) For long-term storage of pure DNA, use **specific cryogenic tubes** to reduce nucleic acid absorption.
7) For storage at 4°C, use **tubes with screw caps** to avoid water evaporation.

DNA STORAGE

The duration and temperature of final storage are factors to be taken into account to ensure long-term DNA quality, if it is not immediately analyzed.

We recommend:

1) Follow the kit manufacturer's instructions on storage conditions.
2) If there are no recommendations:
   a. For short-term storage, isolated DNA can be stored **at 4°C**[3].
   b. For long-term storage, DNA should be stored at -20°C or lower, **ideally in a range between -70°C and -80°C**. If possible, it is recommended to **generate aliquots** to avoid repeated freeze/thaw cycles, which could lead to DNA degradation[2].
3) Implement temperature monitoring and automated alarming system to check storage temperature, to ensure the safety of frozen samples.

DNA SAMPLE REQUIREMENTS

Prior to nucleic acid analysis, it is essential to assess its quality which can directly impact the success of the analysis.

Determining DNA quality involves measuring its concentration, purity (indicator of contamination) and integrity (state of DNA degradation).

We recommend:

1) **Concentration**: Quantify DNA by a sensitive and precise analysis using specialized methods such as fluorescent dye quantification[4,5]. Alternatively, measurement by spectrophotometry is also possible. It should be noted that differences in concentration may be observed for the same sample, depending on the method used. Recommended values depend on subsequent analyses.
2) **Purity**: Measure by spectrophotometry
   a. the A260/280 ratio, an indicator of contamination by proteins or RNA. A ratio of ~1.8[4,5] is generally accepted as 'pure' for DNA, and a range between 1.7 and 2.0 is accepted.

b. the A260/230 ratio, an indicator of contaminants that absorb at 230 nm such as proteins, guanidine HCL (used for DNA isolations), EDTA, carbohydrates, lipids, salts, or phenol[4,5]. A ratio between 2.0 and 2.2[5] is accepted.

Note that the 260/280 ratio may vary according to pH[6]. When comparing the 260/280 ratio for different DNA samples, it is important to ensure that the pH and ionic strength of the elution buffers used are the same. Absorbance at 260 nm and 260/280 values are reproducible when a low-salt buffer is used as elution buffer, but not water[5].

3) **Integrity:** Measure the DNA Integrity Number (DIN) or genomic quality number (GQN), which are metrics obtained by electrophoresis-based technologies. DIN values are distributed on a scale of 1 to 10. A high DIN indicates highly intact DNA, and a low DIN indicates highly degraded DNA sample. A value above 7[7] indicates good quality DNA, but recommended values may depend on subsequent analyses.

PREANALYTICAL VARIABLES TO BE DOCUMENTED - DNA SAMPLES

Below is the list of the key variables to be documented as part of the standardization of data documentation to accompany your DNA sample and record key preanalytical data. For a complete list of variables, please refer to the document "SBP Dataset for Human Liquid Sample"[8].

Process	Variables	Description
Collection	`sample_ID`	Unique identifier of primary sample
	`collection_start_time`	Date and time of sampling
	`sample_type`	Type of sample
	`primary_container_type`	Type of primary container in which the sample is collected
	`container_volume`	Volume of primary container in which sample is collected
	`collection_special_conditions`	Additives or specific conditions applied
	`pre_transport_temp`	Temperature conditions before transport
Transport	`transport_start_time`	Date & time when transport starts
	`transport_temp`	Temperature conditions during transport
Reception	`reception_time`	Date & time when sample arrives at reception
Processing	`processing_start_time`	Date & time when processing starts
	`sample_additive`	Type of sample additive
	`isolation kit`	Isolation kit used for DNA Isolation
	`storage_container`	Storage container type for long term storage
	`freezing_mode`	Freezing mode of the sample
	`freezing_start_time`	Date and time of freezing
Quality measurements	`quantification_method`	Type of quantification method used
	`concentration`	Concentration value obtained (standard unit: ng/µL)
	`DIN`	DIN value obtained
	`A260/A280 ratio`	A260/A280 ratio value obtained
	`A260/A230 ratio`	A260/A230 ratio value obtained
Storage	`storage_start_time`	Date & time when sample is physically stored in the freezer – can be the same as freezing time
	`storage_temperature`	Storage temperature
	`storage_place`	Storage location of the sample
	`freeze_thaw_cycle`	Actual sample status - e.g. "2" = defrosted twice

References

1. ISO 20186-2:2019 - Molecular in vitro diagnostic examinations - Specifications for pre-examination processes for venous whole blood - Part 2: Isolated genomic DNA
2. Yates JR, Malcolm S, Read AP. Guidelines for DNA banking. Report of the Clinical Genetics Society working party on DNA banking. J Med Genet. 1989;26(4):245-250. doi:10.1136/jmg.26.4.245
3. Wu, J., et al. "Stability of Genomic DNA at Various Storage Conditions." International Society for Biological and Environmental Repositories, 2009 Annual Meeting. Vol. 12. 2009.
4. Common minimum technical standards and protocols biobanks dedicated to cancer research, IARC technical publication no. 44, 2017.
5. Lucena-Aguilar G, Sánchez-López AM, Barberán-Aceituno C, Carrillo-Ávila JA, López-Guerrero JA, Aguilar-Quesada R. DNA Source Selection for Downstream Applications Based on DNA Quality Indicators Analysis. Biopreserv Biobank. 2016;14(4):264-270. doi:10.1089/bio.2015.0064
6. Wilfinger WW, Mackey K, Chomczynski P. Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. Biotechniques. 1997;22(3):474-481. doi:10.2144/97223st01
7. Betsou F, Bulla A, Cho SY, et al. Assays for Qualification and Quality Stratification of Clinical Biospecimens Used in Research: A Technical Report from the ISBER Biospecimen Science Working Group. Biopreserv Biobank. 2016;14(5):398-409. doi:10.1089/bio.2016.0018
8. SBP datasets for human liquid samples – Version 2.0

Acknowledgments

We would like to express our sincere gratitude to the experts who have contributed to the improvement of this document through their invaluable revisions and suggestions. Their dedicated efforts have not only enriched its quality and clarity but also strengthened its relevance and reliability.

In particular, we would like to thank for their contribution:

- Tim Frayling, Professor of Human Genetics, UNIGE (Geneva, Switzerland)
- Cedric Howald, Head of the DNA Sequencing Platform, Health 2030 Genome Center (Geneva, Switzerland)
- José Antonio López-Guerrero, Head of the Laboratory of Molecular Biology and Scientific Director of the ISO 20387 certified IVO Biobank, Cell Biology Professor at UCV (Valencia, Spain)
- Katrin Mannik, Head of Genomics Strategy, Health 2030 Genome Center (Geneva, Switzerland).
- Elodie Ristorcelli, Pre-analytical Laboratory Head, BGC (Lausanne, Switzerland)