TISSUE BANK AT THE IU SIMON CANCER CENTER

Standard Operating Procedure (SOP) 008V6.0

Processing and Storage of Extracted Blood DNA

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Approved by:

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Materials:
Sanitizer: 70% ETOH
96-plate: (MicroAmp Fast Optical 96-Well Applied Biosystems cat: 4346906)
Optical Adhesive Covers (Applied Biosystems, 4360954)
Tips: BIO DOT Max™ : 10 uL U2 Filter Tips
PCR workstation Flow Hood: Air Science® PURAIR PCR80884
Pipet: Nichipet Premium NICHIRYO P.5-10 uL J1563761
DNAstable Alpha Numeric Tube Plate (Micronic): Biomatrica® 99901-000 (custom)
TE Buffer, Tris-EDTA, 1X Soln: (Fisher BP24731)
MicroTubes: Micrewtube 2.0ml microtubes sterile (Dot Scientific T332-7S)
Barcode labels: Brady® Thermal Transfer labels THT-68. (Fisher 11-877-51)
Eppendorf Reference Pipet: 10-100ul (Fisher Cat. No.S304664 or Eppendorf Biotools Cat. No.:22470205/EMD)
Pipet tips: 100ul (Fisher Cat. No.05-403-49 or Eppendorf Cat. No.022491733)
Dry Storage Cabinet: Biomatrica® 95904-178
Reusable Desiccant Canister (Fisher 08-594-20)
Hygrometer: VWR (Cat No. 61161-378)

Methods:

DNA is manually extracted from the buffy coat cells at the Indiana University Genetics Biobank (IUGB) lab per SOP IUGB-3-11.03 Sample Processing. Newly extracted DNA in 1XTE buffer is retrieved from IUGB (R3 C158) on the IUPUI Campus and stored at 4°C until ready for aliquoting. Volumes will vary and are specified on the IUGB manifest report. After utilizing 1µg for SNP analysis, remaining DNA is stored both frozen (-80°C) and at ambient temperature.

Genetic Ancestry genotyping (SNP Analysis): Aliquoting is performed in a Laminar Flow PCR workstation which is decontaminated with 70% ethanol. Workstation, pipettes, and tips are decontaminated with UV light for 30 minutes prior to procedure. 1µg of extracted DNA is retrieved for each sample and placed in 96-well plate (MicroAmp Fast Optical 96-Well Applied Biosystems) using P-10 pipet (Nichipet Premium LT) and filtered 10µl tips. DNA vials are placed back in 4°C. Plate/s is/are left under the hood over night to let the samples air dry. The following day the plates are sealed with Optical Adhesive Covers (Applied Biosystems, 4360954).

Plates are shipped to LGC Genomics at Beverly, MA (now Biosearch Technologies) where the samples are re-hydrated for KASP genotyping [A] on a Thermo Fisher QS7 PRO qPCR instrument. As described in Nievergelt *et al*, 2013 [3], the genotyping of 41 SNPs, followed by data analysis using STRUCTURE v2.3.2.1, is used to estimate individual genetic ancestry.

Ambient Storage: DNAstable® matrix tubes in the Alpha Numeric Tube Plate are labeled with barcode labels as follows: Starting in position A1 and moving from left to right through position H12 (Table 1), four tubes are labeled with the same sample barcode and aliquot number D1 through D4. Following this protocol, a single Alpha Numeric Tube Plate can hold DNA from 24 donors. Barcode labels are placed right under the lip at the top of the tube. If the labels are longer than the circumference of the tube, they are to be overlapped in such a way that the data matrix barcode remains visible.

Table 1.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample 1 D1	Sample 1 D2	Sample 1 D3	Sample 1 D4	Sample 2 D1	Sample 2 D2	Sample 2 D3	Sample 2 D4	Sample 3 D1	Sample 3 D2	Sample 3 D3	Sample 3 D4
B	Sample 4 D1	Sample 4 D2	Sample 4 D3	Sample 4 D4								
C												
D												
E												
F												
G												
H												

Concentration of purified DNA (µg/ml) is measured at the IUGB Lab using Dropsense96 (IUGB-3-35.01 DNA Analysis) or Nanodrop (IUGB-3-17 DNA Analysis) and Picogreen, Quantiflour (IUGB-3-47 DNA Analysis) and recorded on the IUGB manifest. Amount to be aliquoted is calculated so as not to exceed 30µg of total DNA per tube in a maximum volume of 50µl. If required, sample is diluted in 1XTE buffer prior to aliquoting. Total volume should be at least 250µl in order to have sample remaining for frozen storage backup. In a decontaminated Laminar Flow PCR workstation, the matrix tube caps are removed and up to 50µl of DNA sample is pipetted and thoroughly mixed following the Biomatrica® DNAstable® Handbook and Quick Reference Protocol [1,2]. **The bottom of each Biomatrica® tube is coated with DNAstable® matrix so care must be taken to thoroughly mix DNA sample with gentle pipetting.** Tubes are left uncapped.

Sample volume, concentration, and total µg for each matrix tube (aliquot) is calculated and recorded.

After aliquoting, matrix tubes are left uncapped at room temperature under the laboratory laminar flow hood for a minimum of 24 hours and up to three days to dry. Once dry (samples should not feel sticky when tapped with a sterile pipette tip) the tubes are capped and the labeled tube plate is stored in a dry storage cabinet. Reusable desiccant canisters are used within the cabinet to keep the humidity level at or below 40%.

Frozen Storage: Remaining DNA in solution is stored at -80°C in one or two aliquots depending on volume and biospecimen manager discretion. If remaining volume >500ul, then sample is split into two equal aliquots. If remaining volume is <500ul, then one aliquot is frozen. The first aliquot may be stored in the original tube as received from IUGB while second aliquot is transferred by pipet to a new pre-labeled 2ml microtube (Dot Scientific T332-7S) working in the laminar flow hood. Both tubes are labeled with appropriate barcode label printed on THT-68 labels.

References:

1. DNAstable® Handbook. March 2013. Biomatrica®. www.biomatrica.com.

2. DNAstable® Sample Stabilization and Recovery Quick Reference Protocol. January 2012. Biomatrica,Inc.

3. Nievergelt CM, Maihofer AX, Shekhtman T, et al. Inference of human continental origin and admixture proportions using a highly discriminative ancestry informative 41-SNP panel. *Investig Genet*. 2013;4(1):13. Published 2013 Jul 1. doi:10.1186/2041-2223-4-13

Electronic Resources:

A. https://www.biosearchtech.com/support/education/kasp-genotyping-reagents/kasp-overview