ST-PETERSBURG STATE UNIVERSITY
THEODOSIUS DOBZHANSKY CENTER FOR GENOME BIOINFORMATICS

GENOME RUSSIA PROJECT

BLOOD SAMPLES COLLECTION, DNA EXTRACTION AND DNA QUALITY CONTROL PROTOCOLS

2015

The object of the blood samples collection is the family trio, i.e. two biological parents and their full aged child. Ideally, we are aiming to collect blood samples from about 20 trios (60 individuals) from each geographic location under study.

The requirements for the selection of study participants

All members of the trio should be:

1. Age not younger than 18 years.
2. Healthy: at the time of sampling should not have serious chronic illnesses (according to the participants, the diagnosis is not required).
3. Ethnically homogeneous, originate from a particular region, including grandparents on both sides.
• In case of doubt or lack of information about at least one of the ancestors of the family cannot be included in the study
4. The members selected for the study of family trio should be biological relatives
• both parents in the trio must be the biological parents of the child
5. Selected to the study families should not have a family relationship between trios
• the member of the family under study must not have parents, children, sisters, brothers, grandparents, cousins, aunts and uncles in other selected for sample collection trios.
6. All participants must provide the information necessary to complete the questionnaire and sign the free-will informed consent.

Collection of blood into the vacuum tube

Blood sampling is performed by a qualified health care professional in a proper facility in accordance with all rules and regulations.

1. Before blood sampling the vacuum tube must be signed in accordance with the established marking of specimens.

2. The blood sample (10 ml, for rural places 5 ml) is collected into the vacuum tube (included in the kit along with the needle and holder) according to the instructions for use.

3. Immediately after collection, the blood sample should be mixed with buffer by turning tubes (10 times) softly upside-down for even distribution of the anticoagulant (K3 EDTA coated on the wall of the tube) in the sample.

If the sample is collected in the rural place go to 5. For cities go to 4.

4. The vacuum tube should be stored at + 4 ° C (+ 2 ° C to 8 ° C) and transported **for not longer than 24 h.**

5. 7 ml of the blood sample must be transferred as quickly as possible in the 7 ml transport tube containing TES buffer
• before transfer to the tube containing **TES buffer the sample can be stored at + 4 ° C** (+ 2 ° C to 8 ° C) for not more than 4 hours

• **Do not freeze the samples!**

6. After collection of the sample it is necessary to make the appropriate notice in the logbook.

Transport in the tube with TES buffer

• 15 ml plastic transport tube (included in the kit) contains 7 ml TES buffer (100 mM Tris, pH 7,5; 100 mM EDTA, 2% SDS)
• Before transfer of blood sample ensure that there is no foam in the transport tube containing TES buffer
• Presence of foam in the tube will prevent the introduction of the blood sample
• Cooling of tubes with TES buffer below the temperature + 10 ° C precipitation is possible.
• When mixed in equal volume with blood, TES buffer disrupts blood cells, inactivates enzymes while DNA goes into the solution.
• The buffer protects the DNA while transporting and storing
• **The transport tubes with TES buffer before putting blood sample to them should be stored at room temperature (do not store at temperatures below + 10 ° C)**
• The samples mixed with TES buffer until the transportation should be stored at a temperature between + 2 ° C to + 8 ° C **(do not freeze!)**

Preparation of the samples for shipment

1. Make sure you have supporting documentation (signed informed consent, completed questionnaires and book keeping notes) for samples collected.
2. Ensure tightness (no leakage) of the tubes.
3. Pack the tubes along with the accompanying documentation in a container which protects the samples against mechanical damage and send with the appropriate conditions with transport companies.
• transportation is covered by the Dobzhansky Center

TES BUFFER FOR DNA:

100mM Tris, 100mM EDTA, 2% SDS; pH 7.5

This buffer, when mixed in equal part with fresh blood (collected an anticoagulant, preferably EDTA), will lyse the red and white blood cells but protect the DNA and inhibit any nucleases and microorganisms. This solution is used in field situations where no Centrifugation or refrigeration is available. Once the samples are back in the lab, refrigeration or freezing is recommended for long-term storage.

(1) From stock solutions:	for 100ml	for 500ml
water	50 ml	250 ml
0.5M Tris HCl pH 7.5	20 ml	100 ml
0.5M Na2EDTA	20 ml	100 ml
20% SDS(sodium dodecyl sulfate)	10 ml	50 ml

Appropriate quantities can be dispensed into vials for transport to the study site. Use a large enough vial to allow room to add an equal volume of blood.

(2) From dry chemicals:

Tris base	(MW=121.2)	1.2 gm/100 cc
EDTA Na2	(MW=372.2)	3.7 gm/100 cc
2% SDS		2.0 gm/100 cc

Add water to final volume of 100cc
(The resulting pH will be around 8.0)

Alternatively, the components can be weighed into plastic vials for transport and later mixed with the appropriate amount of water at the study site. Be careful with this because it requires weighing out microgram amounts of each chemical (enough for 2-5 ml/vial).

Reception of blood samples by the laboratory

1. Open the package with care. Remove the boxes containing biological samples and immediately transfer the biological samples into fridges (+4°C).
2. Obtain all relevant documentation
    a. copy of informed consent
    b. questionnaire
3. Enter sample information into the database
4. Send feedback to sender and report any discordance within 1-3 days of sample reception.

Aliquoting of blood samples

Depending on the time required to transport samples to the laboratory, we have two protocols for laboratory sample processing:

- Protocol A. Remote collection in rural areas. Developed for samples with more than 48 hours delivery time. Laboratory receives whole blood mixed with TES buffer in 15 ml tube (7 ml blood + 7 ml TES).
- Protocol B. Local collection in big cities and St-Petersburg. Developed for samples with less than 48 hours delivery time. Laboratory receives EDTA vacutainer tube with 9 ml whole blood.

A. Remote collection in rural areas

Type of biomaterial received by the laboratory:
One 15 ml tube with 14 ml whole blood in TES (7 ml blood + 7 ml TES)
Storage condition before sample processing:
+4°C for up to 2 weeks
Sample processing yield:
14 aliquots blood with TES, 1 ml each, in barcoded 2 ml criotubes
Aliquots storage condition:
Freezing -80˚C for long term storage – 7 tubes for whole blood with TES
Refrigerated +4˚C for several years storage – 7 tubes for whole blood with TES

Protocol:

1. Mix blood in TES solution in 15 ml tube by rotation tube 5 min at 50 rpm in the rotating tube shaker.
2. Print 14 barcoded labels for aliquots in the informatics management system
3. Prepare 14 X 2ml criotubes for each sample with printed labels
4. Transfer the volume of biological sample (1 ml) from 15 ml tube into the empty 2 ml tube by using one tip per sample
5. Place aliquot tubes in the tube rack for storage according the informatics management system data
6. Transfer the biological samples into freezer (-80°C) and refrigerator (+4°C)
7. Clean the bench with a disinfectant

B. Local collection in big cities and St Petersburg

Type of biomaterial received by the laboratory:

One 9 ml vacutainer tube with 9 ml whole blood with EDTA

Storage condition before sample processing:

+4°C for up to 24 hours

Sample processing yield:

Whole blood - 4 aliquots, 1 ml each, in barcoded 2 ml criotubes

Whole blood with TES - 10 aliquots, 1 ml each (0.5 ml blood in 0.5 ml TES), in barcoded 2 ml criotubes

Aliquots storage condition:

Freezing -80˚C for long term storage – 4 tubes for whole blood and 4 tubes for whole blood with TES

Refrigerated +4˚C for several years storage – 5 tubes for whole blood with TES

Refrigerated +4˚C for DNA extraction - 1 tube for whole blood with TES

Protocol:

1. Print 4 barcoded labels for whole blood aliquots freezing and 10 barcoded labels for blood in TES aliquots freezing in the informatics management system
2. Prepare 14 X 2ml criotubes for each sample with printed labels (4 + 10)
3. Whole blood aliquots freezing. Transfer whole blood aliquots (1 ml) from the vacutainer tube into 4 empty criotubes by using one tip per sample
4. Whole blood in TES aliquots freezing. Add 0.5 ml TES into each empty criotube (x10). Transfer whole blood aliquots (0.5 ml) from the vacutainer tube into criotubes with TES by using one tip per sample. Carefully invert tube 8 -10 times to ensure the mixing of the sample and the lysis buffer inside the tube.
5. Place aliquot tubes in the tube rack for storage according the informatics management system data
6. Transfer the biological samples into freezer (-80°C) and refrigerator (+4°C)
7. Clean the bench with a disinfectant

DNA extraction

1. Print 1 barcoded label for tube with DNA in the informatics management system, place label on the tube
2. Extract DNA from 1 ml aliquot of whole blood in TES according MagCore or Qiagen QIAamp DNA Blood Midi Kit protocols.
3. Elute DNA in 200 μl ddH20 to the labeled tube
4. Aliquot 12 μl in 96 well plate for quality control
5. Store DNA sample and aliquot at -20˚C before quality control

DNA quality control

1. Evaluate DNA concentration by Qubit BR dsDNA protocol with 3 μl DNA
2. Evaluate DNA purity 260/280 ratio by Nanodrop with 2 μl DNA
3. Evaluate DNA integrity by electrophoresis in 0.8% agarose with 5 μl DNA
4. Enter concentration, purity and integrity and volume data into informatics management system
5. Calculate total DNA quantity in the sample.
6. If DNA quantity > 10 μg, DNA purity 260/280 ratio is between 1.8 and 2.0, and DNA integrity is good, QC is passed, go to DNA transport sample preparation and storage step. Else, QC failed, repeat DNA extraction step.

DNA transport sample preparation and storage

1. Print 1 barcoded label for DNA transportation tube in the informatics management system, place label on the tube.
2. Calculate DNA solution volume corresponding 2 μg DNA
3. Aliguot calculated volume of DNA solution to the labeled tube.
4. Precipitate and dry 2 μg DNA aliguot in transportation tube
5. Place main DNA sample to -80˚C freezer for a long-term storage according the informatics management system sample location data
6. Place ready for transportation 2 μg DNA sample to -20˚C freezer according the informatics management system sample location data