Procedure to extract DNA from peripheral blood with FlexiGene DNA kit (Qiagen) and genotyping genomic DNA.

Neuropsychiatric Disease and Treatment

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ABSTRACT

To describe the procedure to extract DNA from peripheral blood with FlexiGene DNA kit (Qiagen), for genotyping studies.

1. **Extract DNA from buffy coat (FLEXIGENE KIT (Qiagen), follow basically manufacturer's instructions).**
2. **Quantification genomic DNA**
3. **Genotyping genomic DNA**

EXTERNAL LINK

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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Protocolos.io Escamilla et al.docx

PROTOCOL STATUS

Working

GUIDELINES

Follow procedures as demonstrated in the Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th Edition https://www.cdc.gov/biosafety/publications/bmbl5/bmbl.pdf

MATERIALS

NAME	CATALOG #	VENDOR
Isopropanol		
Microcentrifuge Tubes		
Filter Tips		
ethanol		
TE buffer		
water		
Incubator		
Microtubes 1.5ml with screw caps		
Pipette Tips		

NAME	CATALOG #	VENDOR
10µl Pipette Tips	022491504	Eppendorf
Micropipetes tips		
barrier pipette tips		
optical multiwell reaction plates and adhesive fi lm covers		
Optical 96-well Reaction Plates		
Microcentrifuge		
Vortex		
Refrigerator		
Molecular Biology Grade Water	51200	Lonza
FlexiGene DNA Kit (250)	51206	Qiagen
TaqMan SNP Genotyping Assays	4362691 (COMT)	Applied Biosystems
TaqMan SNP Genotyping Assays	4351379 (DRD2)	Applied Biosystems
TaqMan SNP Genotyping Assays	4351379 (DR3)	Applied Biosystems

SAFETY WARNINGS

Wear appropriate personal protective equipment (gloves and lab coat).
Follow procedures as demonstrated in the Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th Edition https://www.cdc.gov/biosafety/publications/bmbl5/bmbl.pdf
Samples may contain infectious agent(s). You should be aware of the health hazard presented by such agents and should use, store, and dispose of such samples in accordance with the required safety regulations.

BEFORE STARTING

Wear appropriate personal protective equipment (gloves and lab coat).

1 1) Extract DNA from buffy coat (FLEXIGENE KIT (Qiagen), follow basically manufacturer's instructions)

a) Obtain 5 ml of peripheral blood in vacutainer tubes with EDTA from each patient.

2 b) Centrifuge whole blood at 2000 x g for 10 minutes at 4°C, to obtain approximately 500 µl the leukocyte-enriched fraction (buffy coat).

3 c) Transfer the buffy coat into a 2 ml O-ring Tube with the help of a disposable 3 ml transference pipette.

4 d) Pipet 1250 µl Buffer FG1 to each tube with buffy coat and mix by inverting the tubes 5 times.

5 e) Centrifuge 20 sec. at 10,000 x g in a fixed-angle rotor microcentrifuge.

6 f) Discard the supernatant and leave the tube inverted on a clean sheet of absorbent paper for 1 min, taking care that the pellet remains in the tube.

g) Add 500 µl Buffer FG2/QIAGEN protease (solution FG2/protease) close the tube and vortex immediately until the pellet is completely

homogenized.

h) Centrifuge tube briefly (3-5 s), place it in heating block and incubate at 65°C for 10 minutes.

i) Add 500 µl Isopropanol (100%) and mix thoroughly by inversion until the DNA precipitate becomes visible as threads or a clump.

j) Centrifuge for 3 min at 10,000 x g.

k) Discard the supernatant and briefly invert the tube onto a clean piece of absorbent paper for 1 minute, taking care that the pellet remains in the tube.

l) Add 500 µl 70% ethanol and vortex for 5 seconds to re suspend the pellet.

m) Centrifuge for 3 min at 10,000 x g.

n) Discard the supernatant and leave the tube inverted on a clean piece of absorbent paper for at least 1 minute, taking care that the pellet remains in the tube.

o) Ethanol is completely removed using a sample concentrator, where the samples remain in vacuum at 65°C for 15 minutes.

p) Add 200 µl the solution TE ((10 mM Tris pH 8.0, 0.1 mM EDTA) and incubated for 1 hour at 65° C and stored at 4° C until quantified.

2) Quantification genomic DNA

a) DNA quantification was done using a NanoDrop 2000 Spectrophotometer

b) We realized two independent measurements 2 ul each and obtain the media for each sample. 260/280 and 260/230 ratios were analyzed in combination with overall spectral quality. DNA concentration was obtained as ng/ul, 260/280 ratio was ~1.8 and a 260/280 ratio was of ~2.3 for each DNA sample.

3) Genotyping genomic DNA. We used an ABI 7500 DNA detection system (Applied biosystems).

a) Create a new experiment using the Design Wizard in the 7500 software.

b) Prepare for the run by opening the example experiment file you created in allelic discrimination.

c) Load the plate into the plate holder in the instrument. Ensure that the plate is properly aligned in the holder. Lace the reactions in the precision plate holder.

d) Perform an initial review of the experiment results in the Allelic Discrimination Plot, which contrasts the normalized reporter dye fluorescence (Rn) for the allele-specific probes of the SNP assay.

e) The following protocol: initial desnaturation at 94°C for 15 minutes, followed by 40 cycles of 94°C for 20 seconds and 60°C for 1 minute.

For quality control, the experimenters were completely blind to the case and control status and 10% of the samples were genotyped again to test the reliability and stability of results.

24 f) Verify that the Allelic Discrimination Plot displays: Clusters for the three possible genotypes (Allele 1 homozygous, Allele 2 homozygous, and Allele 1/2 heterozygous) A cluster for the negative controls.