EuroDSD

Standard Operating Procedure WP2 Identification of novel genetic markers for DSD

DNA extraction for array CGH procedures and control samples

We prefer that DNA is extracted from 10 ml of blood in tubes with EDTA. For children this may be reduced to 1 ml. For the DNA extraction any method that provides DNA of high quality and quantity is suitable and we recommend the « salting out » extraction procedure described by Miller SA, Dykes DD, Polesky HF. (A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988 16:1215). This procedure can be performed using the PAXGENE blood collection tubes (Qiagen) and PAXGENE DNA extraction kit (Qiagen). This system reliably provides DNA of good quality and quantity for CGH analysis.

We need a minimal concentration of 100 ng/µl of DNA, although higher concentrations (300-500 ng/ µl) are preferable. The total amount of DNA should be in the range of 1 to 5 micrograms. The DNA must be of good quality. We require that the DNA sample has an OD 260/280 ratio of 1.7 or greater and an OD 260/230 ratio of 1.7 or greater. A small aliquot of genomic DNA should be migrated on a 0.8% agarose gel to verify that there is no degradation. DNA should be provided in standard TE-buffer.

Control or reference DNA can be provided by each of the three centers performing the CGH analysis. Control DNA may be from a single individual but preferably should consist of a pool of either male or female DNA from several individuals. In some special situations control DNA may be provided by the clinical centre to the CGH platform. This could arise in a situation where gene number changes would be compared between pathological tissues (gonad tissue) and normal tissue (blood lymphocytes) from the same individual with a DSD phenotype.

In exceptional circumstances the CGH analysis may also be performed on DNA extracted from paraffin-embedded tissues. In this case we would request that a control DNA sample is provided, extracted from paraffin embedded tissue using the same extraction technique. Long term storage of DNA should be performed at -20°C or -80°C.

Shipment of Samples

Only an aliquot of the original genomic DNA should be pipetted into a new “Eppendorf” vial.

Samples should preferentially be shipped on dry or wet ice (e.g. on “cool-packs”).

EuroDSD ID and the name of the submitting institution (e.g., Rotterdam) but no patient name should also be submitted along with the DNA sample to allow for later contact information.

Samples from Germany, Holland and Sweden should be shipped to –

Prof. Dr. med. Peter Wieacker or Dr. rer.-nat. Susanne Ledig
Westfalian-Wilhelms University
Institute of Human Genetics
Vesaliusweg 12-14
48149 Münster
Germany

Samples from the UK should be shipped to –

Dr John C Achermann
Developmental Endocrinology Research Group
Clinical & Molecular Genetics Unit
UCL Institute of Child Health
30 Guilford Street
London WC1N 1EH

Samples from France and Italy should be shipped to –

Dr Ken McElreavey
Human Developmental Genetics
Institut Pasteur
25 rue du Dr Roux
75724 Paris Cedex 15
France