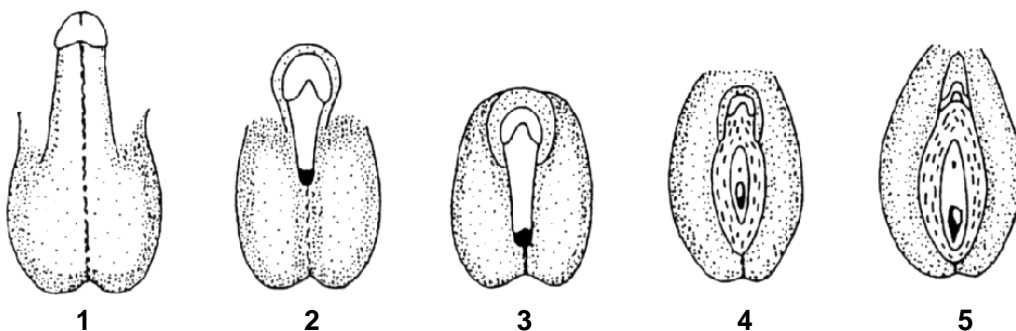


Androgen Memory / Epigenomics Preparation, storage and shipment of fibroblast genomic DNA

- (Culturing of cells is only recommended, if no genomic DNA exists at the site of the sender (protocol see below). The most important predisposition of the project is a very good documentation of the **site of biopsy** since only **labial, labioscrotal** or **scrotal** skin fibroblasts can be used. In particular, foreskin derived fibroblasts are excluded. There is no specific protocol for cell culture except that **confluent fibroblasts** should be harvested for DNA preparation. (*an example protocol can be found below*)
- WP4 only needs fibroblast DNA of samples with the diagnosis **AIS** and with **proven mutations of the androgen receptor gene**.
- For DNA-preparation, we recommend the **Qiagen DNA-preparation kits** (e.g. Genra Puregene Tissue or QIAamp DNA Kits) or any other method that provides DNA of high quality and quantity. We need at least 2 µg of genomic DNA. It is better to have **5 µg of genomic DNA** to enable control experiments, e.g. by Pyrosequencing. The DNA concentration should not be significantly below 100ng/µl. We prefer DNA samples showing an OD 260/280 ratio of 1.7 or greater and an OD 260/230 ratio of 1.7 or greater. If possible, a small aliquot of genomic DNA should be analysed on a 0.8% agarose gel (or by any other appropriate technique) to exclude DNA degradation. Long term **storage** should be performed at **-20°C or -80°C**.
- 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")**.
- The **phenotype** should be indicated according to the **scheme of Sinnecker (1996)**



- **Local 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 retrieval of patient data. The above mentioned new vial (see #4) should be named accordingly.
- In addition, **age at biopsy** and the **passage number** should be indicated, if known.



- **Shipment address is:**
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Example for a fibroblast culturing protocol (taken from a previous manuscript):

Fibroblasts were cultured on 150mm plastic dishes at 37°C with 5% CO₂. To eliminate possible artifacts due to differing states of proliferation, cells were grown to confluency wherein they enter G₀ arrest. They were maintained in phenol-red-free DMEM F12 (Dulbecco's modified Eagle Medium with the nutrient mix F12, Gibco) containing L-glutamine, 15mM Hepes buffer, penicillin / streptomycin (Gibco) and 12.9% of a constant lot of certified fetal calf serum (FCS, Gibco). The pH was adjusted to 7.4 with 1N NaOH and the media was exchanged every 48h. At day 13, the last media exchange was performed and 96h later cells were scraped and mRNA harvested directly.