

Course Summer School 2021

Sample handling

DNA & RNA isolation

Qubit fluorometer

Sample handling

- Clinical samples (important - longterm collecting)
 - on ice, well-marked (number, date, sample, experiment)
 - no names but numbers
 - excel sheet with all possible clinical data (sex, age, BMI...)
 - Usually come in tubes – best is to put them into the plate and make aliquots, make patterns
 - Handle with care – they are precious!
 - Avoid repetition of freezing and unfreezing

Sample handling

- Plasma
 - EDTA – for ecDNA
 - Heparin – for DNase activity, for Biolis

EcDNA and DNase activity

- Plasma, serum, urine, saliva, medium, other fluids...
 - Always centrifuge 1600g 10min, take supernatant a centrifuge 16000g 10 min, 4 degrees, work with supernatant
 - Direct isolation of DNA and DNase activity measurement if possible (without freezing) – always consult with your advisor
- Aliquots (microplates, deepwells), avoid tubes
- Upcoming analysis -20 degrees
- Storage of samples -80 degrees
- Store but save place! – store in plates, properly sealed, do not loose pattern
- Write project summary

Isolation of nucleic acids

- Always discuss the methodology with your advisor beforehand

Isolation of DNA

- Solution based
 - Phenol-chloroform
 - Salting out methods (ethanol, isopropanol)
 - (use flow box)
 - Download the protocol from the internet
 - Widely used method
 - Good for isolation from big amount of tissues

Isolation of DNA

- Solid based
 - Magnetic beads
 - Anion exchange methods
 - Silica resin methods
 - also Qiagen kit is based on this

Isolation of DNA

- Commercial kits – columns (up to 30 samples at once) or plates (2 full plates minimum)
 - Qiagen kit in our lab
 - Always follow the protocol, do optional steps (varies according to the sample type) – you can download it from the webpage
 - Elution volume could be changed according to our needs of yield
 - Do not contaminate!
 - QiaCube is an option (12 samples in one hour)
 - Better for bodyfluids and small samples of tissues

Isolation of RNA

- Solution based
 - Phenol-chloroform
 - Salting out methods (trizol, ethanol, isopropanol)

Isolation of RNA

- Solid based
 - Magnetic beads
 - Anion exchange methods
 - Silica resin methods
 - also Qiagen kit is based on this



Qubit fluorometer

- Based on specific markers for measured substances
- Types of assays
 - High Sensitivity DNA
 - Broad Range DNA
 - RNA
 - Protein
- Follow the protocol
- Be aware of background
- Be careful about RFU of standards

Before your first DNA/RNA isolation

- Always find somebody to help you
 - Baša Konečná
 - Ľubka Janovičová
 - Baška Gromová
 - Or other core IMBM people