Isolation of DNA and RNA (including miRNA) from tissue

DNA and RNA including miRNA are isolated from tissues using the DNA/RNA All prep kit (Qiagen, Hilden, Germany) with a few modifications. Briefly, tissue is lysed in lysis plus buffer using a tissue lyser (Qiagen). DNA is bound to AllPrep DNA spin columns, washed with buffers AW1 and AW2, and eluted with EB buffer. AllPrep DNA spin column flow-through is used for RNA isolation. 1.5 volumes of 100% ethanol are added to the flow-through, RNA is bind to RNeasy mini columns, washed with buffers WT and RPE and eluted with water.

Isolation of RNA (including miRNA) from cells

RNA including mIRNAs is isolated from cells using the miRNeasy Mini Kit (Qiagen) following the manufacturer´s recommendations.

Isolation of miRNAs from serum samples

A combination of phenol/guanidine-based lysis and silicamembrane-based purification is used for the isolation of cell-free RNA from serum samples. Add 1200 µl Tri Reagent to 400 µl serum, voxtex and let incubate for 5min at room temperature. Add 5µl 1:1000 cel-miR-39, -54 and -238 mix (miScript miRNA MIMICs, Qiagen), 6µl 1:10 diluted 20mg/ml glycogen and 320 µl pure chloroform. Invert 6 x and vortex the tube for 15-30s. Let incubate at room temperature for 5min. Centrifuge sample at 12.000 g for 15 min at 4°C. Transfer the aqueous phase (=780 µl) into a fresh 2ml tube. Store samples on ice until centrifuge has reached room temperature. Add 1.5 volume EtOH, vortex and pipet up and down (DO NOT CENTRIFUGE). Add 700 µl sample into a RNeasy Mini spin column (miRNeasy Mini Kit, Qiagen). Centrifuge at 8.000g for 15 s at room temperature and discard flow-through. Add remaining sample on the same column, centrifuge and discard flow-through. Add 700 µl RWT buffer and centrifuge 8.000 g at 15 s at room temperature and discard flow-through. Add 500 µl RPE and centrifuge at 8.000 g for 15 s; discard flow-through. Add 500 µl RPE centrifuge at 8.000 g for 2 min; discard flow-through. Place RNeasy Mini spin column into fresh 2 ml collection tube and dry centrifuge the column for 1min at full speed. Transfer RNeasy Mini spin column to a 1.5ml elution tube and pipet 35 µl RNase free-water directly on the membrane. Incubate 1 min at room temperature, centrifuge for 1min at 8.000 g and store at -80°C.

qPCR-based miRNA screening by TaqMan Low Density Arrays

Total RNA is reverse transcribed to cDNA by priming with a mixture of looped primers and preamplified according to the manufacturer's instructions (Megaplex Pools with Preamplification, Applied Biosystems, Foster City, CA). Subsequently, quantitative real-time PCR amplification of miRNAs using low-density Taqman arrays v2.0 (Invitrogen) is performed using the Applied Biosystems 7900 Sequence Detection System. Cel-miR spike-ins are quantified by single miRNA assays (Applied Biosystems) and are used for normalization. Data filtering and detection of differentially expressed mIRNAs by LIMMA are carried out using custom R scripts.
[Brase et al, Int J Cancer. 2011, 128:608-16.]

Next generation sequencing of smallRNAs

Small RNAs up to 40nt are size-fractionated on a polyacrylamide gel from > 2µg DNase treated RNA (for input amounts <2µg no RNA gel separation is performed). Small RNA libraries are prepared using the NEBNext Small RNA Sample Prep Set (NEB, Frankfurt/M., Germany) as described by the manufacturer, with some modifications (e.g., multiplexing). Amplicons of ~130-150 bp are purified and sequenced on an Illumina HiSeq2000 Instrument (single-read, 50bp) and mapped to the human genome and miRBase using the miRDeep2 package [Friedlander et al., 2012].

For further information contact Daniela Wuttig (d.wuttig@dkfz.de; Tel.: 50-5964)