Collecting DNA from mouse tail

a) Solid Tissues from animal + plant origin (10 mg / 100 mg)

a1) Sample collection and handling

Fresh, frozen, fixed or paraffin-embedded tissue samples as well as mouse tails can be processed with the JETFLEX kit. If tissue samples are to be stored for a longer period of time, they should be stored at –70 to –80°C.

Before entering the JETFLEX procedure, tissue samples are best reduced to small pieces (e.g. by grinding in liquid nitrogen with mortar and pestle). When using fresh tissue samples, keep them on ice until cell lysis buffer (CLB) has been added to minimize any degradation of the DNA by the action of DNases.

a2) Preparation of a DNA-containing lysate from various amounts of solid tissue

1.) Add **up to 10 mg / up to 100 mg** fresh or frozen tissue that was optimally reduced to small pieces into a suitable reaction tube. Keep tube on ice.

When working with plant tissue material, it may be necessary to vary the amount of starting material depending upon species, age, tissue type and genome size. If ever possible, plant tissue samples should be finely ground to disrupt cellular structures before the addition of CLB.

2.) Add **300 µl** (for an initial sample volume of up to 10 mg tissue) or **3 ml** (for an initial sample volume of up to 100 mg tissue) **buffer CLB** to the tissue sample. If the tissue sample wasn't reduced to small pieces, homogenize the sample thoroughly using 30-50 strokes with a suitable tube pestle. Add **20 µl** (for an initial sample volume of up to 10 mg tissue) or **200 µl** (for an initial sample volume of up to 100 mg tissue) proteinase K stock solution (20 mg/ml) and incubate for 1 h to overnight at 58°C until the mixture is fully clear and any tissue structure is fully desintegrated.

CLB will disrupt the cellular structure of the tissue cells and nuclei, thus setting free the genomic DNA.

3.) **OPTIONAL RNase treatment:** Add **10 µl** (for an initial sample volume of up to 10 mg tissue) or **100 µl** (for an initial sample volume of up to 100 mg tissue) of RNase solution (4 mg/ml; provided with the kit) to the lysate of step 2 and incubate at 37°C for another 5 min.

4.) Go ahead to procedure b (Preparation of DNA from nuclear lysates).

b) Preparation of DNA from nuclear lysates

1.) Before processing any cellular lysate from one of the procedures under ‚a)' any further, make sure that it has reached **room temperature** again.

2.) For each **300 µl (3 ml)** of cellular lysate from one of the procedures of part ‚a)' add **150 µl (1.5 ml)** of **protein precipitation buffer PPT**. Mix thoroughly by vortexing for 20 seconds to obtain a homogeneous suspension.

3.) Centrifuge at 13.000 – 16.000 x g for 3 min at room temperature.

The precipitated proteins should form a compact pellet. The supernatant should be clear.

3a) OPTIONAL: The JETFLEX system is designed as a general purpose device to isolate DNA from a multitude of sources. So if the resulting protein pellet after treatment with buffer PPT is small or loose and/or shows a tendency to be co-transfered with the supernatant, proceed with one of the following options:
a1) For a volume of **450 µl** of **protein precipitation mix** (= 300 µl of cellular lysate + 150 µl of protein precipitation buffer PPT, see step 2) add **50 µl of ‚Pellet Compactor'** (provided with each kit) and vortex until a homogeneous suspension is obtained.
For higher volumes of protein precipitation mix the amount of ‚Pellet Compactor' has to be increased proportionally!!!
Then centrifuge at 13.000 – 16.000 x g for 3 min at room temperature. Continue with step 4.
a2) Vortex again and chill the mixture on ice for 5 min. Then centrifuge again for 3 min at 13.000–16.000 x g **in a chilled centrifuge** at 4°C. Continue with step 4.

4.) Transfer the clear supernatant into a fresh tube. Add 1 vol. of isopropanol (2-propanol).

5.) Mix thoroughly by multiple inverting until a homogeneous mixture is obtained. Avoid vortexing.

6.) Centrifuge at 13.000 – 16.000 x g for 3 min at room temperature. The precipitated DNA should be visible as a white pellet.

7.) Decant the supernatant and let residual liquid drain on a sheet of absorbant paper towel for a few minutes. Add **1 ml** (for an initial volume of 300 µl cellular lysate) or **10 ml** (for an initial volume of 3 ml cellular lysate) of **70-80% ethanol** to the tube and wash the DNA pellet by inverting several times.

8.) Centrifuge at 13.000 – 16.000 x g for 1 min. Carefully decant the ethanolic supernatant.

The DNA pellet can sit very loosely at the wall of the tube at this stage. Be very careful not to discard the DNA.

9.) Let residual liquid drain on a sheet of absorbant paper towel for a few minutes. Evaporate residual ethanol by a 10 min incubation at elevated temperature (50-56°C).

One can also allow the DNA pellet to air-dry at room temperature, but it may take significantly longer for all remaining ethanol to fully evaporate.

10.) Add a suitable volume of DNA Dissolving Buffer (TE) to the DNA pellet.

Dissolve the DNA by standing at room temperature overnight. The dissolution process can be enhanced by incubating at 65°C for up to 1 h.

11.) Store the DNA at 4°C. The dissolved DNA should be quantified spectrophotometrically. Pure DNA has a A_{260}/A_{280} ratio of 1.7– 1.9. One OD_{260} corresponds to a DNA concentration of 50 µg/ml. Analysis on an analytical agarose gel should reveal the majority of DNA migrating at a size of ^{3} 50 – 150 kb.

c) Re-purification protocol for still impure DNA's

Pure DNA should dissolve readily in the provided DNA Dissolving Buffer (TE), giving a clear, colorless solution. If the DNA solution still contains insoluble matter, is stained in some way or the A_{260}/A_{280} ratio is <1,70, the DNA is still contaminated with protein or another impurity. This may occur, when e.g. the chemistry of the purification procedure has been overloaded. Still contaminated samples may be further purified easily using the following protocol.

1.) For every **100 µl** of contaminated DNA solution add **500 µl** of **buffer CLB** and mix thoroughly by inverting or pipetting up and down.

If necessary, incubate at 37°C until any particulate matter has dissolved.
IMPORTANT NOTE*: Cool down the sample to room temperature before proceeding to step 2.*

2.) For every **600 µl** of DNA solution/CLB mixture add **300 µl** of **protein precipitation buffer PPT** and vortex vigorously for 20 sec to obtain a homogeneous mixture.

3.) Centrifuge for 3 min at 13.000 – 16.000 x g (or 10 min at 5.000 x g) to pellet any precipitated impurities.

If a pellet is not visible, re-vortex and incubate the sample for 5 min on ice. Then re-centrifuge the sample as described.
If there is still no stable pellet obtained, mix ***900 µl*** *of protein precipitation mix (= 600 µl of cellular lysate + 300 µl of protein precipitation buffer PPT, see step 2) with* ***100 µl of ‚Pellet Compactor‘*** *(provided with each kit) and vortex until a homogeneous suspension is obtained. Centrifuge at 13.000 – 16.000 x g for 3 min at room temperature. Continue with step 4.*

4.) Transfer the supernatant into a clean suitable receptacle without transferring any precipitated particles.

5.) Add 1 vol. of isopropanol (2-propanol) to the transferred supernatant and mix thoroughly by multiple inverting.

The precipitated DNA should become visible as a thread-like matter.

6.) Centrifuge for 1-3 min at 12.000 x g to collect the precipitated DNA at the bottom of the tube. Discard the supernatant and let residual liquid drain from the DNA pellet by placing the tube upside-down on a sheet of absorbant paper towel.

7.) Wash the DNA pellet with a suitable volume of 70-80% ethanol. Centrifuge again for 1-3 min at 12.000 x g to collect the DNA at the bottom of the tube. Discard the ethanolic supernatant.

The DNA pellet may sit very loose at the tube's wall at this stage. Be careful not to discard the DNA.

8.) Let residual liquid drain on a sheet of absorbant paper towel for a few minutes. Evaporate residual ethanol by a 10 min incubation at elevated temperature (50-56°C).

One can also allow the DNA pellet to air-dry at room temperature, but it may take significantly longer for all remaining ethanol to fully evaporate.

9.) Add a suitable volume of DNA Dissolving Buffer (TE) to the DNA pellet. Dissolve the DNA by standing at room temperature overnight. The dissolution process can be enhanced by incubating at 65°C for up to 1 h.

10.) Store the DNA at 4°C. The dissolved DNA should be quantified spectrophotometrically. Pure DNA has a A_{260}/A_{280} ratio of 1.7– 1.9. One OD_{260} corresponds to a DNA concentration of 50 µg/ml. Analysis on an analytical agarose gel should reveal the majority of DNA migrating at a size of ^{3} 50 – 150 kb.