Preparation of DNA

I.Digestion

1. Add 500 µl of Lysis-buffer and 10µl of Proteinase K (10mg/ml) to each tube with tail-cuts (<0,5mm).
2. Put the tubes into a Thermomixer and shake it over night at 56°C.

II. Extraction and purification of DNA

1. Take out tubes of the Thermomixer and centrifuge for 10 min. at 14.000 rpm.
2. Prepare fresh tubes with numbers of samples and add 500 µl Isopropanol to each.
3. Transfer the supernatant of the centrifuged tubes into the tubes with Isopropanol.
4. Mix gently but thoroughly by inverting several times until a flocculent precipitate appears.
5. Centrifuge for 20 min. at 14.000 rpm.
6. Discard the supernatant.
7. Add 400 ml of 70% ETOH to each tube and centrifuge again at full speed.
8. Remove as much of the supernatant as possible by aspiration without disturbing the DNA-Pellet.
9. Add again 400 µl of 70% ETOH to each tube and centrifuge again at full speed.
10. Remove as much of the supernatant as possible by aspiration without disturbing the DNA-Pellet.
11. Dry pellets for 30 min to 1h at RT (headed block or warm room).
12. Add 300 to 600 µl of TE-buffer/H2O to each tube (depends on pellet size) and resuspend (Thermomixer; 56°C at least for 2h).

III. Lysis Buffer:

Chemical	**stock**	**final conc**
TrisHCL pH8,0	1M	50mM
NaCl	5M	100mM
SDS	20%	1%
EDTA	0,5M	100mM

Add EDTA at last !