Genomic DNA Extraction

- **CTAB Extraction Buffer**

Stock	**To 100ml**	**To 200ml**	**To 300ml**	**To 400ml**	**To 500ml**
H2O	73	146	219	292	365 ml
1M TRIS-HCl pH 7.5	10	20	30	40	50 ml
0.5M EDTA pH 8.0	2	4	6	8	10 ml
5M NaCl	14	28	42	56	70 ml
CTAB (1%) add just before use	1	2	3	4	5 g
BME (1%) add just before use	1	2	3	4	5 ml

- Turn on the water bath @ 65 C
- Weight 700mg of liophylized tissue (preferable young), grind it with sand to a fine powder . Fill a 15 ml Falcon Tube until 1,5 ml mark.
- Add 9 ml of EXTRACTION BUFFER . Homogenize to avoid formation of tissue clumps in the tube
- Incubate @ 65 C for 1- 1,5 h homogenizing it every 15' ;
- Take it out and let it cool down for 10'
- Add 5 ml of Chloroform:Octanol (24:1), Invert the tube gently for 5'
- Spin @ 2-3000 rpm for 10'
- Pour off the aqueous supernatant into a new 15 ml Falcon tube ;
- Add 50 ul of RNAse A (10mg/ml) , invert few times and incubate @35 C for 30';
- Add 6 ml of cold isopropanol (see spindle), invert tube few times and hook the DNA precipitated with a glass hook
- Transfer to a disposable tube containing 2 ml of 76% **ETOH + 0.2M NaOAc** for *20'*
- Rinse the DNA in the hook briefly in a microfuge tube containing **ETOH + 0.2M NH_4OAc** ;
- Let the DNA dry in the hook for 10-20';
- Transfer the DNA to a microfuge tube containing 400ul of TE
- Dissolve the DNA overnight using the rocker @ 4 C;
- Next day, centrifuge for 10' to compact solids.
- Remove supernatant to a new microfuge tube and determine the concentration !
- Take 2ul in 998ul H2O , OD @ 260nm x 25 = [ ] in ug/ul
- Adjust the final concentration to 2,5 ug/ul for southern and 50ng/ul for PCR