

Tissue: alcohol fixed, remove all alcohol, some little residue may remain

Buffer 1x CTAB: 0.7 M NaCl, 10 mM EDTA, 50 mM Tris pH 8.0, 1% hexadecyltrimethylammonium bromide CTAB

DNA number	sample number	species	locality	dissolved in $\mu$ l TE	yield ng/ $\mu$ l	ABI reactions
1143	136b	<i>Aegina</i>	<i>citrea</i>	Friday Harbour	400	>10
1144		<i>Ptychogastria</i>		Antarctica	400	>10
1145	Vfr16.01	<i>Oceania</i>	<i>armata</i>	Villefranche	400	>10
1146	Vfr16.02	<i>Oceania</i>	<i>armata</i>	Villefranche	400	>10
1147	Vfr16.03	<i>Oceania</i>	<i>armata</i>	Villefranche	200	10
1148	Vfr16.04	<i>Oceania</i>	<i>armata</i>	Villefranche	400	10
1149	Vfr16.05	<i>Geryonia</i>	<i>proboscidalis</i>	Villefranche	400	>10 dilute 2X
1150	Vfr16.08	<i>Cunina</i>	<i>octoraria</i>	Villefranche	200	<0.5
1151	Vfr16.09	<i>Laodicea</i>	<i>undulata</i>	Villefranche	200	5
1152	Vfr16.11	<i>Zanclaea</i>		Villefranche	300	5
1153	Vfr16.14	<i>Helgicirrho</i>	<i>schulzii</i>	Villefranche	300	5
1154	Vfr16.15	<i>Zanclaea</i>		Villefranche	300	10

- add CTAB 1x buffer <sup>300</sup> 200  $\mu$ l (=1 volume)
- add 10  $\mu$ l Proteinase K 10 mg/ml
- 55°C from 17.30... to 08.30
- add 1 volume of chloroform+isoamylalcohol (=3-Methyl-1-butanol) 24+1, vortex vigorously
- centrifuge 5 min. max. rpm
- upper phase in new eppentube, avoid any contamination with the interphase or lower phase
- add 1  $\mu$ l linear acrylamide 25 $\mu$ g/ $\mu$ l (Sigma GenElute)
- add 2 volumes of ethanol 100%, mix
- 15 min centrifugation at max. rpm
- pipet out liquid, gently wash with 0.2 ml 70 % ethanol
- dry DNA at 55°C 5min
- dissolve in TE buffer as given above (normally 100  $\mu$ l)
- 8  $\mu$ l on gel

100bp ladder 200ng

1143 44 45 46 47 48 49 50 51 52 53 54

