Procedure: cccDNA and Total HBV DNA from liver tissue

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Material:

NP-40-Lysis buffer:	50 mM Tris-HCl (pH 8) 1 mM EDTA 0.2 % NP40 0.15M NaCl
Proteinase K:	20mg/ml in H_20 store at 4°C
Alkaline/SDS:	6 % SDS, 0.1 N NaOH
3M KAc pH 4.8:	60ml 5M KOAc add acetic acid until pH ± 5.8 (or 1:10 dilution is 4.8) fill with H_20 to 100ml
acid Phenol:	H_20 saturated Phenol pH 4.5
Phenol:	H_20 saturated and buffered Phenol pH ≥ 6
Phenol/Chloroform:	1:1 mixture of buffered Phenol and Chloroform or commercial mixture
Chloroform:	standard quality
salt:	3M NaAc pH 7
isopropanol:	standard quality
1 x TE:	10mM Tris-HCl (pH 8) 1 mM EDTA

Method:

cccDNA extraction

add 5.5 ml lysis buffer into 14ml round bottom snap cup tube (place on ice)
break off 100-150mg liver tissue and add to the lysis buffer
homogenize with motorized tissue grinder
transfer 500μl homogenate into 2ml Eppendorf tube (-> total DNA extraction)
add 5ml Alkaline/SDS buffer to the homogenate and mix thoroughly

Incubate at 37˚C for 30min. mix occasionally
add 2.5ml 3M KaAc pH 4.8. Invert several times to mix.Thick precipitate forms.
centrifuge at 4˚C for 15min at 10,000rpm in SW-6 rotor in Sorvall centrifuge
transfer supernatant into 50ml conical Falcon tube (use gauze to filter if necessary)
extract with 1 volume **acid** phenol
centrifuge at RT for 20min at 4,000rpm in table top centrifuge
reextract with Phenol/Chloroform
reextract with Chloroform
transfer supernatant into new 50ml conical Falcon tube
add 2.5 volume EtOH and mix well
precipitateat -20˚C or -70˚C
centrifuge at 4˚C for 30min at 4,000rpm in table top centrifuge
wash pellet
let completely dry
dissolve in 100μl TE
load 10-30μl corrected for OD from total DNA on Southern

<u>total DNA:</u>

add 25μl 20%SDS and 10μl 0.5M EDTA to the 500μl homogenate (from above)
add 25 μl proteinase K
mount ED-holder on rocker and incubate over night at 37˚C
add 500μl Phenol and shake vigorously (vortex)
spin 10 min 14k at RT
carefully transfer supernatant into new 1.7 ml tube and add 500 μl Phenol/Chloroform
mix vigorously (vortex)
spin 10 min 14k at RT
carefully transfer supernatant into new 1.7 ml tube and add 500 μl Chloroform
mix vigorously (vortex)
spin 10 min 14k at RT
carefully transfer supernatant into new 1.7 ml tube containing 55 μl 3M NaAc
add 600 μl Isopropanol
mix vigorously
precipitate nucleic acids at -20˚C for 1h or over night
pellet nucleic acids at 14k, 4˚C for 25 min
discard supernatant
wash pellet with 80% EtOH
wash pellet with 100% EtOH
air dry pellet

dissolve in 200μl TE
measure OD

Critical parameters and Troubleshooting: