Preparation of Genomic DNA – FUNGI

-Grow up fungal cultures, Petri dish for ~3days, o/n ~200ml shake culture, …

-Squeeze mycelial pad into compact mass or filter culture

-Chill mortar containing ~200mg glass beads(100-200um) and a pestle with liquid nitrogen

-Add compressed tissue sample to cold mortar, fill with liquid nitrogen

-Immediately after the liquid nitrogen evaporates, grind tissue into a fine powder with cold pestle

-Promptly scoop ~0.4ml of the fine powder into a 2ml microcentrifuge tube

-Add 0.4ml phenol and 0.8ml H-Buffer, quickly thump into suspension

-Incubate suspension at 60 C for 5min

-Cool on ice, then add 0.4ml chloform:IAA (24:1 v/v)

-Centrifuge for 1min at maximum

-Pull off 0.7ml of aqueous layer and transfer to a fresh 1.5ml microcentrifuge tube

-Add 0.7ml chloroform:IAA , mix and centrifuge 1min at max again

-Pull off 0.5ml aqueous layer and transfer to a fresh 1.5ml tube

-Add 50ul of 3M NaOAC and mix gently

-Add 1ml of 95% EtOH and mix gently

-Place in -20C freezer for 45min to precipitate

-Centrifuge for 10min at max

-Pour off EtOH and wash pellets with 500ul 70% cold EtOH, spin 5min

-Decant EtOH and dry pellets (do not overdry as pellets will not resuspend) and bring up in 50-100ul of TE-8 + RNAse A (10mg/ml, 1ul/50ul TE-8) + (optional) Preteinase K (33ug/ml).

-Incubate genomic preps for 30min to overnight at 37 C

-Add 400 ul of TE and 500 ul of PCI, mix + spin at max for 1min

-Pull off 400ul of aqueous layer to new tube; add 40ul of 3M NaOAC+ 800ul 95% EtOH

-Precipitatae ~15min in -20C freezer

-Spin 10min at max

-Wash with 500ul of EtOH, spin 5min

-Decant EtOH, dry and resuspend in TE-8

-Measure in UV spec to get concentration and run on 0.8% TAE gel to determine quality

-Store genomic preps at 4 C

Fungal Lysis H-Buffer:		
250µL	8.0M	LiCL
800µL	0.25M	EDTA
200µL	1M	Tris (pH 7.5)
500µL	20%	SDS
18.25mL	Distilled	H_2O
20mL		