GENOMIC DNA ISOLATION

Spin down 75 mL culture at 1000xg, 4ºC, 10 min.

Resuspend in 10 mL of Starvation Buffer and transfer to a 15 mL tube.

Spin at 1000xg, 4ºC, 10 min.

Resuspend in 5 mL ice cold TE.

Add 250 uL of 30% sarkosyl (in water).

Mix and add 5 mL phenol. Mix by inversion several times.

Spin 4000 rpm at 4ºC for 1 hour.

Transfer top layer to a new tube containing 5 mL of phenol/chloroform/isoamyl alcohol.

Mix by inversion.

Spin 4000 rpm at 4ºC for 45 minutes.

Transfer top layer to a new tube containing 5 mL of chloroform. Mix by inversion.

Spin at 4000 rpm at 4ºC for 30 minutes.

Keep top layer and add 25 uL of 10 mg/mL RNAse A and incubate for 1 hour at 37ºC.

Store overnight at -20ºC.

Add 25 uL of 10 mg/mL proteinase and incubate at 37ºC for 45 minutes.

Repeat phenol, phenol/chloroform/isoamyl alcohol, chloroform extractions.

Keep top layer and add 750 uL of 10M NH_4Ac and 1.25 mL EtOH.

Spin at 10000 rpm, 4ºC, 30 minutes.

Wash with 5 mL 70% EtOH.

Spin at 10000 rpm, 4ºC. 30 minutes.

Air dry and resuspend in 250-500 uL TE depending on the size of the pellet.