MNase digestion of chromatin

10x phasing buffer (1mL)

200 uL 1M Tris

50 uL 1M DTT

10 uL 100 mg/mL BSA

2 uL 0.5 M EDTA

200 uL 5 M KCl

40 uL 1 M MgAc

498 uL H2O

MNase digestion (25 uL)

2.5 uL 10x MNase buffer (NEB)

1.5 uL 10x phasing buffer

1 uL 5U MNase

20 uL lambda chromatin assembly

10 min, 37deg water bath

syringe filter and use immediately, do not save

Phenol-chloroform extraction

100 uL phenol-chloroform

vortex vigorously

spin down

remove aqueous phase – I try to pipette at least 18 uL

add 200 uL chloroform

vortex vigorously

spin down

remove aqueous phase – I try to remove at least 15 uL

add 5 uL (for 15 uL aqueous phase) 50% glycerol

Run digests on a 1% agarose gel, 1X TAE, room temp, ~120V

I run the no-SDS purple loading dye (ladder lane only) until the pink band is 1/3 of the way to the end of the gel