Chromatin phasing

1x phasing master mix

20 uL 250 mM DTT

100 uL 1 mg/mL BSA

20 uL 5 mM EDTA

50 uL 0.5 M Tris-HCl

100 uL 1.25 M KAc

200 uL 25% glycerol

100 uL 20 mM MgAc

360 uL H2O

10x phasing buffer (1mL)

250 uL 1M Tris-HCl

50 uL 1M DTT

10 uL 100 mg/mL

2 uL 0.5 M EDTA

250 uL 5M KAc

20 uL 1M MgAc

400 uL 100% glycerol

18 uL H2O

Wash buffer (10 mL)

100 uL 1M Tris-HCl

10 uL DTT

H2O to 10 mL

Filter all buffers and the 100 mg/mL BSA stock

Make BSA, DTT, and Silane all fresh as well as all buffer

For zero salt conditions, add H2O in place of KAc and MgAc

Wash and block 96-well plate

1. 120 uL 1:1:1:: EtOH:MeOH:IPA, 2x, dry ~30 min
2. 70 uL 20 mg/mL Silane in MeOH, room temp, 15 min
   1. protect from light from this point on
3. Wash 3 x 120 uL 100% MeOH
4. Block with 100 uL 100 mg/mL BSA, room temp, 15-30 min, in the dark
5. Wash 3 x 120 uL Wash buffer
   1. Remove entire volume the first two washed (pipette out 110 uL)
   2. Leave 5 uL in well after removal of last wash volume (pipette out 115 uL)
6. Add phasing reaction immediately

Phasing reactions (50 uL)

29.5 1x master mix

2 uL 1:1 assembly ratio 2 uM (601-sites) assembly 2.5 uL (0.8:1) 2.86 (0.7:1)

18.5 uL H2O 18 uL H2O 17.64 uL H2O

For the higher concentration assemblies (12mer), the amount added is such that the octamer concentration is constant (here: 80 nM octamer)

30 uL 1x Master mix

20 uL 0.2 uM (601-sites) assembly

For the lower concentration assemblies (lambda), the volume is limiting when using the 1x master mix (here, 80 nM 601-sites). If using the 10x phasing buffer, the volume of assembly added can be titrated to keep the concentration of nucleosomes constant.

Phasing reactions with Snf2h (50 uL) – pg 147, notebook 2

10.5 uL H2O 28.5 uL H2O

10 uL Tris-HCl pH 7.5

3.5 uL 1M KAc

2 uL 50 mM Mg +ATP

2 uL 75 mM MgAc

2 uL 24.3 uM Snf2H (from MK)

20 uL lambda assembly (0.2 uM 601-sites) 2 uL 2uM 601-site assemblies

For assemblies with wildtype octamer, 5 uL 100 nM Yoyo-1 is added to each well to stain DNA