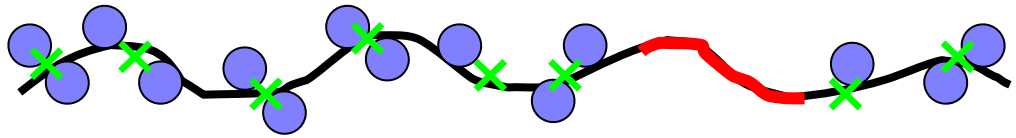
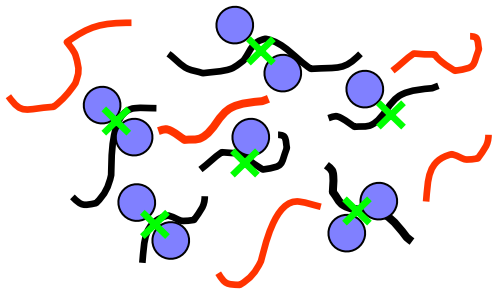
Formaldehyde Assisted Isolation of Regulatory Elements (FAIRE)

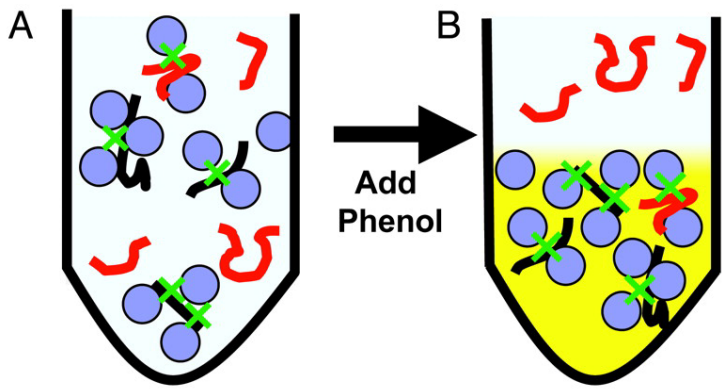
(1) Crosslink with formaldehyde

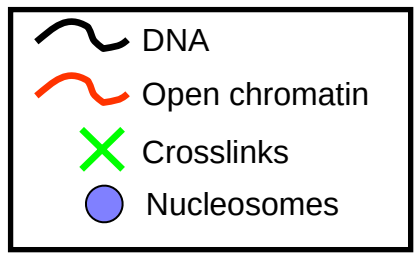


(2) Shear by sonication

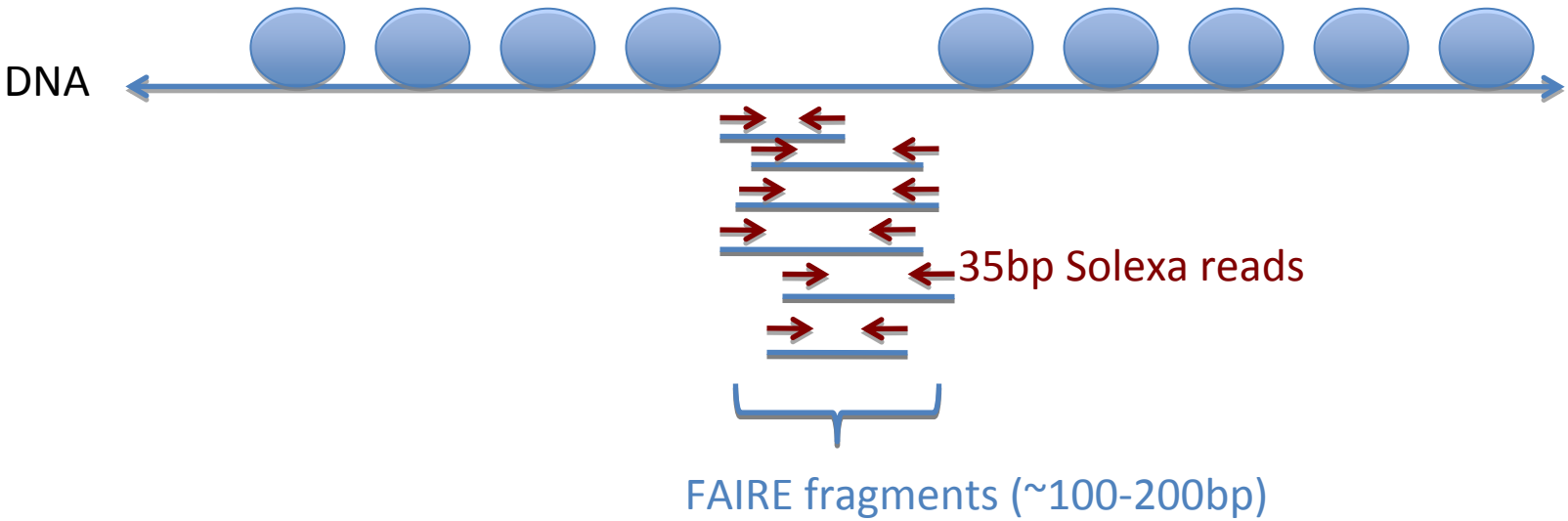


(3) Phenol extraction





FAIRE



35bp reads are aligned to genome and extended to 200bp

FAIRE Cell Culture Protocol

Crosslinking

1. If cells are grown in suspension remove an aliquot to be used as a reference and place on ice. Otherwise, the reference sample can be obtained by removing an aliquot following sonication, reversing the crosslinks, and purifying the DNA.
2. Add 37% formaldehyde directly to media to a final concentration of 1%.
3. Incubate at 25°C for 5 min with shaking 80 rpm.
4. Add 2.5 M glycine to a final concentration of 125 mM incubate 5 min at RT with shaking.
5. Spin at 700 x g for 5 min at 4° C.
6. Wash twice with ice cold 1xPBS, spin at 1000 rpm for 5 min at 4° C.
7. Cells can be snap frozen at this point and stored at -80° C.

Cell lysis (if frozen thaw cells on ice)

1. Resuspend cells in 1 ml of lysis buffer per 0.4g of cells.
2. Add 1 ml 0.5 mm glass beads to rubber sealed 2 ml screw topped tube. Add 1 ml of cells in lysis buffer.
3. Lyse cells in the mini-beadbeater-8 for five 1 minute sessions, ice cells for two minutes between each session.
4. Recover the lysate by puncturing the bottom of 2 ml tube with 25G syringe and drain into 15 ml tube on ice. *Filtered air can be used to expedite recovery.*
5. Add an additional 500 μl of lysis buffer to flush remaining sample.
6. Transfer 300 μl aliquots to 1.5 ml tubes and sonicate in Bioruptor for 15 minutes on HIGH using 30 second pulses and 30 seconds of rest, keep waterbath at a constant 4°C.
7. Spin the extract at 15,000 x g for 5 minutes at 4°C to clear cellular debris. Transfer the supernatant to a new tube.
   Remove an aliquot equivalent to 500 ng genomic DNA and check fragment size on 1% agarose gel.

Phenol/Chloroform extraction

1. Add an equal volume of phenol/chloroform, vortex, and spin at 12,000 x g for 5 minutes, transfer aqueous phase to a new tube.
   NOTE: If aqueous phase is large add 500 μl of TE, vortex, spin down again, and recover aqueous phase.
2. Add an equal volume phenol/chloroform to aqueous phase in fresh tube, vortex, spin down, and transfer aqueous phase to a fresh tube.
3. Add an equal volume of chloroform-isoamyl alcohol (24:1), vortex, and spin 12,000 x g for 5 min.
4. Add 1/10th volume of 3 M Sodium Acetate (pH 5.2), mix by inverting, and add 2X volume of 95% ethanol. Incubate at -20° C 1 hour to overnight.
5. Pellet precipitated DNA at 15,000 x g for 30 min at 4° C and remove supernatant. Wash pellet with 500 μl 70% ethanol, spin at 15,000 x g for 5 min at 25° C. Remove supernatant and dry pellet in speed-vac.
6. Resuspend pellet in 50 μl 10 mM Tris-HCl (pH 7.4).
7. Add 1 μl of 10 mg/ml RNase A and incubate at 37° C for 1 hour. **OPTIONAL:** Incubate crosslinked samples at 65°C overnight to reverse DNA-DNA crosslinks.
8. Cleanup sample using spin column (must recover 75 to 200 bp DNA) or additional phenol/chloroform extraction and ethanol precipitation.

Lysis buffer
2% Triton X-100
1% SDS
100 mM NaCl
10 mM Tris-Cl ph 8.0
1 mM EDTA

Phenol/Chloroform- Sigma #P3803 phenol, chloroform, and isoamyl alcohol 25:24:1 saturated with 10mM Tris, pH 8.0, 1 mM EDTA

Checking fragment sizes after sonication
NOTE: Samples limit vortexing to avoid aditional shearing

1. Add 1 μl of 10 mg/ml of RNase A, flick tube to mix, and incubate at 37°C for 1 hour.
2. Incubate at 65°C overnight.
2. Add 1 μl of 10 mg/ml of Proteinase K, flick tube to mix, and incubate at 37°C for 1 hour.
3. Add 10 mM Tris-HCl (pH7.4) to a final volume of 250 μl. Add an equal volume phenol/chloroform, mix, and spin at 12,000 x g 5 minutes, transfer aqueous phase to a new tube.
4. Add an equal chloroform-isoamyl alcohol (24:1), mix, spin at 12,000 x g for 5 minutes, and transfer aqueous phase to a new tube.
5. Add 1/10th volume of 3M Sodium Acetate (pH 5.2), mix by inverting, and add 2X volume of 95% ethanol, incubate at -20°C for 1 hour
6. Pellet DNA by spinning at 15,000 x g for 10 minutes at 4°C, wash with 500 μl 70% ethanol, and spin at 15,000 x g for 5 min at 25 °C
7. Dry pellet and resuspend in 10 μl 10 mM Tris-HCl (pH 7.4) and run on a 1% agarose gel.
NOTE: An ideal distribution is a smear from 1000 bp to 100 bp with an average size of 500 bp