Wash flies

Reagents:

- 50% Bleach
- Sterile mqH2O
- 80% EtOH
- PBS

1. Collect 2-4 mLs of flies into 15mL Falcon tube.
2. Wash flies with 2-3x volume of 50% Bleach
3. Wash with 1-2x volume of sterile mqH2O
4. Wash with 1-2x volume of 80% EtOH
5. Wash with 1-2xvolume of sterile mqH2O
6. Wash with 1-2xvolume of ice cold 1xPBS

xSDS treatment and lysis:

Reagents:

- HEPES-NIB (20mM HEPES. 10mM NaCl, 3mM MgCl2, 0.1% igepal, 1x protease inhibitors)
- 37% Formaldehyde
- 2.5 M glycine
- NEBuffer 2.1
- 20% SDS
- 10% Triton-X

1. Place flies into dounce homogenizer with 5 mLs of HEPES-NIB (20mM HEPES. 10mM NaCl, 3mM MgCl2, 0.1% igepal, 1x protease inhibitors)
2. Dounce until fully homogenized and incubate on ice for 5-10 minutes.
3. Strain homogenate twice with 40 um cell strainer.
4. Bring up to 10mL with HEPES-NIB.
5. Add 406 uL of 37% formaldehyde rotate at room temp for 10 minutes.
6. Add 800 uL of 2.5M glycine and incubate on ice for 5 minutes.
7. Pellet nuclei by spinning at 500xg for 5 minutes.
8. Aspirate supernatant and wash with 900 uL of 1x NEBuffer 2.1
9. Pellet at 500xg for 5 minutes again.
10. Aspirate supernatant and resuspend with 800 uL of 1x NEBuffer 2.1
11. Add 12 uL of 20% SDS and incubate at 42C with shaking for 30 minutes
12. Add 200 uL of 10% Triton-X and incubate at 42C with vigorous shaking for 30 minutes.
13. Strain through 40 um cell strainer.
14. Make into aliquots and use 10uL to quantify via Hematocytometer.
15. Use previous quantification to dilute an aliquot to ~2,000 nuclei/uL and quantify dilution using Hematocytometer.

Tagmentation:

Load Tn5:

Reagents:

- 100 uM i5/i7 unique transposase oligo
- 100 uM Mosaic End oligo
- 2xAnneal buffer (20mM Tris, 2mM EDTA, 50mM NaCl)

1. Add equal volumes of 100 uM i5/i7 oligo and 100 uM Mosaic end oligo into .2mL tube.
2. Add an equal volume of 2x Annealing buffer (20mM Tris, 2 mM EDTA, 50mM NaCl)
3. Anneal using:

   95C 5 minutes

   25C (-0.1C/s)

   Hold 25C

4. Add equal volumes of Tn5 to oligo and incubate at room temp for 1 hour. (12.5uM Tn5 complex)
5. Add equal volumes of i5 and i7 Tn5 complex and mix via pipetting.
6. Dilute 12.5uM complex to 2.5uM with DEPC H2O.

Nuclei tagmentation:

Per rxn:

Tn5 (2.5uM)	1uL
NIB (10mM Tris, 10mM NaCl, 3mM MgCl2, 1x protease inhibitor)	6uL
5xTAPS-PEG	2uL
Nuclei (2000/uL)	1uL

1. Incubate each rxn at 55C for 15 minutes.
2. Let cool to room temperature then place on ice.
3. Pool all wells together.
4. Estimate concentration of nuclei:

   (2,000 nuclei/well * # wells)/ volume of pool = # nuclei/uL in pool

   Make dilution to 20-25 nuclei/uL

PCR Indexing:

Per rxn:

DEPC H2O	7.75 uL
1% SDS	.5uL
BSA (20mg/mL)	.25uL
I5 Indexing primer (10uM)	2.5uL
I7 indexing primer (10uM)	2.5uL
Nuclei (20-25/uL)	1uL

1. Incubate rxns at 68C for 45 minutes.
2. Add to each rxn:

DEPC H2O	4.25uL
5x KAPA HiFi buffer	5uL
dNTPs	.75uL
Kapa HiFi Polymerase	.5uL

3. Run on following protocol:

    1. 72C 5 minutes
    2. 95C 3 minutes
    3. 98C 20 sec
    4. 65C 15 sec
    5. 72C 30 sec
    6. Repeat from step 2 10x
    7. 72C for 5 minutes
    8. Hold at 4C

Pool and purify:

1. Pool all of samples and purify using NEB Monarch PCR purification kit.
2. Quantify on qubit and QC on tapestation.