Fast-ATAC sequencing.

Reference: Corces et al. Nat Genet 2016 Oct 48(10: 1193-1203

*** This protocol has been optimized for blood cells. We note that digitonin is a gentle detergent and this protocol may not be ideal for cell lines and other cell types that are more resistant to lysis.

*** Test for this protocol used **frozen blood cells** (may have been FACS sorted before freezing), not "fresh after sorting".

*** we used the following start cell input count for this test: 5k, 10k, 25k, 50k

1. Thaw cells, add 1 volume of warm RPMI media (to dilute DMSO) and centrifuge at 500g to pellet cells. Resuspend cells in 1mL of RPMI and count cell concentration
    a. Washing with warm RPMI media might reduce cell death
2. Prepare cell aliquots for the different cell counts above
3. Pellet cells by centrifugation at 500*g* RCF for 5 min at 4 °C in a precooled fixed-angle centrifuge. All supernatant was removed using two pipetting steps, being careful to not disturb the cell pellet, which was not visible.
4. Fifty microliters of transposase mixture (25 µl of 2× TD buffer, 2.5 µl of TDE1, 0.5 µl of 1% digitonin, and 22 µl of nuclease-free water) was added to the cells, and the pellet was disrupted by pipetting (3-4 times plus gentle flicking of tube)
5. Transposition reactions were incubated at 37 °C for 30 min in an Eppendorf ThermoMixer with agitation at 300 rpm.
6. Transposed DNA was purified using a QIAGEN MinElute Reaction Cleanup kit (28204), and purified DNA was eluted in 10 µl of elution buffer (10 mM Tris-HCl, pH 8).
7. Transposed fragments were amplified for 5 cycles, then a 5ul aliquot was used to determine the number of additional cycles to add by qPCR, according to Buenostro et al ATAC-Seq protocol.
8. Libraries were purified by AMPure XP beads
9. Libraries were quantitated using Qubit dsDNA kit, and profile was checked on the TapeStation HS D1000 assay.
10. Libraries were sequenced on a HiSeq 4000 using PE-50 (4 libraries were pooled per lane)