GGR PROTOCOL: ATAC-Seq
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Frozen keratinocytes were thawed at 37˚C, washed with warm media, centrifuged at 500 x g for 5 minutes at room temperature and re-suspended in 5 mL of media supplemented with DNase I (Worthington Biochemical Corporation, Lakewood, NJ) at a final concentration of 200 U/mL. Cells were incubated with DNase I at 37˚C for 15 minutes, gently pipetted over 5 mL of Ficoll-Paque Plus (GE Healthcare) in a 15 mL conical tube, and centrifuged at 500 x g for 25 min. at room temperature with no brake. Cells at the Ficoll-media interface were removed with a sterile transfer pipet and washed with cold 1x PBS, pelleted at 500 x g at 4˚C for 5 min. and re-suspended in 1x PBS.

ATAC-seq was carried out as described in Buenrostro et al 2013 with the following modifications employed to reduce contamination from mitochondrial DNA and improve library quality. 70,000 cells were used per technical replicate reaction. The first wash was performed with 100 µL cold 1x PBS and an additional wash was performed with 1 mL cold RSB buffer (10 mM Tris-HCl pH 7.5, 10 mM NaCl, 3 mM $MgCl_2$), pipetting gently to re-suspend the cell pellet into this buffer, and centrifuging using the same conditions (500 x g, 4˚C, 5 min) to pellet. Tween-20 (Sigma-Aldrich; P9416) was added at a final concentration of 0.1% to both the lysis buffer and the transposition reaction. Final libraries were size-selected to between 150 and 1200 bp to remove PCR primers, primer dimers, and high molecular weight DNA using a 2.5% agarose gel and extracted using a gel extraction kit (QIAGEN) following manufacturer instructions. Sequencing was performed on a HiSeq 4000 (Illumina) using paired-end 50 bp x 50 bp reads.

References:

1. Buenrostro, Jason D., et al. "Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position." *Nature methods* 10.12 (2013): 1213-1218.