**CORE COLLABORATIVE PROJECT**

**Stage 1.ATAC-seq K562 DSP fixation and freeze-thaw**

We will perform 3 replicas of ATACseq of the following procedures in order to compare the data to ATACseq data ATACseq data already acquired in fresh K562:

1. **K562 treatment using fixative DSP:**

K562 (50,000 cells) will be fixed following the core protocol with DSP and ATAcseq performed after:

* 3 days from fixation (3 replicas)
* 7 days of fixation (3 replicas)

On the day of fixation, fixative will be inactivated and cells kept at 4 C until the day when ATACseq needs to be performed.

On the day of ATACseq performance cells will be washed with PBS (twice), lysed (using pre-made and stored Core buffer) and transposed.

After transposition two different approaches have been followed:

1. No de-crosslinking:
   * 5 uL of the transposed material will be used in a 50 uL PCR reaction with the ATACseq protocol conditions and 12 cycles of amplification
   * After PCR the material will be purified with beads and QC (tapestation)
2. De-crosslinking:
   * The transposase in the remaining 45 uL of the transposed reaction will be inactivated using EDTA 500 mM 30 min at 70 C
   * After inactivation de-crosslinking will be performed using DTT 50 mM 30 min 37 C

* After de-crosslinking the approximately 50 uL of reaction will be purified using The Minielute columns as in the ATACseq protocol and eluted in 10 uL
* PCR using the 10 uL of the eluted material
* Bead purification
* QC

1. **Freeze-thaw**

K562 will be frozen following our 2-steps protocol (Consuelo) at a concentration of 5x10^6 cells in 500 uL aliquots.

On the day of performing ATACseq (5 to 7 days after freezing and transferring to liqN) 3 different aliquots (3 replicas)cells will be recovered for 30 min in complete RPMI 10% FCS and ATACseq will be performed in 50,000 cells (lysis using pre-made and stored Core buffer and transposed) up to elution of the transposed material in 10 uL.

-Afterwards PCR amplification, beads purification and tapestation QC will be performed

**Stage 2.ATAC-seq K562 additional libraries**

We will perform 3 replicas of ATAC-seq of the following procedures in order to complete the data from Stage 1 and be able to draw more accurate conclusions from K562 cell line.

K562 will be provided by Core. They will have 2 flasks available for the experiment at a cell density of ~700 cells/uL in ~50 mL per flask (a total of 70M cells). Sorting will be done in collaboration with the Core (Laura).

1. **K562 fresh (x3):**

-Cells will be sorted based on viability using DAPI in the sorter SH800 from SONY.

-55,000 cells will be sorted in low binding tubes and counted with the hemocytometre to double check

-ATAC-seq will be performed following the standard protocol

-PCR using the 10 uL of the eluted material

-Bead purification

-QC

1. **Freeze-thaw (x3)**

-K562 will be frozen following our 2-steps protocol (Consuelo) at a concentration of 10x10^6 cells in 1 mL aliquots.

-We will be freezing 4-5 aliquots (40-50 M cells)

-On the day of performing ATACseq (5 to 7 days after freezing and transferring to liqN) 3 different aliquots (3 replicas) cells will be recovered for 30 min in complete RPMI 10% FCS

-Cells will be stained with DAPI and 55,000 cells will be sorted based on viability (protocol to confirm)

-ATACseq will be performed in 50,000 cells up to elution of the transposed material in 10 uL.

-PCR amplification

-Beads purification

-Tapestation QC

1. **K562 treatment using fixative DSP for 24 hours+transposase inactivation+de-cross linking (x3):**

-From the fresh K562 cultures 55,000 cells will be sorted and counted again

-Cells will be fixed using the DSP protocol and left for 24 hours at 4̊ C after the DSP inactivation.

- On the day of ATACseq performance cells will be washed with PBS (twice), lysed and transposed. following the standard protocol

-Transposase inactivation:

* The transposase in the 50 uL of the transposed reaction will be inactivated using EDTA 500 mM 30 min at 70 C

-De-crosslinking:

* After inactivation de-crosslinking will be performed using DTT 50 mM 30 min 37 C
* After de-crosslinking the approximately 50 uL of reaction will be purified using The Minielute columns as in the ATAC-seq protocol and eluted in 10 uL

-PCR using the 10 uL of the eluted material

-Bead purification

-QC

1. **K562 treatment using fixative DSP for 24 hours+transposase inactivation+w/o de-cross linking (x3):**

-From the fresh K562 cultures 55,000 cells will be sorted and counted again

-Cells will be fixed using the DSP protocol and left for 24 hours at 4̊ C after the DSP inactivation.

- On the day of ATAC-seq performance cells will be washed with PBS (twice), lysed and transposed following the standard protocol

-Transposase inactivation:

* The transposase in the 50 uL of the transposed reaction will be inactivated using EDTA 500 mM 30 min at 70 C
* After de-crosslinking the approximately 50 uL of reaction will be purified using the Minielute columns as in the ATAC-seq protocol and eluted in 10 uL

-PCR using the 10 uL of the eluted material

-Bead purification

-QC