Protocol to pool inputs for ChIP

NMBU – 14052021

Pool of inputs

Context

Each single tissue sample (BodyMap), pool of embryos (DevMap) and cell culture plate (ImmunoMap) will be used to generate multiple ChIP libraries (H3K4me1, H3K4me3 etc). In theory, each sample/pool/plate should also be used to generate 1 input control (total sonicated chromatin) representing background/control without immunoprecipitation. To reduce costs, we have decided that across the AQUA-FAANG project we will create and sequence POOLED INPUTs where samples are grouped by tissue, sex and sexual maturity (BodyMap), developmental stage (DevMap), treatment (ImmunoMap) etc.

As an example, for BodyMaps, each input will contain DNA from the 2 fishes used as biological replicates representing a specific gender, maturity state, and tissue (exception done for BodyMap gonads: 3 fishes pooled). For other work packages/activities, you must consider the appropriate pooling strategy.

A specific BodyMap pooling strategy is described below.

How to proceed – for each couple of fishes used (same sex, same maturity, same tissue)

1. From the 2 input controls obtained following the protocol of ChIP from NMBU, determine which one of the two is the least concentrated
2. Take out 5 µl of this diluted input. Calculate the corresponding mass (ng)
3. Take out the corresponding volume for the second input containing the same mass of sonicated chromatin
4. Add it to the volume from step 2 and complete to 10 µl with low TE buffer

The pooled input is ready for library preparation
For more than 2 inputs, calculate accordingly

Example

Input 1 = 30 ng/µl
Input 2 = 50 ng/µl

Pool input = 5 µl of input 1 (150 ng) + 3 µl of input 2 (150 ng) + 2 µl of low TE buffer

Sequencing

After the same library preparation, it is recommended to sequence twice more the input or pool input compared to the ChIP libraries.