**“Tethered” in situ Hi-C**

Kalhor et al. (2012) introduced a variant of Hi-C that they called “Tethered Conformation

Capture” (TCC), in which proteins are biotinylated prior to restriction so that crosslinked chromatin can be tethered to

streptavidin beads. Fill-in of restricted fragment ends and blunt-end ligation is then performed on beads. They reasoned

that this would limit interactions between non-crosslinked fragments.

While tethering might have a significant impact on chromatin in dilution, we reasoned that tethering proteins to beads

prior to ligation should have no effect on our in situ protocol, as chromatin is already constrained by the intact nucleus. We

adapted the TCC protocol in order to develop a tethered variant of our in situ protocol and confirm that it does not have an

impact on library quality.

After step 10 of the Lysis and Restriction Digest section above, we mixed the suspension with 20μl of 25 mM EZlink

Iodoacetyl-PEG2-Biotin (IPB) (Pierce Protein Biology Products, 21334) and rocked at room temperature for 60 minutes.

We then mixed the sample with 260μl of NEBuffer2 and 45μl of 10% Triton X-100, incubated on ice for 10 minutes and

then at 37oC for 10 minutes. Next, we added 20μl of NEBuffer2, 1μl of 1M DTT, 86μl of water, and 100U of MboI and

incubated at 37oC overnight to digest the chromatin.

The next day, we passed the sample through a 2mL Zeba spin desalting column (Thermo Scientific, 89889) in order to

remove any unreacted IPB.

The steps between attachment to MyOne Streptavidin T1 beads (Invitrogen) and detachment from the beads were

performed as in TCC (Kalhor et al., 2012), with the exception that the dNTPs used in the fill-in step were the same as the

ones that we use in our in situ Hi-C protocol, and the ligation was either performed in 5mL (as in TCC) or in 1mL (with all

volumes scaled down). In both cases, 5μl of 400 U/ μl T4 DNA Ligase (NEB, M0202) was added during ligation. After

detachment of the library from the T1 beads, the library was completed using the standard in situ Hi-C protocol beginning

with step 19.