3. “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 37$_{o}$C for 10 minutes. Next, we added 20µl of NEBuffer2, 1µl of 1M DTT, 86µl of water, and 100U of MboI and incubated at 37$_{o}$C 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.