I.a.4. In situ Hi-C in agar plugs: After lysis (as in the usual in situ Hi-C protocol, step 11), nuclei were resuspended in 100µl
2X NEBuffer2 and mixed with 100µl molten 2% NuSieve agarose (Lonza, 5009) and allowed to solidify into an agarose
plug. The nuclei embedded in agar were restricted overnight in 500µl 1X NEBuffer2 with 100U of MboI at 37$_o$C.
After restriction, the buffer was discarded and the agar plug was washed twice with 1ml of 1X NEB T4 DNA ligase buffer
for 30min at 37$_o$C. The buffer was discarded and the agar plug was submerged in 0.5ml fill-in reaction mix:
398µl of water
50µl of 10X NEB T4 DNA ligase buffer
37.5µl of 0.4mM biotin-14-dATP
1.5µl of 10mM dCTP
1.5µl of 10mM dGTP
1.5µl of 10mM dTTP
10µl of 5U/µl DNA Polymerase I, Large (Klenow) Fragment
The library was incubated for 1.5 hours at room temperature. After incubation, 2000U of T4 DNA Ligase were added to the
reaction and the library was ligated at room temperature for 4 hours.
After ligation, the buffer was discarded and the agar plug was washed twice with 1ml of 1X NEB β-agarase I buffer (NEB, B0392) for 30min at 37$_o$C. The buffer was removed and the agarose was melted by incubation at 68°C for 10 minutes. Liquid agarose was equilibrated at 42°C for 15 minutes. The agarose was digested with 4U of β-Agarase I (NEB,
M0392) at 42°C for 1 hour. Next, we reversed the crosslinks. All subsequent steps were performed following the standard
in situ Hi-C protocol beginning at step 18.