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Supplementary information

SPRITE: a genome-wide method for mapping higher-order 3D interactions in the nucleus using combinatorial split-and-pool barcoding

In the format provided by the authors and unedited

SUPPLEMENTARY METHODS

Cell culture and crosslinking for adherent cells

1. Seed and culture adherent cells on 15 cm plates under recommended conditions. We typically freeze 5-10 million cells per pellet in a 1.7 mL microcentrifuge tube.
2. Pre-chill one bottle (≥100mL) of 1 x PBS (without Magnesium and without Calcium) at 4 °C, keep one bottle (≥100mL) of 1 x PBS at room temperature. Store scraping buffer at 4 °C.
3. Aspirate media from plates.
4. Wash cells gently with 10 mL of room temperature 1x PBS.
5. Remove PBS.
6. Add 10 mL of 2 mM DSG crosslinking solution to a 15 cm plate.
7. Rock **gently** side-to-side at room temperature for 45 minutes.
8. Wash cells with 10 mL room temperature 1 x PBS.
9. Add 10 mL of freshly prepared 3% formaldehyde solution.
   ✖CRITICAL: We strongly recommend making a working solution of 3% formaldehyde **fresh** every time by opening a new ampule to minimize methanol conversion. Do not use an ampule opened more than ~30 min before crosslinking the cells.
10. Rock gently at room temperature for 10 minutes.
11. Add 2 mL of fresh 2.5 M glycine stop solution ✖.
    ✖ CRITICAL: Ensure that the 2.5 M glycine stop solution was made within a month from its use.
12. Rock gently at room temperature for 5 minutes.
13. Discard formaldehyde supernatant in an appropriate waste container. From here, keep cells at 4 °C.
14. Carefully wash with 10 mL of cold 1x PBS, gently rocking plates for 1-2 minutes.
15. Repeat wash step two more times, discarding PBS in formaldehyde liquid waste.
16. After the last wash, add 7 mL of Scraping Buffer (ice cold 1x PBS + 0.5% (w/v) BSA) to each 15-cm plate.
17. Scrape cells from plate and transfer to a 15mL falcon tube.
18. Centrifuge at 1000 x *g* at 4 °C for 5 min to pellet cells.
19. Discard supernatant and resuspend cells in 1 mL cold scraping buffer to break up the pellet. Add scraping buffer until cell concentration is ~10M cells per 1mL.
20. Aliquot 10 million cells into microcentrifuge tubes and spin at 2000 x *g* for 5 min at 4 °C.
21. Remove supernatant and discard.
22. Flash freeze cell pellets in liquid nitrogen.
    Pause Point: Store pellets at -80 °C for up to 5 years.

Legends

Supplementary Methods: An alternative approach to crosslinking in solution (described in the main procedure) is to perform crosslinking on adherent cells within a tissue culture plate. A detailed step-by-step protocol is included in the Supplementary Methods.