

Experimental parts for immunoprecipitating BrdU-labeled DNA from early and late S phase fractions are essentially identical to the conventional method.⁷⁾ WGA was performed using SeqPlex DNA Amplification Kit (Sigma, SEQXE-10RXN).

Ethanol precipitation

1. Add 2.5 µg glycogen to each sample (immunoprecipitated early and late replicating DNA from 10,000 cells).
2. Precipitate DNA with 2 volume of ethanol and 1/10 volume of 10M NH₄OAc.
3. Centrifuge at 15,000 rpm for 30 min at 4°C.
4. Rinse with 70% ethanol.
5. Resuspend DNA in 12 µl of H₂O (SEQXE_W4502).

Pre-amplification

6. Add 2 µl of 1X Library Preparation Buffer (SEQXE_LP100).
7. Incubate at 95°C for 2 min in thermal cycler.
8. Cool on ice.
9. Add 1 µl of Library Preparation Enzyme (SEQXE_E0531).
10. Incubate in thermal cycler as follows:
 - 16°C 20 min
 - 24°C 20 min
 - 37°C 20 min
 - 75°C 5 min
 - 4°C Hold

Amplification

11. Prepare 60 µl of amplification mix as follows:
 - 43.5 µl of H₂O (SEQXE_W4502)
 - 15 µl 5X Amplification Master mix (SEQXE_A5112)
 - 1.5 µl DNA Polymerase for SeqPlex (SEQXE_SP300)
12. Add 60 µl of amplification mix to each sample.
13. Incubate in thermal cycler as follows:
 - 94°C 2 min
 - 94°C 15 sec
 - 70°C 5 min
 - 70°C 30 min
 - 4°C Hold24 Cycles
14. Purify samples using the Macherey-Nagel Gel and PCR purification kit.

Primer-removal

15. Prepare 2.1 µg purified products from step 14 in 66.65 µl of H₂O.
16. Add following reagents to each sample.
 - 8.0 µl 10X Primer Removal Buffer (SEQXE_SR401)
 - 1.6 µl Primer Removal Solution (SEQXE_SR400)
 - 3.75 µl Primer Removal Enzyme (SEQXE_SR402)
17. Incubate in thermal cycler as follows:
 - 37°C 60 min
 - 65°C 20 min
 - 4°C Hold
18. Purify samples using Macherey-Nagel Gel and PCR purification kit.
19. Use 1.0 µg purified DNA for subsequent library construction.

Fig. S1 Detailed whole genome amplification (WGA) procedures.