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 NH_4OAc.
3. Centrifuge at 15,000 rpm for 30 min at 4°C.
4. Rinse with 70% ethanol.
5. Resuspend DNA in 12 μl of H_2O (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 H_2O (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
    } 24 Cycles
    70°C 30 min
    4°C Hold
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 H_2O.
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.