

Experimental parts for immunoprecipitating BrdU-labeled DNA from early and late S phase fractions are essentially identical to the conventional method.<sup>7)</sup> 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<sub>4</sub>OAc.
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<sub>2</sub>O (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<sub>2</sub>O (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	24 Cycles
94°C 15 sec	
70°C 5 min	
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<sub>2</sub>O.
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.