1. Y-shaped adapters bind DNA via sticky-end ligation

2. PCR

PE1 5′**CAAGCAGAAGACGGCATACGAGATCGGT** CTCGGCATTCCTGCTGAACCGCTCTTCCGATCT binds blue sequence above.
PE2 5′ **AATGATACGGCGACCACCGAGATCT** ACACTCTTTCCCTACACGACGCTCTTCCGATCT binds the complement of red sequence above.

3. Paired-end Sequencing

Paired-end read 1 sequencing primer: 5' ACACTCTTTCCCTACACGACGCTCTTCCGATCT 3' matches red adapter sequence.
Paired-end read 2 sequencing primer: 5' CGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCT 3' is complementary to blue adapter sequence.

Figure S1 GBS adapter scheme and sequences. (1) Y-shaped adapters (red/blue) include barcode sequences and a three-base 3′ overhang (CWG) complementary to that left by the ApeKI digestion of genomic DNA (black). The degenerate nucleotide W represents A or T. (2) During PCR amplification, primers PE1 and PE2 add sequences (bold) to the ends of adapter-ligated DNA. These sequences facilitate binding to the flow cell. After the PCR, each double-stranded DNA fragment has a different adapter sequence on each end, and can bind the flow cell. (3) During sequencing, primers bind to DNA strands such that each sequence read begins with the barcode followed by the cut site.