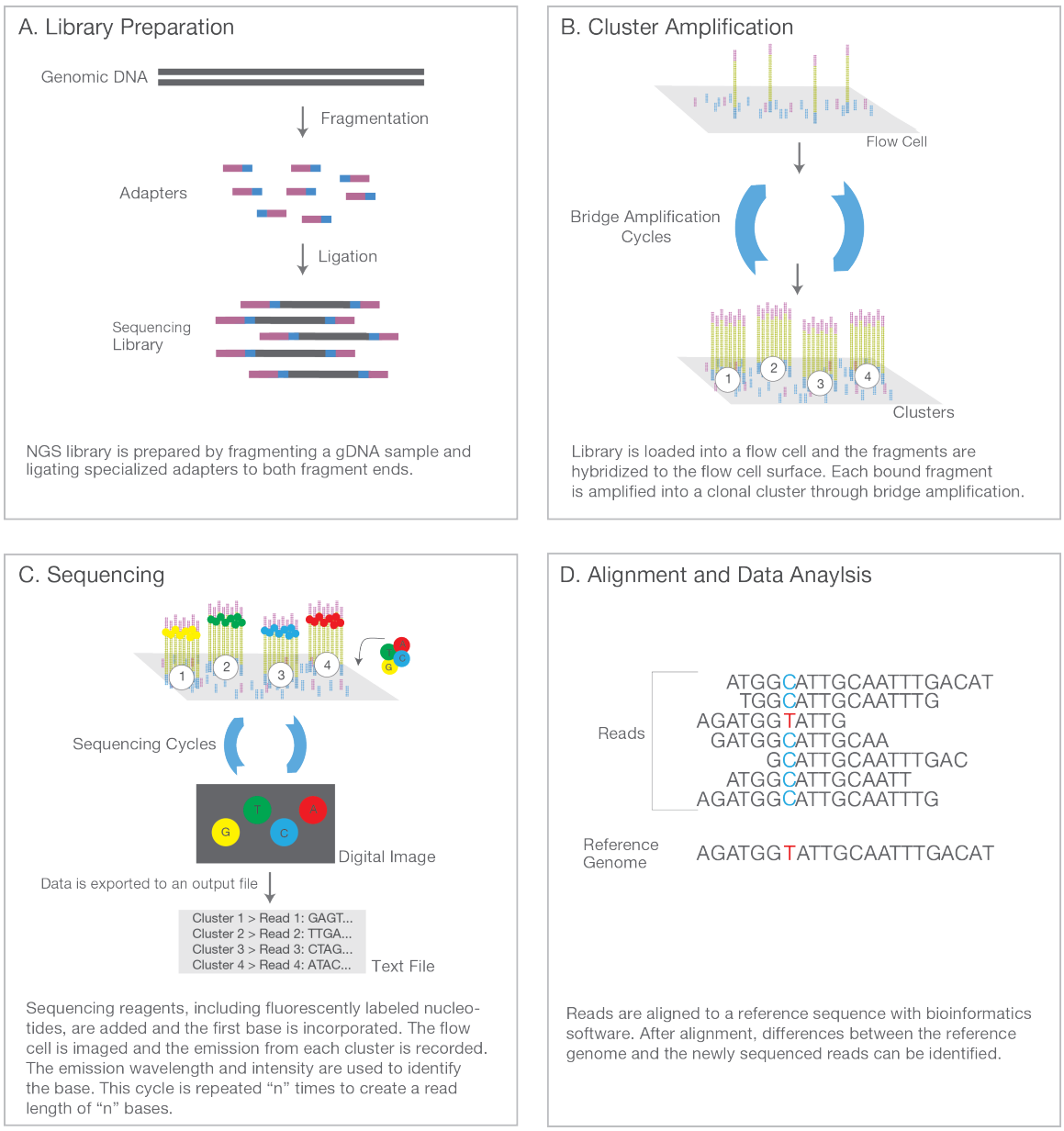

Figure 3: Next-Generation Sequencing Chemistry Overview—Illumina NGS includes four steps: (A) library preparation, (B) cluster generation, (C) sequencing, and (D) alignment and data analysis.

c. Advances in Sequencing Technology

Paired-End Sequencing

A major advance in NGS technology occurred with the development of paired-end (PE) sequencing (Figure 4). PE sequencing involves sequencing both ends of the DNA fragments in a library and aligning the forward and reverse reads as read pairs. In addition to producing twice the number of reads for the same time and effort in library preparation, sequences aligned as read pairs enable more accurate read alignment and the ability to detect indels, which is not possible with single-read data.[8] Analysis of differential read-pair spacing also allows removal of PCR duplicates, a common artifact resulting from PCR amplification during library preparation. Furthermore, PE sequencing produces a higher number of SNV calls following read-pair alignment.[8,9] While some methods are best served by single-read sequencing, such as small RNA sequencing, most researchers currently use the paired-end approach.