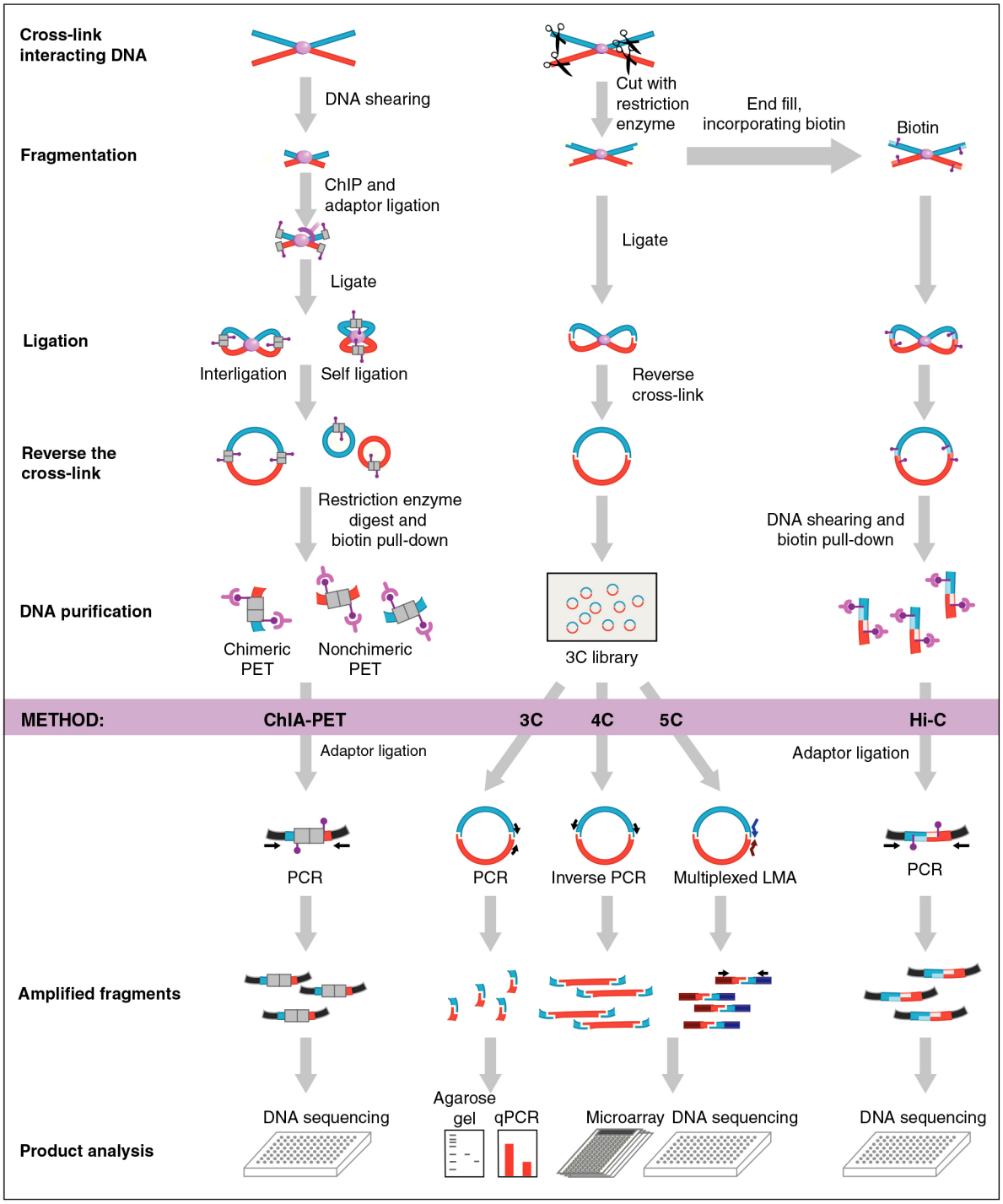

Figure 5. Overview of 3C-based methods. All 3C-based methods rely on covalently linking spatially proximal chromatin segments. Through a series of steps chromatin is then fragmented and religated and ligation products are detected using PCR, microarrays, or deep sequencing. In ChIA-PET (chromatin interaction analysis with paired-end tag sequencing), chromatin fragmentation is achieved by shearing and ligation junctions are marked by adaptors that contain recognition sites for type I restriction enzymes. Redigestion of ligation products with such enzymes yields small ligation, junction-containing molecules that can be analyzed by deep sequencing. 3C, 4C, and 5C use restriction enzyme digestion to fragment cross-linked chromatin. Religation of DNA then produces ligation products that can be directly analyzed by PCR (3C), inverse PCR (4C), or ligation-mediated amplification (LMA; 5C). Hi-C is as 3C, but includes a step to incorporate biotinylated nucleotides before religation. This facilitates purification of ligation junctions that are then analyzed by deep sequencing. (Adapted from Sanyal et al. 2011.)