



**Figure S3:** Fractional read depth in the Polypolish algorithm.

**A:** hypothetical read alignments for three 100 bp reads to a 1200 bp assembly. Read A aligned to a single location, read B to two locations and read C to four locations.

**B:** Polypolish read depths resulting from the alignments. Each alignment contributes  $1/n$  depth, where  $n$  is the total number of alignments for that read. This results in a mean depth of 0.25 for the entire assembly, the same value which would be obtained if each read only had a single alignment.