



Butterfly's minimum support requirement for path extension during transcript reconstruction. To extend a previously constructed path (thick dark black line) that ends at a given node (u), there requires minimum read or pair support (shown as thin blue lines) that should be compatible with the path (not traversing nodes that disrupt the existing path), extends to a connecting node (v), and exhibits sufficient overlap with the current path. Relevant Butterfly parameters include the minimum read support threshold ('-R', default: 2) and the required path overlap distance ('--path_reinforcement_distance', default: 75). In the above example, there are at least two reads that support an extension from node u to node v , and extend backward from node u along the current path overlap, meeting the default required --path_reinforcement_distance (default is 75 bases for PE fragments, and 25 bases for SE reads), the extension of path $\dots u$ to node v , generating path $\dots uv$, would be sufficiently supported. Using strict parameters (high minimal read support and long reinforcement distance) might prevent the extensions of paths resulting in a partially reconstructed transcript, with breaks at insufficient coverage regions. However, using permissive parameters might fuse several distinct transcripts that have assembled together, possible due to overlapping un-translated regions (UTRs).