



**Defining minimum edge thresholds during initial Butterfly graph pruning.** Butterfly defines the minimum relative incoming and outgoing k-mer edge support of a given node via the parameters ‘--edge-thr’ and ‘--flow-thr’, respectively. Edges with read (k-mer) support below these thresholds are pruned from the graph. The edge and flow threshold values have a substantial impact on the complexity of the resulting pruned graph, with higher pruning resulting in lower Butterfly runtimes. However, over pruning of the assembly graph might lead to segmented transcript reconstruction at low coverage regions, and lower sensitivity for detection of variant transcripts. Shown is an illustrative graph, where each node is a sequence, and directed edges connect sequences from 5’ to 3’.  $w_i$  denote the read support for each edge. For example, the values of  $w_1$ - $w_6$  and  $w_8$  are used to calculate two edge and two flow values by the stated formulae, which if below defined thresholds, will lead to pruning of the edge from u to v.