Here displayed is an outline of the updated Tuxedo Protocol (Excluding Differential Expression Analysis by Ballgown as the tools used depend on experiment i.e. no. of samples – see the Nature Protocol: http://www.nature.com/articles/nprot.2016.095). The samples – paired end reads were taken by Hisat2 (grey) and mapped to a pre-built index of the RN4 and RN6 genomes. StringTie (green) was then used for assembly with the aid of a RefSeq annotation. Assembled reads were merged and used as a guide for StringTie -eB to calculate abundance estimation, stored in a gtf annotation file. This gave values for transcript expression levels in FKPM (Fragments per Kilobase per Million reads) and TPM (Transcripts Per Million).