**A User’s guide for RNA-seq FAQ**

1. **RNA-seq mapping: when do you do de novo assembly versus align to genome versus align to transcriptome?**
2. **What are different features of alignment algorithms that we can consider in choosing one versus another?**
3. **How would one benchmark alignment algorithms?**
4. **What are metrics of performances that are commonly used?**
5. **When would one use a splice-aware mapping algorithm? When would you not use a splice-aware aligner? What are some splice-aware aligners? What are parameters to consider when using a splice-aware aligner?**
6. **How should I sort my BAM file?**
7. **What are PCR duplicates? How would you recognize that and when do you remove PCR duplicates? And when do you not?**
8. **When do you allow multi-mapping reads? Would you allow multimapping in DNA ? In RNA? When is it more crucial?**
9. **What is the difference between FPKM and RPKM? FPKM and TPM? What is the advantage of using TPM?**
10. **When do we use raw counts versus FPKM for expression estimation?**
11. **How much sequencing do we need to do?**
12. **How many replicates do we need?**