RNAseq

* Whole transcriptome sequencing

Applications:

* DGE
* Variant detection and allele specific expression
  + SNPs
* Small RNA profiling
  + Post transcriptional regulation
* Alternative splicing patters

Advantages:

* Can be applied to non-model organisms
* Gives functionally relevant info about what is being expressed at a given time point
* Allows you to detect differences among populations (gene expression and SNPs)

Limitations:

* RNA is less stable
  + Harder to isolate sufficient, high quality RNA
* Need to be mindful of tissue and condition specific expression
  + Though you can leverage this in your experimental design

Workflow:

* Experimental design
* Isolate RNA
* Quality assessment (gel/qubit/bioanalyzer)
* rRNA depletion (select for molecules with poly A tails to enrich for mRNA)
* Fragmentation
* cDNA synthesis
* Add adapters (amplification element, primary sequencing site, barcode for multiplexing)
* Amplify using PCR
* Sequence on the platform of your choice