Which genes are differentially regulated upon treatment?

⇓

QuantSeq!

GENE EXPRESSION PROFILING

Organism human

- well annotated
- many ressources

how many replicates?

- large treatment & control groups
- how to accomodate sample number and read depth?

how many reads/sample?

- genes of interest are lowly expressed
  → confirmed in pilot sequencing
- higher read depth per sample required
  ↳ use 3'mRNA-Seq?
  ↳ multiplex more samples ✓

Sample type blood

- mRNA analysis
  ↓
  poly(A) selection to focus on poly-adenylated transcripts
- globin removal?
  ↑

extraction

- pre-treatment "red blood cell lysis" can remove globin

pro

- easy
- cheap
- quick
- can be used for WTS also

con

- samples need to be fresh
  → non-frozen
  → non-fixed

WTS

↓

- transcript-level information
- isoform information obtained
- globin needs to be removed before library prep
  ↳ using red blood cell lysis?
  ↳ using globin depletion?
- higher read depth required → fragment on full transcript length

3'mRNA-Seq *

↓

- gene-level information ✓
- easy and fast to quantify
  → easy data analysis ✓
- globin transcripts can be blocked ✓
  ↓
  ! Samples can be stored (stabilized) → easier to collect large numbers
- lower read depth requirement compared to WTS-Seq