ChIP-seq Q & A

(1) What is the effective way to determine the minute amount of DNA inChIP sample?

- Invitrogen Qubit High Sensitivity Assay
- qPCR : 4 serial dilution of sample and standard DNA; at least duplicates per dilution (note: pre-amplification or presence of linkers can distort qPCR profiles)

(2) What is the minimal amount of DNA required for subsequent processes?

- recommend submitting 30 ng of ChIP-DNA to ensure success rate; minimal 15 ng in 40 ul

(3) What is the optimal read length (36/75/100) you would suggest?

- 100 bp for poor ref genome, 75 bp for high precision mapping to good ref., 36 bp if sample is very close to ref genome and if ref. genome seq. is very complete and well annotated
- Signle-Read (SR) is prevalent for ChIP-seq; but may choose Paired-end compatibility upon adaptor ligation for seq. flexibility (not critical if having good ref. genome)

(4) Can ChIP-seq libraries be barcoded?

- The current ChIP-seq library prep kit is only compatible with non-barcoded SR adaptor as well as PE adaptor upon user's preference. Illumina will release barcoded version of the library prep kit in late 2012.

(5) What is the charge for everything beyond, including library prep?

- Each library prep: $23,100
- Sequencing charge (per lane):

single-read sequencing 36 bp	42,000
single-read sequencing 75 bp	54,0 00
paired-end sequencing 36 bp	71,000
paired-end sequencing 75 bp	97,000

- Output raw reads and stats to users; no further analyses are included

(6) ChIP-seq sample requirement:

- OD260/280: 1.7-1.95 (spec)
- concentration: DNA $\geq$ 0.5 ng/ul (by fluorometer)
- quantity: $\geq$ 30 ng (min. 15 ng in 40 ul)
- agarose gel pattern: smear between 100-500 bp, major at around 250 bp
- qPCR using gene-specific primers, providing the test result
- Agilent 2100 BioAnalyzer data (recommended)
- indicate if sample are **amplified** or carry any **linkers** and adaptors from users (affect library qPCR result)
- Notify fragment size preference for ChIP targets (if known)

ChIP-seq Libraries

- Prior notice:
  - Indicate whether samples are pre-amplified or carry any linker sequences
  - Compatible with Paired-end sequencing or not
- Total ligation/PCR Libraries:
  - ChIP DNA
  - Mock (need one for each 2nd Ab used) or Total INput
- BioA HS DNA:
  - ChIP (8-50 ng/ul)
  - Mock
  - EB (background; 0.1-0.5 ng/ul)
- Library qPCR: (EZtype PCR mix?)
  - ChIP
  - Mock
- TA cloning and sequencing: by user (20-30, to verify source of insert)
  - ChIP
  - Mock (optional)
- Sequencing lanes:
  - ChIP
  - Mock