2020-07-29

MISO LIMS Training

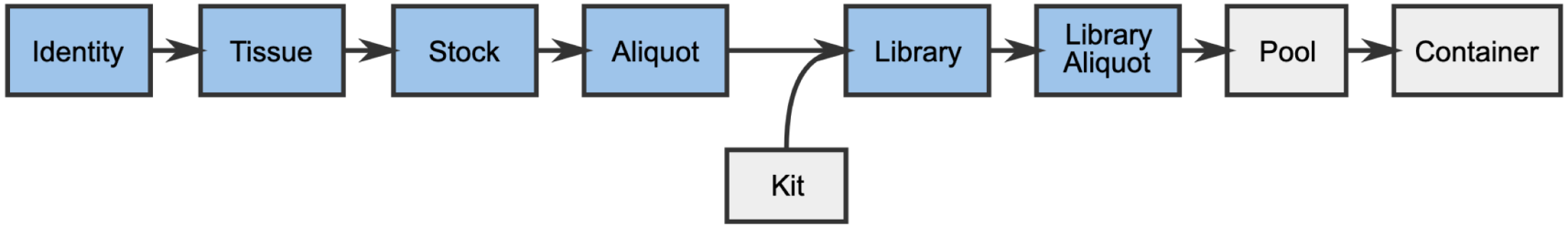
Sequencing

OICR
Ontario Institute
for Cancer Research

Outline

- Pools
- Pool Orders
- Sequencing Orders
- Sequencing Containers
- Sequencer Runs
- Service Records

General MISO Flow



Pools

- A pool is made up of one or multiple library aliquots that are ready to be loaded into a sequencing container partition for sequencing
- Aliquots may be added at different proportions
- QC information may be added to pools
- A pool containing multiple aliquots is called multiplexed
  - Indices are used to demultiplex the libraries later on
  - Duplicate or near-duplicate indices cause problems
  - MISO may prevent you from creating pools containing problematic indices
  - Index Distance Tool can be used to check index compatibility

Pool Orders

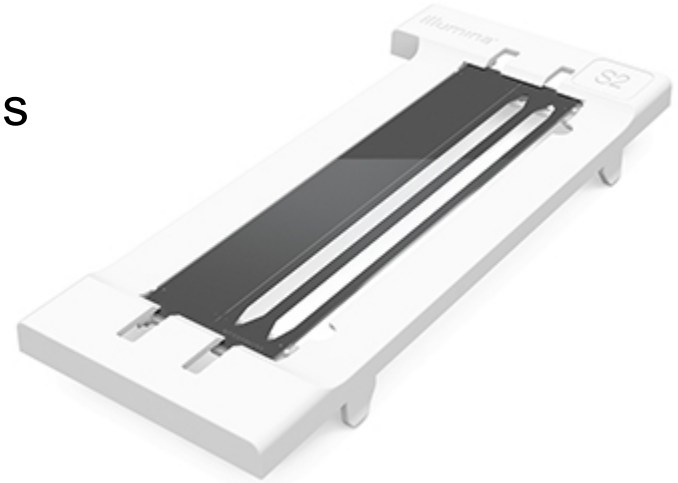
- A pool order tells the sequencing team that a set of libraries is ready to be pooled and sequenced
- Specifies which library aliquots to include and at what proportions
  - Library aliquots derived from the ones specified are also accepted
- May specify sequencing requirements
- Fulfilling a pool order requires
  - Linking a pool that contains all of the specified aliquots (or derivatives)
  - Linking a sequencing order that matches the sequencing requirements of the pool order (if specified)
- Pool orders are optional – useful if one team hands libraries off to another team for pooling

Sequencing Orders

- A sequencing order tells the sequencing team that a pool is ready to be sequenced
- Specifies
  - Instrument type
  - Sequencing parameters such as chemistry and read length
  - Number of partitions (lanes) required
- Automatically marked as fulfilled when matching runs are completed

Sequencing Containers

- The part that one or more pools are loaded into for sequencing
- Terminology is platform-dependant
  - Illumina, Oxford Nanopore: Flow cells
  - PacBio: 8Pac
- Some containers have multiple partitions
  - Illumina: Lanes
  - PacBio: SMRT cells
- Each partition holds one pool



Sequencer Runs

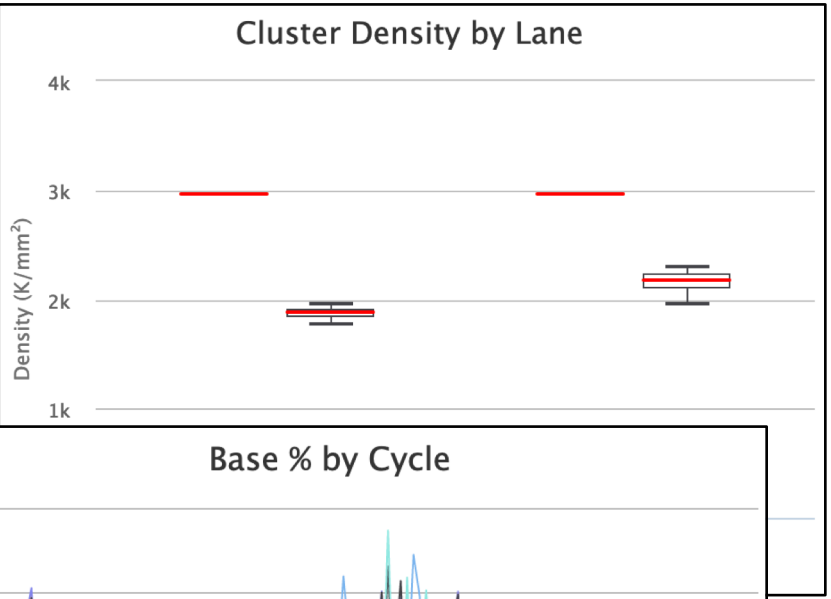
- Represent work done, or being done, by a sequencer
- A sequencing container is loaded into the sequencer for a particular run
- Status is normally one of
  - Running
  - Completed
  - Failed
- QC can also be set per lane. Indicates:
  - Whether the lane fulfills an order
  - Whether analysis should proceed

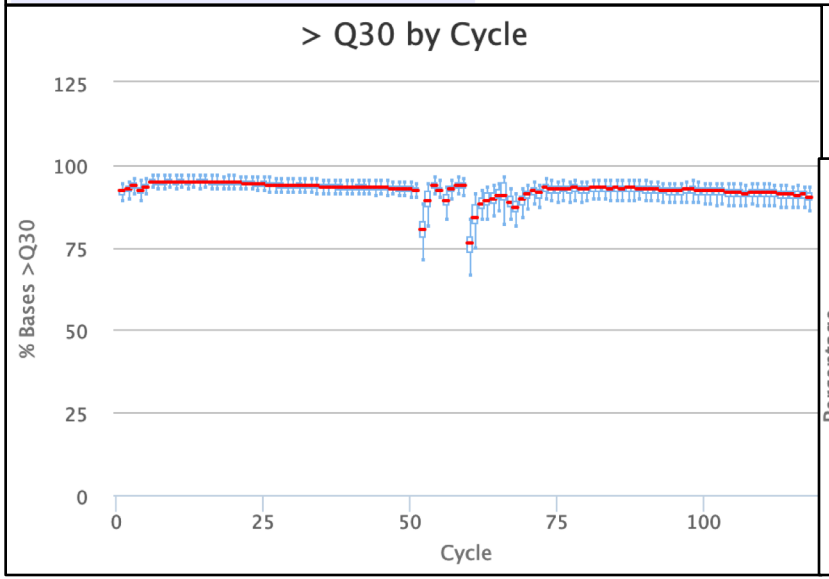
Run Scanner

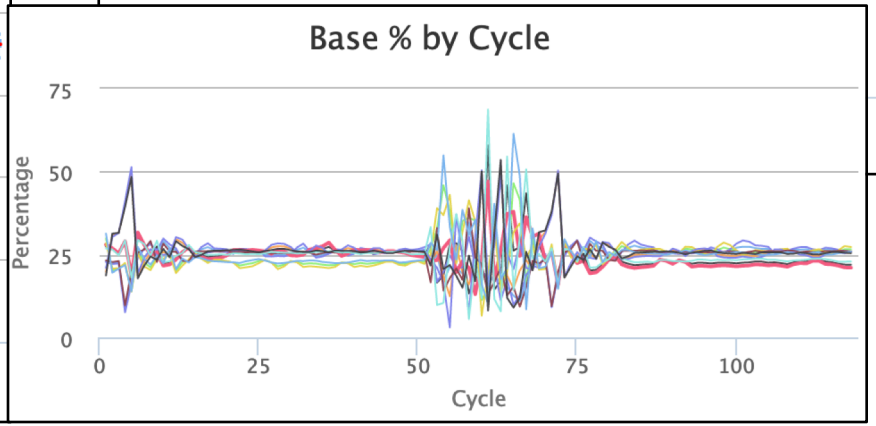
- Separate software
- Scans run directories to collect run information
- View in browser or access via API
- Integration with MISO
  - Automatically detect and create new runs in MISO
  - Containers also created if necessary
  - Data collected depends on the instrument
    - May include progress information and metrics
  - Data updates regularly as run progresses
    - Status automatically transitions to from Running to Completed/Failed

Metrics for an Illumina Run

% > Q30	92.20 %
Base Mask	y51,I8,I8,y51
Clusters	5,761,400,832
Clusters PF	3,936,211,712
Cycles	118 / 118
Ends	Paired

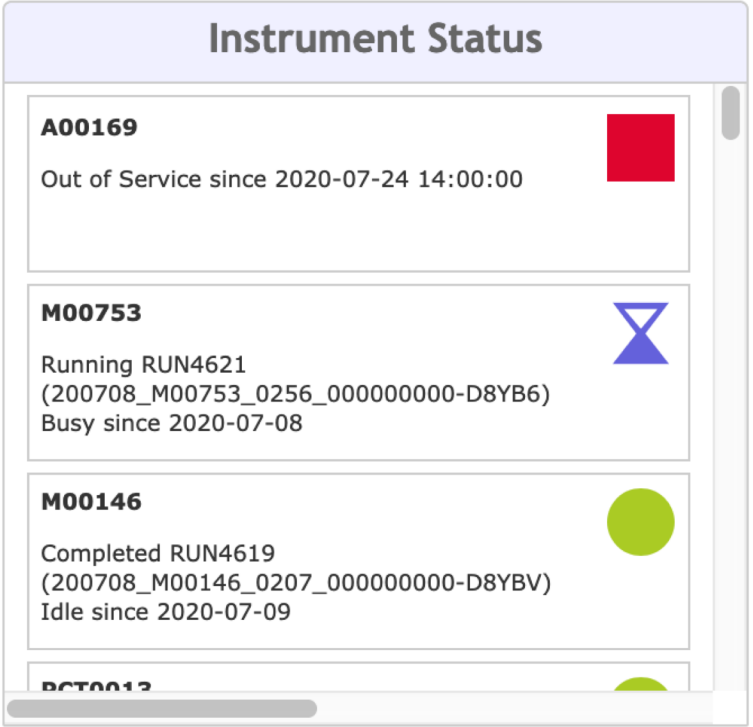






Service Records

- Service records can be added to an instrument
- Detail service that was done
- Files can be attached
- Indicate whether the instrument is out of service
  - Shown on instrument status widget



Exercise

Complete the following tutorial using Chrome or Firefox:

- Tutorial 8: Sequencing

https://miso-lims.github.io/walkthroughs/

Funding provided by the
Government of Ontario.