

2020-07-29

MISO LIMS Training

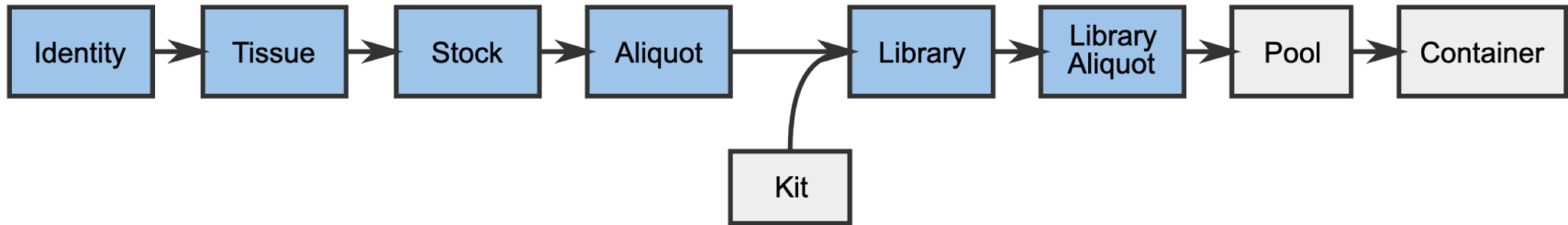
Sequencing



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

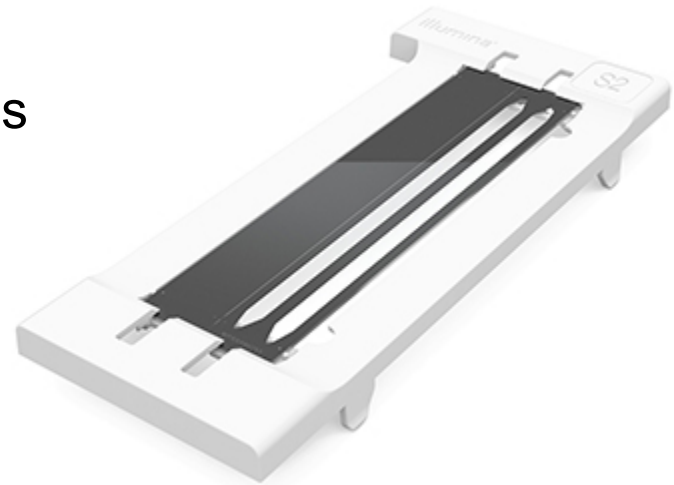
- A pool order tells the sequencing team that a set of libraries is ready to be pooled and sequenced
- Specifies which library aliquots to include and at what proportions
 - Library aliquots derived from the ones specified are also accepted
- May specify sequencing requirements
- Fulfilling a pool order 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

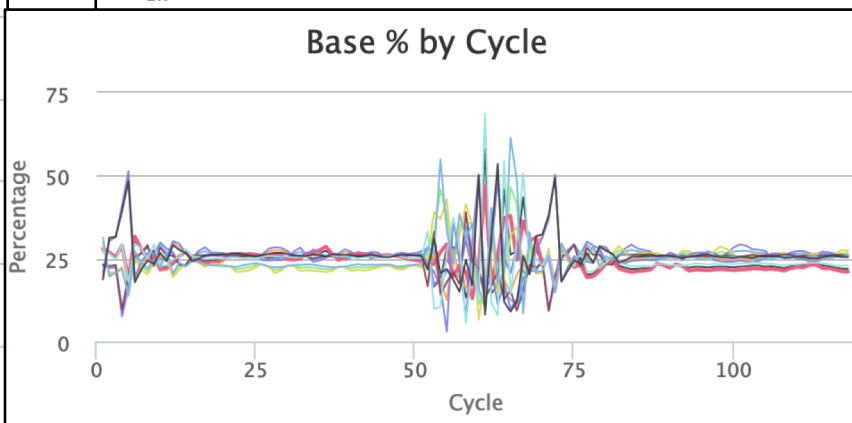
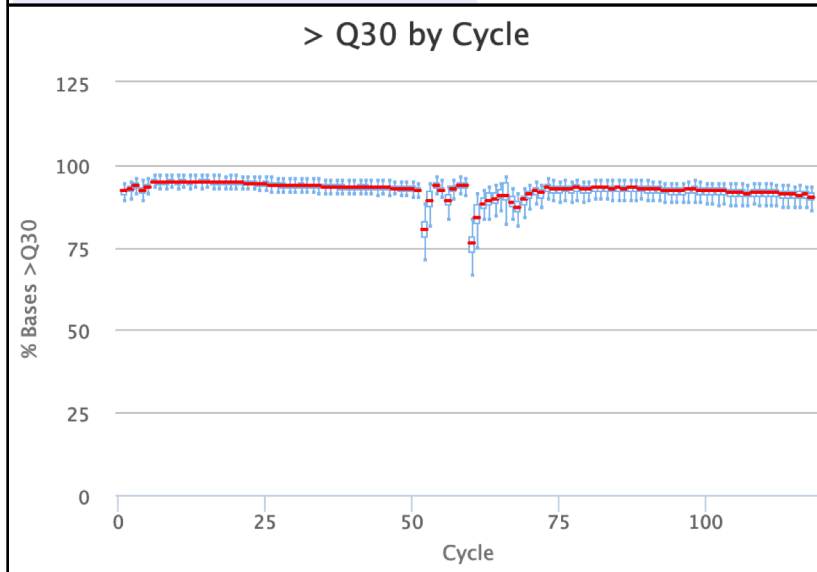
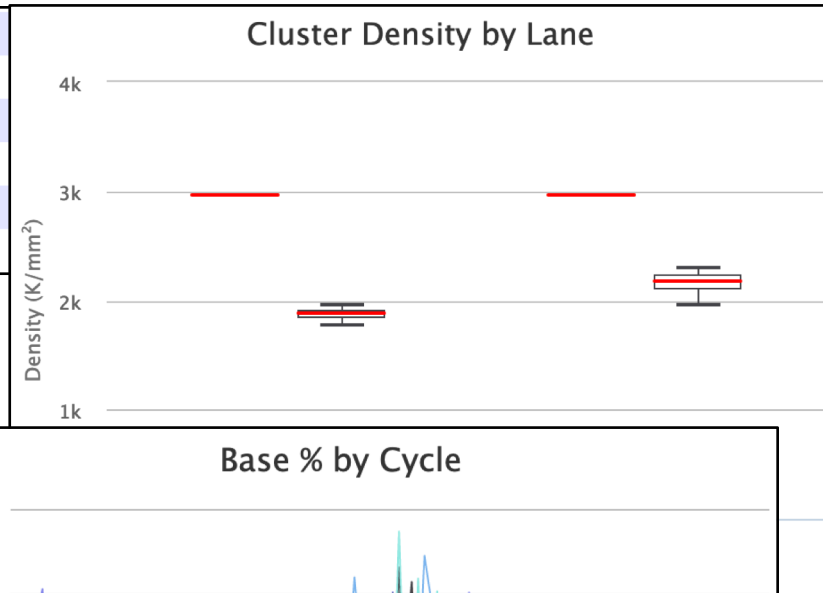


Run Scanner

- 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

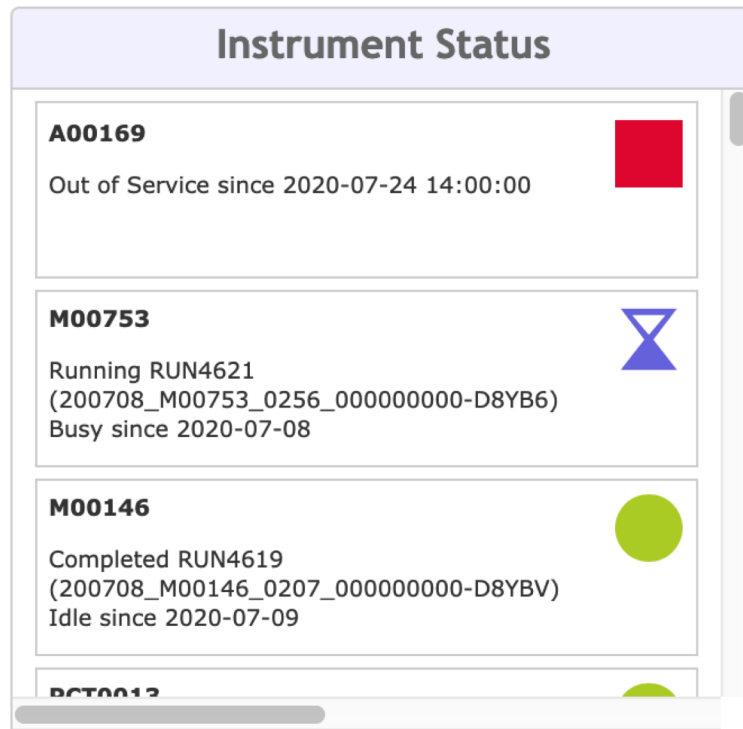
Metrics for an Illumina Run

% > Q30	92.20 %
Base Mask	y51,l8,l8,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



The image shows a screenshot of an 'Instrument Status' widget. The widget has a light blue header with the title 'Instrument Status'. Below the header, there are three instrument records, each in a white box with a thin border. The first record is for instrument A00169, which is 'Out of Service since 2020-07-24 14:00:00' and is represented by a red square icon. The second record is for instrument M00753, which is 'Running RUN4621 (200708_M00753_0256_000000000-D8YB6)' and 'Busy since 2020-07-08', represented by a blue hourglass icon. The third record is for instrument M00146, which is 'Completed RUN4619 (200708_M00146_0207_000000000-D8YBV)' and 'Idle since 2020-07-09', represented by a green circle icon. A fourth record for instrument BCT0012 is partially visible at the bottom, with a green circle icon. The widget has a vertical scrollbar on the right side.

Instrument ID	Status	Details	Icon
A00169	Out of Service	Out of Service since 2020-07-24 14:00:00	Red Square
M00753	Running	Running RUN4621 (200708_M00753_0256_000000000-D8YB6) Busy since 2020-07-08	Blue Hourglass
M00146	Completed	Completed RUN4619 (200708_M00146_0207_000000000-D8YBV) Idle since 2020-07-09	Green Circle
BCT0012			Green Circle

Exercise

Complete the following tutorial using Chrome or Firefox:

- Tutorial 8: Sequencing

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



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