Bionano Access™ Dashboard and Chip Metrics Guidelines

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Table of Contents

Revision History

REVISION	NOTES
A	Initial release.
B	Document updated for Solve 3.7 release: • Update dashboard format and units • Add chip metrics graphs section
C	Updated document template. Updated Analysis Graphs and Run Troubleshooting sections.
D	Updated to include agnostic information intended for both Saphyr and Stratys instruments.
E	Minor updates including changing “QC Metrics” to “Run Metrics”

Dashboard

This document provides guidelines on interpreting the quality metrics shown on the Bionano Access™ Dashboard. The Bionano Access Dashboard displays real-time graphic representations of ongoing Saphyr® or Stratys™ runs, updating throughput and quality metrics over time as the system scans. Dashboards from completed runs are archived within Bionano Access, so the user may refer back to data collection of a particular sample.

The dashboard consists of three main sections: run information, analysis graphs, and a run metrics table. It is a useful tool for evaluating the quality of the run—particularly how it proceeds over time, and how well molecule quality is sustained over long periods of data collection.

Run metrics generated in the dashboard are similar to those in the Molecule Quality Report (MQR). In **Figure 1** below, they are calculated as a weighted average per scan, updating over the course of the run. Once the run is complete, the dataset is imported into Bionano Access and a summary Molecule Quality Report is calculated. For the map rate and all related metrics (effective coverage, PLV, NLV), the dashboard uses molecules >150 kbp, whereas the MQR uses molecules > 150 kbp and minsites = 9. Label density is calculated using the entire population of molecules, not just molecules > 150 kbp.

Figure 1. Run metrics.

Run Information

- **Chip:** Chip serial number and lot number of a given run.
- **Run ID:** System-generated run identifier specific to a given run at a particular instrument.
- **Chip Name:** Chip name as entered by the user at Bionano Access.
- **Instrument:** Instrument serial number.
- **Min Length:** The cutoff of DNA length. Only DNA molecules with equal or longer length of the cutoff are counted in the Run Graphs.

- **Min Labels:** The cutoff of number of labels per molecule. Only DNA molecules with minimum or more number of labels are counted in the Run Graphs.
- **Start Time:** The time when the run was initiated. **NOTE:** this is relative to when data processing occurs in Access, not to when the chip is placed in the instrument.
- **End Time:** The time when the run was completed. **NOTE:** this is relative to when data processing completes in Access, not to when the chip is removed from the instrument.

Analysis Graphs

In **Figure 2**, the top panel displays DNA per scan (Gbp). This shows the total amount of DNA (>150 kbp) per scan in the flowcell as the run proceeds. The X and Y axes will scale to the data. The bottom panel shows the Map Rate (%), which is the percentage of molecules that map to the reference genome in each scan as the run proceeds. The X axis will scale as scans accumulate.

Users may click on either graph to zoom in, and click again to zoom out. If desired, a user can hide a flowcell line in the graphs by deselecting the checkbox in**Figure 3**.

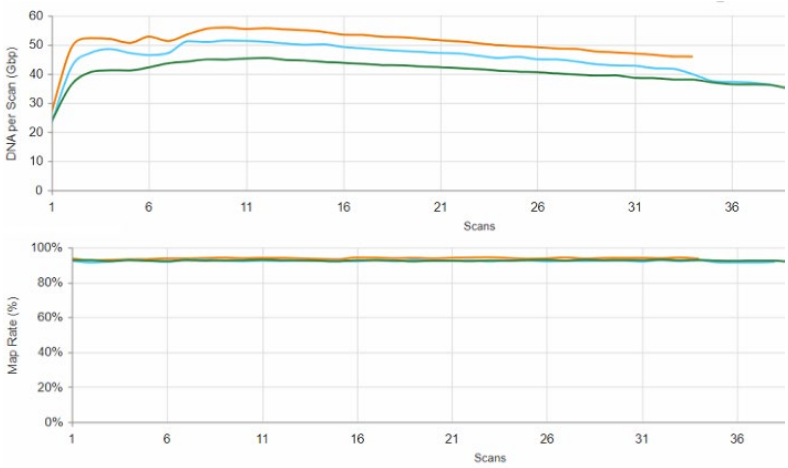


Figure 2. Analysis graphs.

Run Metrics Table

The Run Metrics table is shown in **Figure 3** below. The average values are weighted averages based on the amount of DNA detected per scan.

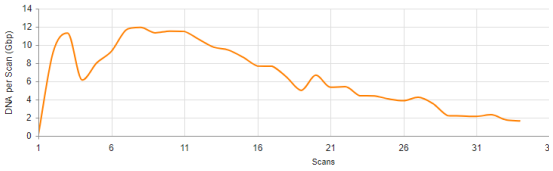
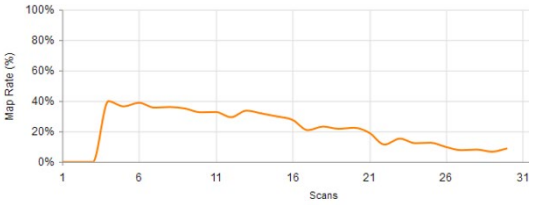
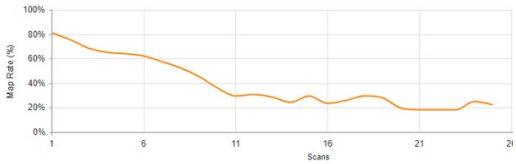
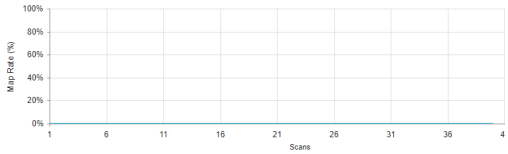
		For Run ID: 81feadf3-ae91-4e09-9961-66cfa755330f									All runs for ch C7VICIGNPPQJB
Plot	Flowcell	Prep	Avg N50 >=150kbp	Avg N50 >=20kbp	Avg Label Density per 100kbp	Avg Map Rate	Est Effective Coverage >=150kbp	Avg PLV	Avg NLV	DNA Collected >=150kbp	Scan Count*	Cumulative DNA >=150kbp
	1	Project: Nabsys Sample: GM24385 Channel: Green 01 Enzyme: DLE-1 Reference: hg38_DLE1_0kb_0labels.cmap	298 kbp	248 kbp	15.14	93.7%	521.13 X	5.8%	6.6%	1718.490 Gbp	Received: 34 Mapped: 34	Collected: 1718 Gbp Target: 1700 Gbp Percent: 101.09 %
	2	Project: Nabsys Sample: GM24385 Channel: Green 01 Enzyme: DLE-1 Reference: hg38_DLE1_0kb_0labels.cmap	318 kbp	268 kbp	15.17	92.1%	512.03 X	5.5%	6.8%	1716.129 Gbp	Received: 38 Mapped: 38	Collected: 1716 Gbp Target: 1700 Gbp Percent: 100.95 %
	3	Project: Nabsys Sample: GM24385 Channel: Green 01 Enzyme: DLE-1 Reference: hg38_DLE1_0kb_0labels.cmap	310 kbp	249 kbp	15.11	92.3%	514.82 X	5.5%	7.4%	1721.730 Gbp	Received: 43 Mapped: 43	Collected: 1722 Gbp Target: 1700 Gbp Percent: 101.28 %

Figure 3. Run Metrics table.

- **Plot:** The line color used in the plot for each flow cell. By deselecting the checkbox(s), a certain flowcell's data is hidden in the graph. Stratys chips have only one flowcell per chip.
- **Flowcell:** The flowcell on the chip.
- **Prep:** The names of the project, sample, channel, enzyme, and provided reference, as designated and entered by the user at Bionano Access.
- **Avg N50>=150kbp:** The molecule N50 for all molecules that are ≥ 150 kbp in length. Given a set of molecules, the N50 is defined as the sequence length of the shortest molecule at 50% of the total molecule length.
- **Avg N50>=20kbp:** The molecule N50 for all molecules that are ≥ 20 kbp in length.
- **Avg Label Density per 100 kbp:** Average number of labels per 100 kbp for total DNA molecules that are ≥ 150 kbp in length.
- **Avg Map Rate:** The average percentage of molecules (≥ 150 kbp in length) that map to the reference. If no reference is provided, the metric is 0%.
- **Est Effective Coverage >=150 kbp**: The estimated effective coverage is calculated as follows: Avg Map Rate * Total DNA throughput/ length of the provided reference.
- **Avg PLV (Average Positive Label Variance):** Percentage of molecule labels absent in the reference.
- **Avg NLV (Average Negative Label Variance):** Percentage of reference labels absent in molecules.
- **DNA Collected >=150 kbp:** The total amount of DNA ≥ 150 kbp that is collected for each flowcell during the run.
- **Scan Count:** "Received" number reflects the number of scans that are submitted by the Instrument Control Software (ICS) to Bionano Access. "Mapped" number reflects the number of scans that have been converted to molecule data and had metrics generated.
- **Cumulative DNA >=150 kbp:** "Collected" reflects the total amount of DNA that is collected in the flowcell across all runs of the chip."Target" reflects the target throughput. "Percent" is the percentage of total collected throughput against the target throughput.

Run Troubleshooting

Problem	Possible Causes	Recommended Actions
1. No data is shown in the Bionano Access dashboard.	Configuration issue in either ICS or Access.	Contact Bionano Technical Support (support@bionanogenomics.com).
2. No or low DNA throughput for molecules ≥ 150 kbp, starting from scan 1 to scan 5 (e.g., <10 Gbp per scan for human samples for Saphyr P/N 60239, <20 Gbp per scan for human samples for Saphyr P/N 60325 or Stratys P/N 60534).	Labeled DNA is <4 ng/µl (Direct Label and Stain - DLS) or <3 ng/µl (Nick, Label, Repair, and Stain - NLRS).	Repeat quantitation of labeled sample. Ensure labeled DNA concentration is within range: 4-16 ng/µl (DLS), 3-10 ng/µl (NLRS) (CV<0.30).
	Low N50.	See Row 10 of this table.

Problem	Possible Causes	Recommended Actions
3. Decrease in throughput over time. 	Chip clogging.	Check for labeled DNA homogeneity (CV<0.30). If DNA is not homogeneous, keep the DNA at room temperature overnight. Before loading on a new flowcell, use a regular p200 tip to pipette the labeled DNA up and down 2-3 times.
4. More than 20% decrease in map rate over time.  	DNA sticks to the chip channels.	Check for labeled DNA homogeneity (CV<0.30). If DNA is not homogeneous, keep the DNA at room temperature overnight. Before loading on a new flowcell, use a regular p200 tip to pipette the labeled DNA up and down 2-3 times.
5. Map rate is zero or consistently low (<40%). 	No reference or wrong reference provided.	Wait for the run to complete. After the Molecule object is imported to Access, edit the object so the correct reference is used.
	Low quality reference.	If possible, provide a better (more complete and/or more accurate) reference after run completion (see above).
	Lower than expected label density.	See Row 6 of this table.
	Wrong color selected.	Contact Bionano Technical Support (support@bionanogenomics.com).
	Low throughput (<1 Gbp/scan).	See Row 2 of this table.
6. Average label density is lower than the expected label density (*in-silico* digestion). Greater than 3 labels/100 kbp difference between observed label density and expected label density would be considered out of range.	Inhibitory substances in the raw gDNA.	Increase Proteinase K digestion during DNA extraction and labeling, if applicable.
	Incorrect enzyme to DNA ratio in the labeling reaction (i.e., too much DNA and/or too little enzyme).	Ensure correct volumes of both DNA and enzyme are added to the labeling reaction.
	Others for DLE-1 labeled samples.	Please refer to Troubleshooting section of the *Bionano Prep Direct Label and Stain Protocol* (CG-30206).

Problem	Possible Causes	Recommended Actions
7. Average label density is higher than the expected label density. Greater than 3 labels/100 kbp difference between observed label density and expected label density would be considered out of range.	Incorrect enzyme to DNA ratio in the labeling reaction (i.e., too little DNA and/or too much enzyme).	Ensure correct volumes of both DNA and enzyme are added to the labeling reaction.
8. High average NLV. NLV >20% (NLRS) or NLV >15% (DLS). *Only evaluate NLV when the map rate is consistent throughout the run and the average map rate is >40%.	Low enzyme activity, or inhibitory substances in the raw gDNA.	Check expiration of labeling enzyme and consider labeling a known control to evaluate enzyme labeling efficiency. Increase Proteinase K digestion during DNA extraction and labeling, if applicable.
9. High average PLV. PLV >15% (NLRS) or PLV >10% (DLS). *Only evaluate PLV when the map rate is consistent throughout the run and the average map rate is >40%.	Non-specific labels in the sample.	Repeat labeling and membrane adsorption. Wet the underside of DLS membrane with 1X DLE-1 buffer up to 10 minutes before sample application. Seal wells to prevent evaporation. Follow recommended incubation times.
10. Fragmented DNA (short molecules). Filtered (>150 kbp) N50 is < 230 kbp, or unfiltered (>20 kbp) N50 is < 130 kbp.	Poor quality of starting material.	Refer to Bionano released DNA extraction protocols for guidance.
	Improper handling of purified gDNA.	Avoid vortexing, rapid pipetting, or excessive pipetting with standard bore tips and use commercial wide-bore tips when appropriate.

Chip Metrics Graphs

Bionano Access enables users to view trends in chip run performance over time, from the **Chips** module under the **Metrics** tab. This view is useful for evaluating run consistency across many samples and flowcells over several weeks to months.

The chip metrics consist of six graphs (**Figure 4**): Total DNA (>=150 kbp), N50 (>=150 kbp), Average label density (>=150 kbp, Map rate (%), DNA per scan (Gbp), and Longest molecule (kbp). The x-axis for each graph is the date of data collection, and users may toggle to view a run performance period of 30, 60, or 90 days. By clicking on a graph, a user can zoom in, and then click again to zoom out.

Each datapoint represents one flowcell run on a Saphyr or Stratys instrument. Hovering over a given datapoint will display a tooltip of the corresponding y-axis value, run ID, and flowcell. Clicking on a data point will bring the user to the dashboard corresponding to that flowcell.

Figure 4. Chip metrics graphs

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Technical Assistance

For technical assistance, contact Bionano Technical Support.

You can retrieve documentation on Bionano products, SDS's, certificates of analysis, frequently asked questions, and other related documents from the Support website or by request through e-mail and telephone.

TYPE	CONTACT
Email	support@bionano.com
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