Bionano Access®: Dashboard Guidelines

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Revision History

Revision	Notes
A	Initial Release

Chapter 1: Introduction

This document provides guidelines on interpreting the quality metrics shown on the Bionano Access Dashboard.

Bionano Access Dashboard: Displays an update of a Saphyr® run, providing throughput and quality metrics of the data/run. The dashboard is a graphic representation for DNA per Scan and the Map Rate per Scan. It also includes run information, run graphs, and QC metrics table. Therefore, the dashboard is a very useful tool for evaluating the quality of the run. By supplying a reference and monitoring the map rate per scan, average map rate, positive label variance, and negative label variance, it is possible to check the alignment of the labeled DNA to the reference.

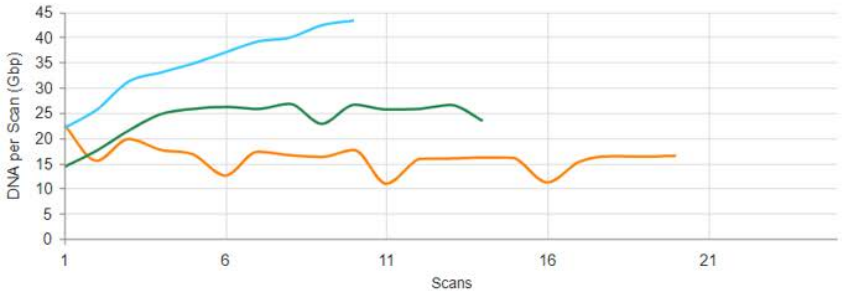
Plot	FC	Prep	Total DNA This Run (Gbp)	Total DNA This Flowcell (Gbp)	Avg N50 >=150kbp (Mbp)	Avg N50 >=20kbp (Mbp)	Avg Label Density (per 100kbp)	Avg Map Rate	Est Effective Coverage	Avg PLV	Avg NLV	Throughput Target*	Scans
	1	Sample: Sample1 Channel: Green 01 Enzyme: DLE-1 Type: Single Reference: hg19_DLE1_0kb_0labels	1354.681	1354.681	0.334	0.268	15.05	90.3%	395.36 X	3.9%	9.8%	423.34% of 320 Gbp	Recd: 21 Done: 21
	2	Sample: Sample 2 Channel: Green 01 Enzyme: DLE-1 Type: Single Reference: hg19_DLE1_0kb_0labels	1342.596	1342.596	0.331	0.264	15.00	90.7%	393.25 X	3.7%	9.8%	419.56% of 320 Gbp	Recd: 21 Done: 21
	3	Sample: Sample3 Channel: Green 01 Enzyme: DLE-1 Type: Single Reference: hg19_DLE1_0kb_0labels	1336.962	1336.962	0.321	0.251	15.12	90.7%	391.56 X	3.7%	9.7%	417.80% of 320 Gbp	Recd: 23 Done: 23

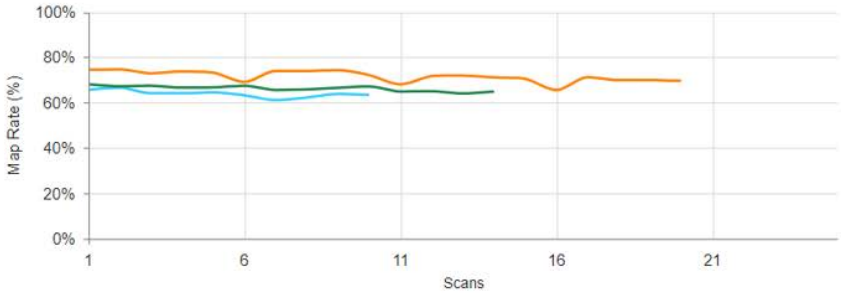
Run information

Chip:	Y4Q6R6ONPN6H7NWU / LOT#: 13300	Cohorts Per Scan:	8
Run ID:	15dbc263-045b-4832-ae71-58605337fde6	Min Length:	150 kbp
Project:	E3	Min Labels:	0
Experiment:	Test	Start Time:	4/18/2019, 3:23:26 AM
Instrument:	Saphyr SN#	End Time:	4/18/2019, 6:38:22 PM

- **Chip:** Saphyr Chip serial number and lot number of this run.
- **Run ID:** System-generated run identifier specific to this run at the particular instrument.
- **Project:** Project name as entered by the user at Bionano Access.
- **Experiment:** Experiment name as entered by the user at Bionano Access.
- **Instrument:** Instrument serial number.
- **Cohorts Per Scan:** The number of subgroups that each scan is divided into for real-time analysis. One scan contains multiple cohorts. Run metrics are generated for each cohort.
- **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 Saphyr).
- **End Time:** The time when the run was completed (note: this is relative to when data processing occurs in Access, not to when the chip is placed in the Saphyr).

Run Graphs*





[left] DNA per scan (Gbp): This shows the total amount of DNA (>150 kbp) per scan in the flowcell as the run goes. This value is the average of each cohort for each scan.
[right] Map rate (%): This shows the percentage of molecules that map to the reference genome in each scan as the run goes.
(*Note: You can click on a graph to zoom in and click on again to zoom out.)

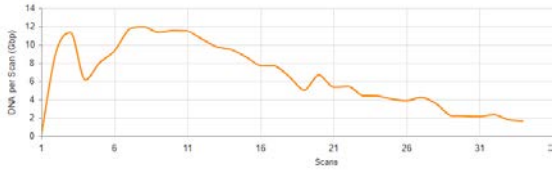
QC Metrics Table*

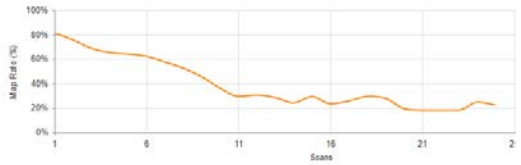
Plot	FC	Prep	Total DNA This Run (Gbp)	Total DNA This Flowcell (Gbp)	Avg N50 >=150kbp (Mbp)	Avg N50 >=20kbp (Mbp)	Avg Label Density (per 100kbp)	Avg Map Rate	Est Effective Coverage	Avg PLV	Avg NLV	Throughput Target*	Scans
☑	1	Sample: 94522 Base SP 2 Channel: Green 01 Enzyme: DLE1 Type: Single Reference: Rat_DLE1	322.487	322.487	0.292	0.173	17.21	71.9%	80.26 X	7.9%	9.8%	100.78% of 320 Gbp	Recd: 20 Done: 20
☑	2	Sample: 94516 Mod SP 7 Channel: Green 01 Enzyme: DLE1 Type: Single Reference: Rat_DLE1	347.873	347.873	0.221	0.121	17.53	63.6%	77.12 X	8.7%	8.6%	108.71% of 320 Gbp	Recd: 10 Done: 10
☑	3	Sample: 94522 Mod SP 3 4.9.19 Channel: Green 01 Enzyme: DLE1 Type: Single Reference: Rat_DLE1	332.811	332.811	0.222	0.133	16.83	66.1%	76.68 X	9.0%	8.3%	104.00% of 320 Gbp	Recd: 14 Done: 14

- **Plot:** The line color used in the plot for each flow cell. By selecting the checkbox(s), a certain flowcell's data is shown in the graph.
- **FC:** The flowcell on the chip.
- **Prep:** The names of the sample, channel, enzyme, type, and provided reference, as designed and entered by the user at Bionano Access.
- **Total DNA This Run (Gbp):** The total amount of DNA that is collected for each flowcell during the run.
- **Total DNA This Flowcell (Gbp):** The total amount of DNA that is collected in the flowcell across all runs of the chip.
- **Avg N50>=150kbp (Mbp):** 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 (Mbp):** 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**: 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 reference.
- **Avg NLV (Average Negative Label Variance):** Percentage of reference labels absent in molecules.
- **Throughput Target:** Percentage of total collected throughput against the target throughput.
- **Scans:** "Recd" number reflects the number of scans that are submitted by Saphyr Control Software to Bionano Access. "Done" number reflects the number of scans that have been converted to molecule data and had metrics generated.

(*****Note: The average values in the QC Metrics Table are weighted averages. They are weighted based on the volume of DNA detected).

Chapter 2: Troubleshooting

Problem	Possible Causes	Recommended Actions
1. No data is shown in the Bionano Access dashboard.	Configuration issue in either ICS (Instrument Control Software) 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).	Labeled DNA is <4 ng/µl (DLS or Direct Label and Stain) or <3 ng/µl (NLRS or Nick, Label, Repair, and Stain).	Repeat quantitation of labeled sample. Ensure labeled DNA concentration is within range: 4-12 ng/µl (DLS), 3-10 ng/µl (NLRS) (CV<0.25).
	Low N50.	See Section 10.
3. Decrease in throughput over time. 	Chip clogging.	Check for labeled DNA homogeneity (CV<0.25). 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.

Problem	Possible Causes	Recommended Actions
4. More than 20% decrease in map rate over time.  	DNA sticks to the chip channels.	Check for labeled DNA homogeneity (CV<0.25). 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 was 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 Section 6.
	Wrong color selected.	Contact Bionano Technical Support (support@bionanogenomics.com).
	Low throughput (<1 Gbp/scan).	Section 2.

Problem	Possible Causes	Recommended Actions
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. Increase drop dialysis time during DNA extraction.
	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 (30206).
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%.	Inhibitory substances in the raw gDNA.	Increase Proteinase K digestion during DNA extraction and labeling, if applicable. Increase drop dialysis time during DNA extraction.
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 on the DNA.	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 < 220 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.

Technical Assistance

For technical assistance, contact Bionano Genomics 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@bionanogenomics.com**
Phone	**Hours of Operation:** **Monday through Friday, 9:00 a.m. to 5:00 p.m., PST** **US: +1 (858) 888-7663**
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