PromethION 24 (PC24B251) Final report

30 Dec 22, 01:31 — 06 Jan 23, 22:57 · PM0199_20221229 · PROM0134_Meyer_GVTP-ONT_12292022 · 3G

Protocol run ID: 1dd95e14-3a64-4132-bb85-a7a7aef4f5d1

Oxford NANOPORE Technologies

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Run summary

DATA OUTPUT

Estimated bases	Data produced
114.64 Gb	**1.31 TB**
Reads generated	Estimated N50
16.72 M	**10.64 kb**

RUN DURATION

Elapsed time

72 hours of 72 hours

Run status

Finished

View unit abbreviations used in this report

BASECALLING

Reads called	Bases called (min Q score: 10)	
100%	**101.15 Gb**	**15.2 Gb**
	Pass	Fail

Run configuration

RUN SETUP

Flow cell type	FLO-PRO114M
Flow cell type alias	FLO-PRO114M
Flow cell ID	PAI23763
Kit type	SQK-LSK114

RUN SETTINGS

Specified run length	72 hrs
Active channel selection	On
Pore scan freq.	1.5 hrs
Reserved pores	On
Minimum read length	200 bp
Read splitting	On
Basecalling	Super-accurate basecalling
Modified basecalling	Off

DATA OUTPUT SETTINGS

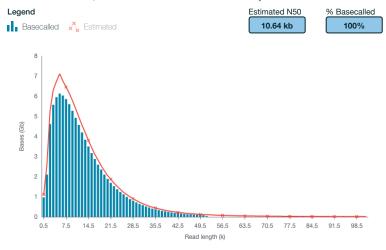|	
FAST5 output	vbz_compress
FAST5 reads per file	4000
FASTQ output	gzip_compress
FASTQ reads per file	4000
BAM output	Off
Bulk file output	Off
Data location	/data/./PM0199_20221229/ PROM0134_Meyer_GVTP-ONT_12292022/20221229_ 1731_3G_PAI23763_1dd95 e14

SOFTWARE VERSIONS

MinKNOW	22.10.7
Bream	7.3.5
Configuration	5.3.8
Guppy	6.3.9
MinKNOW Core	5.3.1

Sequence output

READ LENGTHS · OUTLIERS REMOVED

The read length graph shows the total number of bases vs the read length. The longest 1% of strands are classified as outliers, and excluded to allow focus on the main body of data.
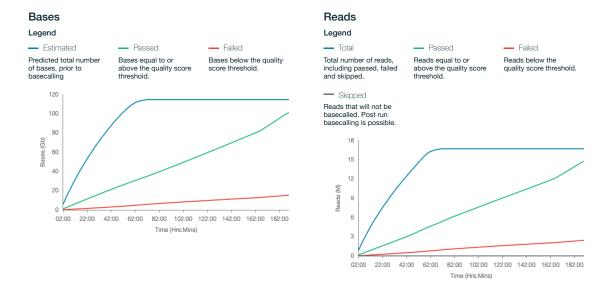
Legend

		Estimated N50	% Basecalled
Basecalled	Estimated	**10.64 kb**	**100%**

OUTLIERS

The longest 1% of strands are classified as outliers, and aggregated into groups to show their relative amounts.

Read length (kb)	Aggregated reads (Mb)
48 - 176	989.38
176 - 304	21.33
304 - 432	0.35
432 - 436	0.44

CUMULATIVE OUTPUT

The cumulative output shows the total amount of bases or reads sequenced over time by your device.

Bases

Legend

— Estimated	— Passed	— Failed
Predicted total number of bases, prior to basecalling	Bases equal to or above the quality score threshold.	Bases below the quality score threshold.

Reads

Legend

— Total	— Passed	— Failed
Total number of reads, including passed, failed and skipped.	Reads equal to or above the quality score threshold.	Reads below the quality score threshold.

— Skipped

Reads that will not be basecalled. Post run basecalling is possible.
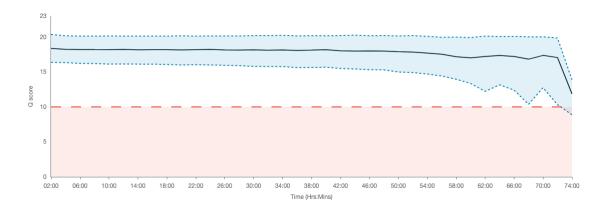
QUALITY SCORE

The quality score is calculated as basecalling is performed on your device. Reads that fall below the minimum value of 10 will be classified as failed reads. You can alter the accepted minimum quality score in MinKNOW.

Legend

— **Mode**

The most frequent quality score of reads in the run.

⬚ **Spread**

The spread of quality scores, found by calculating full width half maximum.

- - - **Min. quality score**

Minimum quality score to be accepted as a passed read.

Troubleshooting

Quality score low
This can be due to the translocation speed being out of the accepted range, which can correlate to low quality scores. If you see that the translocation speed is out of the accepted range in the below graph, please see the Flow Cell refuelling page linked here for further troubleshooting.
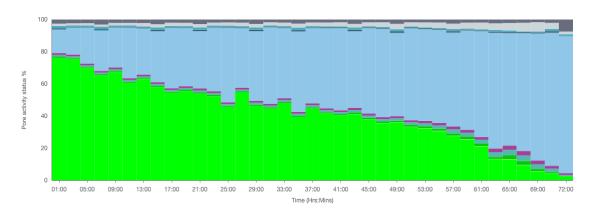
Run health

PORE ACTIVITY

The Pore activity graph shows the performance of your sample as it is being sequenced during a run.

Legend

● **Sequencing**
Pore currently sequencing

● **Adapter**
Pore currently sequencing adapter

● **Pore available**
Pore available for sequencing

● **Unavailable**
Pore unavailable for sequencing

● **Active feedback**
Channel ejecting analyte

● **No pore**
No pore detected in channel

● **Out of range-high**
Current is positive but unavailable for sequencing

● **Multiple**
Multiple pores detected. Unavailable for sequencing.

● **Saturated**
The channel has switched off as current levels exceed hardware limitations

● **Out of range-low**
Current is negative but unavailable for sequencing

● **Zero**
Pore currently unavailable for sequencing

● **Channel disabled**
Channel is disabled and awaiting another pore scan

● **Unclassified**
Pore status unknown

Troubleshooting

General
Some commonly seen issues are excess pores classified as Recovering, Open Pore, or Free Adapter. To find out what advice is applicable for your run, visit the user guide.

PORE SCAN

A Pore scan is performed at configurable time intervals to determine the current status of pores within channels on a Flow Cell. For this run a Pore scan is performed every 1.5 hrs.
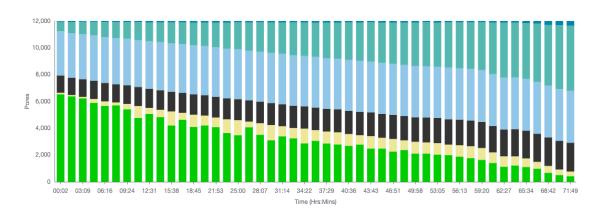
Legend

- 🟢 **Single Pore** — Pore in channel available for sequencing
- 🟡 **Reserved Pore** — Pore in reserve, will return to available when required
- ⚫ **Saturated** — Possible contamination in the sample
- 🔵 **Zero** — No current is passing through this pore, possibly due to bubbles on the membrane
- 🟢 **Unavailable** — Pore inhibited from sequencing
- 🔵 **Inactive** — Pore no longer suitable for further sequencing

Troubleshooting

High proportion Unavailable
Possible contaminants in library blocking the pore. Consider using the Flow Cell Wash Kit, and reloading a library.
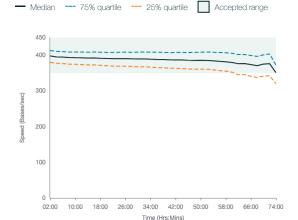
High proportion Inactive
If localised to one area of the Flow Cell, this could indicate that an air bubble has been introduced during the flushing/loading steps. If inactivity is spread across the Flow Cell this could be caused by improper loading of the library, please refer to the user guide for further support.

TRANSLOCATION SPEED

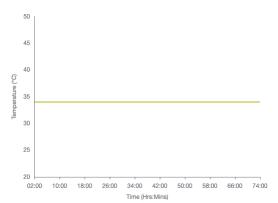The translocation speed is the rate at which DNA/RNA travels through pores as it is being sequenced.

Legend

— Median   --- 75% quartile   --- 25% quartile   ▢ Accepted range

Troubleshooting

Low speed
Check that the Flow Cell is within the target temperature range.

Note
Low-quality and short reads are not included in this graph.

TEMPERATURE

The temperature of the Flow Cell over the run time.

Legend

— Measured   — Target

Troubleshooting

Out of range
Check that the Flow Cell is correctly seated and firmly pushed down into the device. Ensure ambient temperature is always within the specified range for your device in the user guide.

Air flow should be good but not excessive. Excessive amounts of cool air blowing on the device could prevent it from reaching target temperature.

Run log

SYSTEM MESSAGES

System messages are a record of the events that occurred in the time covered by this report.

🚫 Errors

None

⚠️ Warnings

None

ℹ️ Events

Disk space · 30 Dec 22, 01:31
Disk /data has 20500 GB space remaining

Waiting for temperature · 30 Dec 22, 01:32
Waiting up to 300 seconds for temperature to stabilise at 34.0°C

Message · 30 Dec 22, 01:37
300 seconds have elapsed. Experiment commencing.

Starting · 30 Dec 22, 01:37
Starting sequencing procedure

Mux scan starting · 30 Dec 22, 01:37
Performing Pore Scan

Mux scan result · 30 Dec 22, 01:40
Pore scan for flow cell PAI23763 has found a total of 6648 pores. 2549 pores available for immediate sequencing

Message · 30 Dec 22, 01:50
Setting temperature to reach 33.76°C

Message · 30 Dec 22, 02:00
Setting temperature to reach 33.6299983215332°C

Message · 30 Dec 22, 02:20
Setting temperature to reach 33.76999725341797°C

Mux scan starting · 30 Dec 22, 03:11
Performing Pore Scan

Mux scan result · 30 Dec 22, 03:13
Pore scan for flow cell PAI23763 has found a total of 6479 pores. 2513 pores available for immediate sequencing

Mux scan starting · 30 Dec 22, 04:44
Performing Pore Scan

Mux scan result · 30 Dec 22, 04:47
Pore scan for flow cell PAI23763 has found a total of 6342 pores. 2449 pores available for immediate sequencing

Mux scan starting · 30 Dec 22, 06:18
Performing Pore Scan

Mux scan result · 30 Dec 22, 06:21
Pore scan for flow cell PAI23763 has found a total of 6184 pores. 2240 pores available for immediate sequencing

Mux scan starting · 30 Dec 22, 07:52
Performing Pore Scan

Mux scan result · 30 Dec 22, 07:54
Pore scan for flow cell PAI23763 has found a total of 6004 pores. 2198 pores available for immediate sequencing

Mux scan starting · 30 Dec 22, 09:25
Performing Pore Scan

Mux scan result · 30 Dec 22, 09:28
Pore scan for flow cell PAI23763 has found a total of 5926 pores. 2291 pores available for immediate sequencing

Mux scan starting · 30 Dec 22, 10:59
Performing Pore Scan

Mux scan result · 30 Dec 22, 11:01
Pore scan for flow cell PAI23763 has found a total of 5814 pores. 2118 pores available for immediate sequencing

Mux scan starting · 30 Dec 22, 12:32
Performing Pore Scan

Mux scan result · 30 Dec 22, 12:35
Pore scan for flow cell PAI23763 has found a total of 5656 pores. 1953 pores available for immediate sequencing

Mux scan starting · 30 Dec 22, 14:06
Performing Pore Scan

Mux scan result · 30 Dec 22, 14:09
Pore scan for flow cell PAI23763 has found a total of 5516 pores. 2174 pores available for immediate sequencing

Mux scan starting · 30 Dec 22, 15:40
Performing Pore Scan

Mux scan result · 30 Dec 22, 15:42
Pore scan for flow cell PAI23763 has found a total of 5375 pores. 2070 pores available for immediate sequencing

Mux scan starting · 30 Dec 22, 17:13
Performing Pore Scan

Mux scan result · 30 Dec 22, 17:16
Pore scan for flow cell PAI23763 has found a total of 5254 pores. 1709 pores available for immediate sequencing

Mux scan starting · 30 Dec 22, 18:47
Performing Pore Scan

Mux scan result · 30 Dec 22, 18:49
Pore scan for flow cell PAI23763 has found a total of 5149 pores. 2053 pores available for immediate sequencing

Mux scan starting · 30 Dec 22, 20:21
Performing Pore Scan

Mux scan result · 30 Dec 22, 20:23

Pore scan for flow cell PAI23763 has found a total of 5008 pores. 1792 pores available for immediate sequencing

Mux scan starting · 30 Dec 22, 21:54
Performing Pore Scan

Mux scan result · 30 Dec 22, 21:57
Pore scan for flow cell PAI23763 has found a total of 4944 pores. 1901 pores available for immediate sequencing

Mux scan starting · 30 Dec 22, 23:28
Performing Pore Scan

Mux scan result · 30 Dec 22, 23:30
Pore scan for flow cell PAI23763 has found a total of 4800 pores. 1850 pores available for immediate sequencing

Mux scan starting · 31 Dec 22, 01:01
Performing Pore Scan

Mux scan result · 31 Dec 22, 01:04
Pore scan for flow cell PAI23763 has found a total of 4666 pores. 1614 pores available for immediate sequencing

Mux scan starting · 31 Dec 22, 02:35
Performing Pore Scan

Mux scan result · 31 Dec 22, 02:38
Pore scan for flow cell PAI23763 has found a total of 4600 pores. 1534 pores available for immediate sequencing

Mux scan starting · 31 Dec 22, 04:09
Performing Pore Scan

Mux scan result · 31 Dec 22, 04:11
Pore scan for flow cell PAI23763 has found a total of 4495 pores. 1980 pores available for immediate sequencing

Mux scan starting · 31 Dec 22, 05:42
Performing Pore Scan

Mux scan result · 31 Dec 22, 05:45
Pore scan for flow cell PAI23763 has found a total of 4374 pores. 1670 pores available for immediate sequencing

Mux scan starting · 31 Dec 22, 07:16
Performing Pore Scan

Mux scan result · 31 Dec 22, 07:19
Pore scan for flow cell PAI23763 has found a total of 4239 pores. 1430 pores available for immediate sequencing

Mux scan starting · 31 Dec 22, 08:50
Performing Pore Scan

Mux scan result · 31 Dec 22, 08:52
Pore scan for flow cell PAI23763 has found a total of 4149 pores. 1724 pores available for immediate sequencing

Mux scan starting · 31 Dec 22, 10:23
Performing Pore Scan

Mux scan result · 31 Dec 22, 10:26
Pore scan for flow cell PAI23763 has found a total of 4018 pores. 1625 pores available for immediate sequencing

Mux scan starting · 31 Dec 22, 11:57
Performing Pore Scan

Mux scan result · 31 Dec 22, 11:59
Pore scan for flow cell PAI23763 has found a total of 3972 pores. 1383 pores available for immediate sequencing

Mux scan starting · 31 Dec 22, 13:30
Performing Pore Scan

Mux scan result · 31 Dec 22, 13:33
Pore scan for flow cell PAI23763 has found a total of 3858 pores. 1597 pores available for immediate sequencing

Mux scan starting · 31 Dec 22, 15:04
Performing Pore Scan

Mux scan result · 31 Dec 22, 15:07
Pore scan for flow cell PAI23763 has found a total of 3753 pores. 1474 pores available for immediate sequencing

Mux scan starting · 31 Dec 22, 16:38
Performing Pore Scan

Mux scan result · 31 Dec 22, 16:40
Pore scan for flow cell PAI23763 has found a total of 3695 pores. 1471 pores available for immediate sequencing

Mux scan starting · 31 Dec 22, 18:11
Performing Pore Scan

Mux scan result · 31 Dec 22, 18:14
Pore scan for flow cell PAI23763 has found a total of 3570 pores. 1422 pores available for immediate sequencing

Mux scan starting · 31 Dec 22, 19:45
Performing Pore Scan

Mux scan result · 31 Dec 22, 19:48
Pore scan for flow cell PAI23763 has found a total of 3479 pores. 1540 pores available for immediate sequencing

Mux scan starting · 31 Dec 22, 21:19
Performing Pore Scan

Mux scan result · 31 Dec 22, 21:21
Pore scan for flow cell PAI23763 has found a total of 3409 pores. 1364 pores available for immediate sequencing

Mux scan starting · 31 Dec 22, 22:52
Performing Pore Scan

Mux scan result · 31 Dec 22, 22:55
Pore scan for flow cell PAI23763 has found a total of 3288 pores. 1382 pores available for immediate sequencing

Mux scan starting · 01 Jan 23, 00:26
Performing Pore Scan

Mux scan result · 01 Jan 23, 00:28
Pore scan for flow cell PAI23763 has found a total of 3200 pores. 1268 pores available for immediate sequencing

Mux scan starting · 01 Jan 23, 01:59
Performing Pore Scan

Mux scan result · 01 Jan 23, 02:02
Pore scan for flow cell PAI23763 has found a total of 3096 pores. 1405 pores available for immediate sequencing

Mux scan starting · 01 Jan 23, 03:33
Performing Pore Scan

Mux scan result · 01 Jan 23, 03:36
Pore scan for flow cell PAI23763 has found a total of 2988 pores. 1243 pores available for immediate sequencing

Mux scan starting · 01 Jan 23, 05:07
Performing Pore Scan

Mux scan result · 01 Jan 23, 05:09
Pore scan for flow cell PAI23763 has found a total of 2941 pores. 1265 pores available for immediate sequencing

Mux scan starting · 01 Jan 23, 06:40
Performing Pore Scan

Mux scan result · 01 Jan 23, 06:43
Pore scan for flow cell PAI23763 has found a total of 2888 pores. 1234 pores available for immediate sequencing

Mux scan starting · 01 Jan 23, 08:14
Performing Pore Scan

Mux scan result · 01 Jan 23, 08:17
Pore scan for flow cell PAI23763 has found a total of 2778 pores. 1222 pores available for immediate sequencing

Mux scan starting · 01 Jan 23, 09:48
Performing Pore Scan

Mux scan result · 01 Jan 23, 09:50
Pore scan for flow cell PAI23763 has found a total of 2731 pores. 1167 pores available for immediate sequencing

Mux scan starting · 01 Jan 23, 11:21
Performing Pore Scan

Mux scan result · 01 Jan 23, 11:24
Pore scan for flow cell PAI23763 has found a total of 2656 pores. 1128 pores available for immediate sequencing

Mux scan starting · 01 Jan 23, 12:55
Performing Pore Scan

Mux scan result · 01 Jan 23, 12:58
Pore scan for flow cell PAI23763 has found a total of 2525 pores. 1065 pores available for immediate sequencing

Mux scan starting · 01 Jan 23, 14:29
Performing Pore Scan

Mux scan result · 01 Jan 23, 14:31
Pore scan for flow cell PAI23763 has found a total of 2197 pores. 917 pores available for immediate sequencing

Mux scan starting · 01 Jan 23, 16:02
Performing Pore Scan

Mux scan result · 01 Jan 23, 16:05
Pore scan for flow cell PAI23763 has found a total of 1902 pores. 720 pores available for immediate sequencing

Mux scan starting · 01 Jan 23, 17:36
Performing Pore Scan

Mux scan result · 01 Jan 23, 17:38
Pore scan for flow cell PAI23763 has found a total of 1901 pores. 847 pores available for immediate sequencing

Mux scan starting · 01 Jan 23, 19:10
Performing Pore Scan

Mux scan result · 01 Jan 23, 19:12
Pore scan for flow cell PAI23763 has found a total of 1746 pores. 786 pores available for immediate sequencing

Mux scan starting · 01 Jan 23, 20:43
Performing Pore Scan

Mux scan result · 01 Jan 23, 20:46
Pore scan for flow cell PAI23763 has found a total of 1490 pores. 717 pores available for immediate sequencing

Mux scan starting · 01 Jan 23, 22:17
Performing Pore Scan

Mux scan result · 01 Jan 23, 22:19
Pore scan for flow cell PAI23763 has found a total of 1186 pores. 511 pores available for immediate sequencing

Mux scan starting · 01 Jan 23, 23:50
Performing Pore Scan

Mux scan result · 01 Jan 23, 23:53
Pore scan for flow cell PAI23763 has found a total of 920 pores. 394 pores available for immediate sequencing

Mux scan starting · 02 Jan 23, 01:24
Performing Pore Scan

Mux scan result · 02 Jan 23, 01:27
Pore scan for flow cell PAI23763 has found a total of 769 pores. 343 pores available for immediate sequencing

Message · 02 Jan 23, 01:38
The sequencing run has finished, but basecalling may continue

UNIT ABBREVIATIONS

Byte	B	**Base**	b	**Minutes**	mins
Kilobyte	KB	**Kilobase**	kb	**Hours**	hrs
Megabyte	MB	**Megabase**	Mb		
Gigabyte	GB	**Gigabase**	Gb		
Terabyte	TB	**Terabase**	Tb		