UCDAVIS

Lisa Cohen <ljcohen@ucdavis.edu>

Chat transcript

LiveChat <support@livechatinc.com> Mon, Aug 1, 2016 at 11:56 AM
Reply-To: ljcohen@ucdavis.edu
To: ljcohen@ucdavis.edu

Chat transcript

First Name Lisa
Last Name Cohen
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Organisation: UC Davis

Cerri Mon, 08/01/16 11:16:27 am America/New_York
Hello. How may I help you?

Lisa 11:16:45 am
Hi, I have several technical questions related to our MinION

Cerri 11:17:17 am
Lisa, thank you for your message. I can certainly raise a case with our technical team.
Someone will be in contact with you as soon as possible to discuss

Lisa 11:17:30 am
I was hoping to get answers right away
on the phone perhaps, is there someone that I could call?

Cerri 11:18:13 am
I can see if one of my colleagues is available.

Lisa 11:18:35 am
thank you so much
Here are my questions: https://github.com/ljcohen/dib_ONP_MinION/tree/master/Ectocooler

Cerri 11:19:55 am

Ok, thank you. I'm going to transfer you to Richard now.

Technical Support 11:20:15 am
Hi Lisa

Lisa 11:20:24 am
Hello!
Thank you for answering my questions
We just did our burnin of Mk1B last week...I sent the results to support but have not heard back yet. We did a run over the weekend but the harddrive filled up and the reads were much shorter than expected. 11:21:11 am
So, I need to clean the harddrive and figure out what happened to the DNA, if it was extractions or library prep
What files can I delete? 11:22:03 am

Technical Support 11:22:19 am
How much space do you currently have on your hard drive?

Lisa 11:22:30 am
13 GB
out of 220 GB
in the device directory, there are 'uploads' and 'downloads' directories 11:23:25 am

Technical Support 11:23:29 am
Ok I think given the current potential throughputs from a flow cell with a good library prep we need to free up a lot of space.

Lisa 11:23:42 am
how much space should be free for 1 run?

Technical Support 11:23:49 am
The uploaded files are the un basecalled versions
the download folder are the basecalled files 11:24:03 am
you could delete the uploaded.
if you want to rebasecall the downloaded versions will work without issue

Lisa 11:24:35 am
yes, Ok I will delete the uploads

Technical Support 11:25:15 am
The SSD is only required by the MinION when reads are being streamed. Once they have completed they can be moved to any other hard drive (even a HDD) without issue
its probably worth setting this up. - Do you have a HDD on your computer?

Lisa 11:25:47 am

We are working on getting an external harddrive

Technical Support 11:26:28 am
I would definitely look to do that as you can then set Metrichor up to move all reads off of the SSD and onto the HDD during basecalling helping to free up space.

Lisa 11:26:57 am
yes, that is a good idea

Technical Support 11:27:21 am
Im happy to talk you through the process once you have the HDD setup and ready to use.

Lisa 11:28:05 am
ok, thank you - that will take us some time to locate as we are currently in a field course with limited resources, but we are working on it
ok
i deleted the uploads now, which freed about 15 GB
what is the difference between 'pass' and 'fail' downloads?

Technical Support 11:28:49 am
The main difference between pass and fail is the quality of the read itself.
We use a Q score of >9 as a pass

Lisa 11:29:09 am
i set the wrong app on Metrichor for our run to 'barcodes' and since we don't have barcodes, the reads were downloaded into the 'fail' directory, I believe
is taht the case?
(I'm hoping because all of the reads for that run were directed into the 'fail' directory)

Technical Support 11:30:00 am
That could well be the case - I suspect you might see unable to classify barcode (or something similar - I cant remember the exact exit status) within the fast5 file

Lisa 11:30:18 am
yes, there was a directory called 'unclassified'
some of the 'fail' reads were sent to 'unclassified' and others were just in the 'fail'

Technical Support 11:30:50 am
that will be the reads that may have passed but were unable to be classified.

Lisa 11:30:57 am
ok, great
can I delete the 'pass' fast5 from the burnin? 11:31:15 am

Technical Support 11:31:26 am

either way the best thing is to remove all these files (both fail and pass) back into the reads folder and run the basecalling again with the correct workflow
You can delete these as long as you dont want to look or analyse them again

Lisa 11:32:00 am
ok

Technical Support 11:32:32 am
you probably want to try and free up a bit more space as well - 30Gb isnt a huge amount for a run.

Lisa 11:32:46 am
yes, I'm trying!
how much space do you recommend for 1 run? 11:33:00 am

Technical Support 11:33:04 am
Is there much MinKNOW data?
It really depends on the sample prep and flow cell quality but personally I would look at >100Gb (hence it would be good to use Metrichor to move the files off the SSD)

Lisa 11:33:50 am
the 'MinIon_device' directory has 117 GB
are files stored on Metrichor? 11:34:06 am

Technical Support 11:34:19 am
not currently - they are deleted shortly after basecalling

Lisa 11:34:24 am
I see

Technical Support 11:34:46 am
when you say MinION directory do you mean the data folder?

Lisa 11:34:51 am
yes

Technical Support 11:35:39 am
The best way to free up space is really going to be in here then. Is most of that in the reads folder and/or downloaded folder?

Lisa 11:36:07 am
yes, in 'downloaded'
So, to re-do the basecalling for the run that stopped because of space, where I started the wrong App, you're suggesting to move all of the fast5 from 'fail' and 'unclassfied' into the 'reads' directory, then start Metrichor again? 11:37:14 am

Technical Support 11:37:21 am
yeahy exactly that.
its a lot easier to rebasecall with the correct workflow rather than workout what might have happened

Lisa 11:37:52 am
the 'reads' directory is located at the same tree level as 'uploaded' and 'downloaded', is that the one you're referring to?

Technical Support 11:38:37 am
Yeah thats the one (for default settings - C:\data\reads)

Lisa 11:39:38 am
inside the 'reads' directory, there is a 'downloads' directory with files named, e.g. telemetry and agent
when I move files, I should just put them in 'reads', not in this 'downloads' directory? 11:40:15 am

Technical Support 11:41:42 am
In the standard setup MinKNOW outputs reads to C:\data\reads. Metrichor then takes reads from this location and basecalls them. Basecalled files are out put into the downloads folder (C:\data\reads\downloads) and the original reads are moved to the uploaded folder (C:\data\reads\uploaded).
As long as you have the standard setup then yes all you need to do is move all the ffast5 files out of pass, fail and unclassified and back into the reads folder. 11:42:22 am

Lisa 11:43:16 am
ok, doing that now

Technical Support 11:43:25 am
Yeah no worries.

Lisa 11:44:06 am
Also, I had a question about the number of pores in teh flowcells, they seem to fluctuate from day to day
when they arrived last week, we received a flowcell with 0 pores
can this be replaced?
Another flowcell I ran QC and it had ~900 pores, the next day it had ~600 pores

Technical Support 11:44:48 am
0 pores usually results from an air bubble in the inlet channel blocking the current flow from the common electrode.

Lisa 11:44:58 am
they are being stored at 4degC, why is there fluctuation?
ok, how can I removed the air bubble? 11:45:24 am
it is too late? this was last week when it arrived

Technical Support 11:45:47 am
starting with the 0 pore flow cell I recommend that you try removing ~15ul of buffer from the inlet port as this is enough to remove any air bubbles in the inlet channel without uncovering the array.
I would then run the platform QC again. I would also keep an eye on the MinION temperature 11:46:08 am
If this is a bit high it can result in pores being miss classified.
It shouldnt be too late to try this.

Lisa 11:46:55 am
ok, I will try removing 15ul of fluid from the flowcell with 0 pores

Technical Support 11:47:42 am
For the pore fluctuations - there can over a course of time be a drop off in pore numbers as a result of air bubbles but I wouldnt expect this in such a quick period of time
Was there any visible air bubble in this flow cell (or the one with 0 pores)? 11:48:01 am
Air bubbles will certainly cause a drop off in pore numbers as a result of membrane disruption 11:50:39 am

Lisa 11:52:13 am
there was a bubble that I tried to remove by pipetting a small amount

Technical Support 11:52:38 am
Is that in the 0 pore flow cell or the other one? Did this bubble pass over the array?

Lisa 11:52:45 am
the 0 pore flow cell
there were no visible bubbles on the flowcell that dropped from ~900 to ~600 11:53:00 am
are you located in teh US or the UK?

Technical Support 11:53:31 am
In the UK

Lisa 11:54:55 am
I have a ride now to the store to get an external harddrive so need to sign off. You have been SOOO helpful, thank you so much. I'm uploading reads to Metrichor now, and think I can handle switching teh downloads directory to the external HHD when I return. Is there tech support in teh US who I can contact if I need help, as I think your day will be finished by the time I get back in a few hrs?

Technical Support 11:55:00 am
Thats fine - I will get one of our US technical support team to get in touch (or you can email me on richard.ronan@nanoporetech.com and I will get back to you tomorrow)

Lisa 11:55:59 am

thank you, Richard. I would very much appreciate being intouch with US tech support team as well. They will get in touch via email?

My email is ljcohen@ucdavis.edu 11:56:04 am

Technical Support 11:56:30 am

yeah thats correct - I assume you are already setup on our system so I can get your details from there.

Lisa 11:56:38 am

yes, thank you!

have a good day, bye Richard

Duration: 40m 26s

Chat started on: https://store.nanoporetech.com/

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