UCDAVIS

Lisa Cohen <ljcohen@ucdavis.edu>

Chat transcript

LiveChat <support@livechatinc.com> Tue, Aug 2, 2016 at 11:30 AM
Reply-To: ljcohen@ucdavis.edu
To: ljcohen@ucdavis.edu

Chat transcript

First Name Lisa
Last Name Cohen
E-mail: ljcohen@ucdavis.edu
Organisation: UC Davis

Anita Tue, 08/02/16 10:25:36 am America/New_York
Hello. How may I help you?

Lisa 10:26:33 am
Hello, I was chatting with Richard Ronan yesterday about some technical issues. Would you be able to transfer me to him now? I have a run going right now and not sure whether to stop it.

Anita 10:27:34 am
Thank you for your message
Let me just check this now for you
Great i will now transfer you to Richard 10:30:18 am

Technical Support 10:30:41 am
Hi LIsa

Lisa 10:31:22 am
Hello, thank you
I have a run going right now, downloading to an external harddrive through Metrichor 10:32:00 am
like you mentioned yesterday
ID 112459, if you want to look at it
it started ~9 hrs ago, but there are only ~1500 reads as far as I can tell 10:33:27 am
this seems low

there were ~1000 pores on the flow cell
not sure how to tell if it's still going? how do I tell when it has finished? 10:34:26 am

Technical Support 10:34:44 am
Thats does seem quite like a low throughput given the number of pores
do you know how many channels were reported by the MUX scan?
you may be able to see if you scroll up the MinKNOW messages 10:35:31 am

Lisa 10:36:01 am
it only goes down to group_3 97 active pores
group_4 active 21 pores

Technical Support 10:36:31 am
Is it that these have moved off the top?
for 97 in Group 3 I would imagine the MUX scan has been ok. 10:37:02 am
Could you also check the channels states on the physical layout tab of the GUI?
what colour are they?

Lisa 10:37:55 am
the notifications list runs out, I dno't have groups 1 or 2, although I remember it going through these mux scans and the numbers looked normal
channel states? 10:38:21 am
there are 378 zero (light blue)

Technical Support 10:38:39 am
Given the number of channels in Group 3 and 4 I imagine 1 and 2 would have been quite a bit higher indicating the channel is ok.
How many classified as single pore, strand or unavilable?

Lisa 10:39:16 am
8 unavailable
26 single pore
3 strand
15 saturated
what do these mean?

Technical Support 10:40:22 am
unavilable is stalled chemistry - this will be removed periodically by the software
single pore is a pore without chemistry, strand is a pore with chemistry
on a good run these should be high with most in the strand status

Lisa 10:41:13 am
the top notifications are "Lost 1 data points in the last 4184 seconds" at 9:16 AM and "advancing to group 2" at 7:56 AM

Technical Support 10:41:47 am

sturated are channle that were passing too much current so switched off to save the flow cell - generally wells where the membraqne has been disrunpted

light blue means that no current is being passed by these channels - this usually means they are exhausted. 10:42:16 am

Lisa 10:42:23 am

since the strand number is only 2, that means there are only 2 channels with sequences?

Technical Support 10:43:03 am

that means only 2 strands are currently sequecing. Yoiu should expect the single pore channels to move to the strand status at some point when they capture the DNA

Lisa 10:43:13 am

so, should I stop the run? I'm not sure what the problem is.

Technical Support 10:43:51 am

From looking at what you ahev said I would say the first thing to invesitgate is the library prep.

Lisa 10:44:02 am

I see

Technical Support 10:44:05 am

Have other runs been ok and simply this one at a low performance?

Lisa 10:44:10 am

the yield was a bit low, 4.41 ng/ul

Technical Support 10:44:25 am

that could cause an issue with throughput

Lisa 10:45:59 am

when I loaded, I doubled the volume, so 75 ul RBF1, 51 ul NFW and 24 pre-sequencing mixul

Technical Support 10:46:13 am

that should help although not ideal

Lisa 10:46:22 am

I see

Technical Support 10:46:45 am

do you know what step you lost more than expected of your library?

Lisa 10:47:34 am

I was emailing with Concetta yesterday and decided not to do a gtube shearing but add FFPE

I don't know what step the DNA was lost

Technical Support 10:48:02 am

and your starting DNA concentration was still 1ug?

Lisa 10:48:30 am

I added 1,872 ng to the FFPE reaction

Technical Support 10:48:41 am

ok - plenty of DNA

Lisa 10:48:45 am

yes

Technical Support 10:48:57 am

just to check have any errors been reported on the MinKNOW GUI?

Lisa 10:49:30 am

just 1 data point in the last 4184 seconds

according to the notifications

Technical Support 10:49:54 am

ok thats perfectly fine.

How did your burn in look? 10:50:12 am

Lisa 10:50:20 am

this is the FFPE protocol I followed, with bead cleanup:

https://www.neb.com/protocols/2015/01/16/protocol-for-use-with-other-user-supplied-library-construction-reagents-neb-m6630

burnin was ok, metrichor id: 112029

Technical Support 10:51:39 am

I assume on the burn in you did shear?

with a g-tube

Lisa 10:52:24 am

yes

Technical Support 10:52:52 am

I wonder if it would be worth running your own library with a shearing step to start with and then try the non-shearing one

Lisa 10:53:06 am

ok

Technical Support 10:53:21 am
its possible that the DNA has sheared in the final bead step (or during loading for instance)
if the DNA is sheared and loses the nanopore specifric adapters then this would not be sequenced
Did you add DNA CS?

Lisa 10:53:50 am
yes

Technical Support 10:54:02 am
Is that coming through in the analysis?
if thats showing and your DNA not that would definitely indicate that this could be the case.

Lisa 10:54:40 am
I don't know how to tell whether it is coming through
Will it say in the metrichor report? 10:55:12 am

Technical Support 10:55:25 am
Looking at the Metrichor QC data - about 1/3rd of your strands have a 2D

Lisa 10:55:50 am
which page is the QC on?
I'm on the metrichor report now
"Basecalling 2D" 10:56:14 am

Technical Support 10:56:15 am
I was looking at the Basecall 1D tab
now Im on the 2D and I can see of the 1/3rd that are 2D very few were successful
26 in total

Lisa 10:57:11 am
what does that mean?

Technical Support 10:57:18 am
and of those 1 is detected as calibration strand
That would indicate that the whole library may well have failed rather than just your DNA (although there can be some corss talk if your library for instance caused the pores to block) 10:58:08 am
I think the best thing though is to go back to a sheared library and then once that is running well try a non sheared version

Lisa 10:58:44 am

ok, so the library was bad

Technical Support 10:59:18 am
That would be my starting point when it comes to working things out - it certainly seems most likely

Lisa 10:59:34 am
any ideas for what to fix?
I did exactly the same protocol as the lambda burnin, except did not shear and added FFPE

Technical Support 11:00:14 am
I think with the unsheared method there are a host of things that could cause the DNA to shear and lose the nanopore sepcific adapters after this FFPE step.

Lisa 11:00:26 am
Ok
So, I will shear with the same settings as the lambda burnin, 6000 rpm 2 x 1 min

Technical Support 11:00:52 am
once the adapters are added any shearing of these will result in the strands being un readable by the nanopore (or at least 1D only)
yeah I think thats the best starting point 11:01:02 am

Lisa 11:01:24 am
then do the NEB FFPE with bead cleanup, followed by the NEB end-repair/dA tailing

Technical Support 11:01:34 am
yeah thats sounds sensible

Lisa 11:01:52 am
will there be some lost during the FFPE bead cleanup?

Technical Support 11:02:09 am
possibly - its worth quantifying to find out
you should aim for 1ug of DNA post FFPE 11:03:36 am

Lisa 11:03:37 am
the end of the FFPE beadcleanup is supposed to be 32 µl elution, but instead I elute with 47 and add 45 ul to the end-prep steps
ok, aim for 1 ug post FFPE
Ok, I will do this again 11:04:19 am

Technical Support 11:04:45 am
the FFPE step with all quantities can be foun - https://community.nanoporetech.com/protocols/genomic-dna-sqk-nsk007/v/gde_

9002_v7_revc_16may2016-449/dna-repair

Lisa 11:04:57 am
great, thank you
Can I ask you another question about MinKnow software? 11:05:06 am

Technical Support 11:05:17 am
of course you can

Lisa 11:06:17 am
I installed and used poretools to make fastq from our last run. but when I went to minKnow to start the QC for the run last night, the QC script did not work because poretools had changed the registry and was pointing to another version of Anaconda

Technical Support 11:06:30 am
I have heard of that happening previously to other users

Lisa 11:06:32 am
I found the answer on the forum somewhere to uninstall MinKnow completely and had to reinstall it
but I want to use poretools
this seems unreasonable to have to keep uninstalling and installing

Technical Support 11:07:19 am
Yeah I can see that would be annoying

Lisa 11:07:43 am
I'm not a Windows user typically. in linux I would create a conda env to separate my versions of Anaconda
is there no way to use poretools? 11:08:03 am
and MinKnow?

Technical Support 11:08:21 am
I would need to look into this and find out

Lisa 11:08:26 am
thank you
is there another way to make fastq?

Technical Support 11:09:06 am
There are quite a few different tools that can extract the fastQ from the Fast5 files
hang on I will find a link

Lisa 11:09:24 am
thank you

Technical Support 11:10:27 am

its old community but - https://wiki.nanoporetech.com/pages/viewpage.action?title=Community+discussions+on+data+analysis&spaceKey=DS

Lisa 11:10:51 am

that link tells me that I do not have access

Technical Support 11:11:38 am

Could you try this link and click through the see here at the bottom - https://community.nanoporetech.com/posts/community-discussions-on-d-102

Lisa 11:12:39 am

when I click on the links on that page, the same thing happens that I don't have access

Technical Support 11:13:14 am

I will try a cut and paste

poRe

R package for the visualization and analysis of nanopore sequencing data (Mick Watson)

Poretools

A toolkit for working with Oxford nanopore data (Nick Loman)

Contents

Julia FAST5>FASTQ converter

Details on working with Julia to convert FAST5 to FASTQ files (Keith Robison)

Contents

RichPoreToolKit

An R-bioconductor package that can process all FAST5 files from Oxford Nanopore Devices (David Eccles)

Bash Script

To extract fastq files from the Fast5 files (Mahesh Panchal)

IONiserR

IONiseR provides tools for the quality assessment of Oxford Nanopore MinION data.

HPG Pore

A toolkit to explore and analyze nanopore sequencing data

thats not great. I will summarise and email (or download a pdf version of this page)

Lisa 11:13:53 am

great, thank you

Ok, I will get going and start another library now 11:14:14 am

thank you for your help

we are using almost all of our flowcells to troubleshoot, can these be re-used or is it possible for your send us replacements? we were sent flowcells last week with bubbles and zero pores measured during QC 11:15:29 am

Imentioned this to Concetta and will get her the flowcell ID numbers, but I am in a course and need these flowcells this week to actually collect data

by Fri

Technical Support 11:16:24 am

getting them replaced this week will be very tight - if you could get the flow cells IDs to Concetta ASAP I will see what logisitcs can do

Lisa 11:16:32 am

thank you

Technical Support 11:16:38 am

we only ship to US on Thursday's I believe

If you could send those IDs to Concetta now I will give her a phone call in 10minutes and discuss what we might be able to do 11:17:20 am

Lisa 11:18:21 am

that would be great, thank you - I am writing them down now and will email her and cc you

Technical Support 11:19:20 am

excellent - I will have a word with logistcs straight away then

we should be able to do Friday but I will let you know

speak shortly - I will reply to your email with the link for the tools (in pdf version) 11:21:07 am

Lisa 11:29:51 am

thank you, just sent

will look for email with link for tools 11:30:30 am

Duration: 1h 5m 2s

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