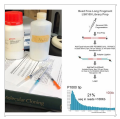

Jan 29, 2020

Rocky Mountain adventures in Genomic DNA sample preparation, ligation protocol optimisation / simplification and Ultra long read generation

John Tyson[1]

[1]Snutch Lab, UBC, Vancouver, BC, Canada

1 Works for me dx.doi.org/10.17504/protocols.io.7euhjew

High molecular weight DNA extraction from all kingdoms Long Read Club

John Tyson

ABSTRACT

We have been playing quite a bit with native genomic DNA sequencing for a project we have running on Epigenetic modifications of Rat models of neurological disease. While we are still rapidly iterating on tweaks etc. to some new protocols around increasing our production efficiency of ultra-long reads (100kb+) using LSK109 ligation kit components and PEG/NaCl mediated DNA precipitation, we are settling into a workflow now that I think is worth sharing and has been generating very good results for us. That said remember this is a little “wild west” and perhaps for the more adventurous at this stage, use at your own risk etc. etc. and perhaps help with some tweaks of your own ;o). I’ll say now that I have been optimizing this on a very easy Rat cell line grown in culture and realise some people don’t have this luxury, however I think this is a very good jumping off point and allowed us to iterate on a consistent starting material and will myself be venturing off into tissue extractions now.

There are currently three main areas to highlight and detail changes within a sample to sequence workflow that we think has allowed us to push the efficiency and yields of sequence data out. Firstly DNA extractions using Phenol/Chloroform and spooling out of HWM DNA to generate the starting DNA sample. Secondly input material shearing and modifications to the LSK109 ligation protocol that have yielded high efficiency libraries, and production of greater ultra-long reads numbers. Lastly the use of flowcell refueling and DNAseI clearing of the flowcell surface to remove “blocking” DNA and allow fresh library addition after a surface “reset”.

I’m detailing all we have here is an attempt to allow people to fully understand what is going on as much as possible and so we can all use this information to make intelligent changes to the protocols etc. if you are so inclined. Commercial “black box kits” and solutions while great for defined on mass purpose and reproducibility make this process more opaque and often overly complex and expensive. A good example of this is the addition of just NaCl after the ligation reaction to precipitate the adapted DNA showing how knowing what you have provides simplification and economic sense for the end user in some situations, but more on that later……

Final thoughts….

One of my interests around nanopore sequencing and moving sequencing back to small labs and beyond with the MinION device is also mitigating the sample preparation costs while not sacrificing performance. Using cheap needles, Polyethylene Glycol, salt and old school molecular biology techniques we are seeing uncompromising performance for both yield and read lengths that I think even the “big” guys will use :o)). I will reproduce this post and methods at www.longreadclub.org soon so they will be easier to find and develop going forward, and we will try and provide protocols on www.protocols.io as well.

Things on the to do list include:

- Further optimisation of PEG/salt parameters for size selection / short read elimination at different stages of library preparation.
- Refinement of shearing to provide increased 100kb+ reads from HMW DNA
- Look at replacing Phenol/Chloroform with salting out in initial HMW Genomic DNA preparation.

Who knew that PEG and salt would be a route to cheap ultra-long reads, we all just need to keep tweaking away in an open fashion and who knows where we can get. Anyway that’s enough from me for now, happy Nanopore adventuring!

EXTERNAL LINK

https://www.longreadclub.org/mountain-protocol/

SAFETY WARNINGS

Please see SDS (Safety Data Sheet) for hazards and safety warnings.

Files


Phenol/Chloroform Genomic DNA extraction from Tissue Culture cells
by John Tyson


Modified LSK109 ligation prep with needle shear and bead clean up
by John Tyson


DNaseI and flowcell clearing for increasing long read yields and multi-sample sequencing
by John Tyson


Size Selective Precipitation of DNA using PEG & Salt
by John Tyson


Bead-free long fragment LSK109 library preparation
by John Tyson