DNA extraction from gram negative bacteria for ONT MinION sequencing

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Abstract

This is the protocol I have developed to genterate High'ish' Molecular Weight gDNA from gram negative bacterial speices to use for ONT MinION sequencing.

This protocol was intially developed for the high throughput extraction of bacterial gDNA for Illumina short read sequencing. To perform our high throughput work we utislise Beckman Coulter Biomek liquid handling robots and the Beckman Coutler gDNA extraction kit Genfind v2. This kit utilises magnetic pariticles and is relatively gentle. We have found that gDNA extracted using this method has a high'ish' molecular weight. On a fragment analyser the GNDA is typically a clean single peak >60 kb (the limits of the fragment analyser). ONT MinION libraries generated wiht this gDNA typically have N50s in excess of 20 kb and the data is suitable for the assembly automated of almost bacterial genomes we have tested to date. This protocol will not give you the ultra long 'whale' reads that others have reported but it is a good compromise between HMW DNA, speed and ease of use and capacity to scale up to high throughput.

NOTE

We have found that gDNA extracted using this method often have underepresentation of smaller plamids (<20kbp). We are not sure exactly why this happens but possible reason are; the circular replicons do not bind as readily to the magnetic particles and so are excluded from the extraction or the plamids remain circule and so are not availabe to adapter ligation in the library prep. I will upload an additonal protocol for gDNA extraaction that focuses on enrichment of plasmid sequences.

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Protocol

Bacterial culturing

Step 1.

1) Inoculate single bacterial colony into 2 ml of appropriate growth media

2) Incubate overnight 37°C with shaking

3) Transfer 1.5 ml of overnight culture into microfuge tube

4) Pellet bacteria

5) Remove supernatant

NOTE

At this stage bacterial pellet can be sored at -80°C for gDNA extraction at a later date with no compromise to extraction

Bacterial Lysis

Step 2.

1) Dissolve lypholised RNase A American Bioanalytical #AB14043) in 50% glycerol and 10 mM Tris (pH 8.0) to a final concentration of 100 mg/ml

(stock can be sorted at -20°C for future use)

2) Dissolve lypholised Genfind proteinase K in Genfind proteinase K buffer to a final concentration of 96 mg/ml

(stock can be sorted at -20°C for future use)

3) For each bacterial ioslate to be extracted make a lysis master mix containing 400 ul Genfind lysis buffer, 9 ul Genfind proteinsae K and 1 ul RNase A (see step 1)

4) Add 400 ul of the lysis master mix to each bacterial pellet

5) Gently tip mix 10 times to enusre the bacterial pellet is well resuspended

6) Incubate at 37°C for 30 minutes

7) Procedd to DNA extraction

DNA extraction

Step 3.

1) Invert the Genfind binding buffer 20 times to ensure complete resuspension of the magnetic particles

2) Add 300 ul of the Genfind binding buffer to each of the samples and gently tip mix 10 times, avoid

generating bubbles.

The magentic particles will most likely clump together at this stage due to the high concentration of DNA in the sample.

3) Incubate samples at room temperature for 5 minutes

4) Place the sample on a magentic rack for 5 minutes or until solution completely clears

5) With the tubes on the magnet remove the supernatant avoiding the magentic particles

6) Remove samples from the magnet

7) Add 800 ul of Genfind wash buffer 1 to each of the samples

8) Gently tip mix each sample 10 times.

The idea is to try and resuspend the magentic beads but this will most likely not be possible as they will clump together.

I try to make sure the clumps of beads move in and out of the pipette tip with each mix

9) Place the sample on a magentic rack for 5 minutes or until solution completely clears

10) Repeat steps 7-10 for a total of two washes with Genfind wash buffer 1

11) Add 500 ul of Genfind wash buffer 2 to each of the samples

12) Gently tip mix each sample 10 times.

The idea is to try and resuspend the magentic beads but this will most likely not be possible as they will clump together.

I try to make sure the clumps of beads move in and out of the pipette tip with each mix

13) Place the sample on a magentic rack for 5 minutes or until solution completely clears

14) Repeat steps 11-13 for a total of two washes with Genfind wash buffer 2

15) Briefly centrifuge samples to bring all liquid to the bottom of the tube

16) Place the sample on a magentic rack for 5 minutes or until solution completely clears

17) Remove all traces of wash buffer 2 with a pipette, but do not allow beads to fully dry out.

18) Add 50-200 ul of 10 mM Tris pH 8.0 to each sample

(I usually start with 100 ul but it will depend on the groth dynamics of your baccteria)

19) Remove samples from the magnet

20) Gently tip mix each sample 10 times and incubate at 37^{o}C for 5 minutes then tip mix again.

To ensure elution of DNA off the particles it is crucial that the magentic particles are completely resuspended.

If it is not possble to entirle resuspend particles then add more 10 mM Tris.

21) Place the sample on a magentic rack for 5 minutes or until solution completely clears

22) Tranfer supenatant to a clean microfuge tube. Try to avoid carryover of magnetic particles into the supernatant.

NOTE

After extraction DNA should be stored at 4°C to minimise any damage. Storage at -20°C will result in ice crystal formation that will shear you DNA.

I try to do my DNA extractions on the smae day as my library prep but have stored DNA at 4°C for a couple of weeks and still amde sucessful libraries.

DNA QC

Step 4.

1) Visualise sample to estimate the molecular weight. We have a Fragment Analyzer and run a gDNA 1-60knp gel.

Once you have extracted gDNA from the same species a number of times it is possibly not necessary to run a "gel" wiht each subsequent extraction. However, I would run QC gels each time you tackle a new bacterial speices

2) Quantify your sample with a fluorimter er. QUANTUS of Qubit.

3)Analyse 1 µL in a UV spectrophotometer (e.g. Nanodrop).