Worm Genomic DNA Prep

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Hawaiian Mapping Method (pooled samples)

IMPORTANT: Make sure there is no contamination of any kind in your DNA sample (or else you will be sequencing the contamination instead of your sample).

Pick F2 mutant progeny onto 60mm plates and let them give progeny until plate is full of worms (F3's, F4's) and starved for no more than one day. Aim to pick 50 F2's onto individual plates as described in Doitsidou et al. 2010 in order to achieve the tightest possible mapping region when using the CloudMap pipeline (Minevich et al. 2012). When pooling washed off worms into a 15ml Falcon, aim to have equal amounts of worms from each of the F2 progeny plates in order to not bias the DNA in the pool toward any one F2 progeny sample. Additional steps below.

Suggestion: Always try to pick 5 to 10 F2's more than your desired number in case some plates become contaminated and you need to substitute.

Since not all F2 plates might be ready at the same time, wash ready plates into one Eppendorf tube as follows:

1) Rinse ready plates with M9 and collect into an Eppendorf tube

2) Spin for 2-3 min.

3) Rinse with M9 2-3 times

4) Leave for 2 hours in M9 at 20 degrees on a NUTATOR mixer to get rid of food in the gut

5) Rinse with M9 2-3 times

- repeat step 5 for more confidence if necessary

6) Spin tube and remove as much M9 as possible without disturbing the pellet of worms formed at the bottom of tube

7) Leave tube at -80 degrees overnight or until ready to proceed

8) Thaw and combine all Eppendorf tubes into one 15ml Falcon tube

- When combining the individual Eppendorf tubes, do not use M9 to wash and transfer the thawed pellets; Simply pipet out the thawed pellet and transfer/combine into the 15ml Falcon tube

Non-mapped samples (not pooled):

IMPORTANT: Make sure there is no contamination of any kind in your DNA sample (or else you will be sequencing the contamination instead of your sample).

Seed 3-4 big plates (150x15mm) by chunking small, starved plates. Grow until plate is full of worms and starved for no more than one day!

Wash the plates as follows:

1) Rinse plates with M9 and collect into a 15ml Falcon tube

2) Spin for 2-3 min.

3) Rinse with M9 2-3 times

4) Leave for 2 hour in M9 at 20 degrees on a NUTATOR mixer to get rid of the food in the gut

5) Rinse with M9 2-3 times

- repeat step 5 for more confidence if necessary

6) Spin tube and remove as much M9 as possible without disturbing the pellet of worms formed at the bottom of tube

a. There should be approximately 500µl of worms. If you have fewer worms than this, it may still be OK to continue with the protocol, as the most important point is that you have 1µg DNA input as the final product. Use your best judgment to decide whether to continue if you have less than 500µl of worms at this step.

7) Leave tube at -80 degrees overnight or until ready to proceed

Genomic Prep from a few hundred worms (e.g. homozygous recessive lethal mutants)

IMPORTANT: Make sure there is no contamination of any kind in your DNA sample (or else you will be sequencing the contamination instead of your sample).

If your mutant of interest does not allow you to grow a big amount of worms (e.g. homozygous recessive lethal worms), you can still prepare genomic DNA by picking a few hundred worms. You should pick anywhere between 200 and 600 worms (the more the better), perform 1 – 2 washes with M9, remove as much of M9 as possible, and freeze at -80C right away.
When starting the genomic extraction please follow instructions for (DNA Prep using Gentra Puregene Kit (Qiagen); *Protocol: DNA Purification from Tissue Using the Gentra Puregene Tissue Kit*, page 39,) *if processing 5-10 mg tissue.*

Suggestion: Genomic extraction from a few hundred worms will give you a very small DNA pellet, which is extremely hard to visualize and easy to loose, so we recommend you use *Novagen Pellet Paint NF Co-Precipitant (cat# 70748-3).* Make sure you add the *Pellet Paint* before you add the isopropanol. The *Pellet Paint* will color the DNA pellet blue, which will make it easier to process. Once you dissolve the DNA pellet, the solution will still look blue but this will not affect your sequencing.

Genomic Prep Protocol

DNA Prep using Gentra Puregene Kit (Qiagen)
(Protocol: DNA Purification from Tissue Using the Gentra Puregene Tissue Kit (cat# 158622, 158667, 158689) Page 39)

IMPORTANT: DO NOT perform phenol extraction at any point because this will interfere with library preparation and cluster formation, which will result in lower coverage!!!

1) Unfreeze tube and add 3ml of Cell Lysis Solution

2) Sonicate – **OPTIONAL!!!**

   a. Aplitude – 20%

      Pulse: ON for 01 sec.

      OFF for 01 sec.

      Total time: 30 sec.

   b. If you choose not to Sonicate, make sure worms lyse well during next step

3) Add 15µl ProteinaseK (20mg/ml) and incubate at 55 degrees for 3 hours or until worms entirely lysed

   a. invert worms periodically

4) Let the lysate cool down at room temperature

5) **Add 15µl RNase A Solution and incubate at 37 degrees on a NUTATOR mixer for a minimum of 1 hour!**

6) Cool for 3 min. on ice

7) Add 1ml Protein Precipitation Solution

   a. cool for 5 min. on ice

8) Vortex vigorously for 20 sec. at high speed

a. cool for 5 min. on ice

9) Centrifuge for 10 min. at 2000xg

a. transfer supernatant

10) Add 3ml isopropanol and mix by inverting 50 times

a. **OPTIONAL** - Add 3µl of glycogen or Pellet Paint (Novagen, cat# 70748) if you don't see DNA precipitating

b. **OPTIONAL** – Incubate at -20 for 1 hour

11) Centrifuge for 3 min. at 2000xg

a. Remove supernatant

12) Add 3ml 70% ethanol and invert several times to wash DNA pellet

13) Centrifuge for 3 min. at 2000xg

a. Remove supernatant

b. Air dry pellet

14) Add 150µl of DNA Hydration Solution, pipette up/down, transfer to a clean eppendorf tube

15) Incubate at 65 degrees for a minimum of 30 min. or until DNA is dissolved

16) Measure DNA concentration

17) Load 10µl on a gel; should see one band at range >10kb. There should be no RNA

ASSESSING DNA QUALITY

- **Absorbance measurements at 260 nm are commonly used to assess DNA quality:**
    - The ratio of absorbance at 260 nm to absorbance at 280 nm is used as an indication of sample purity, and values of 1.8–2.0 are considered indicative of relatively pure DNA.
    - Both absorbance measurements can be compromised by the presence of RNA or small nucleic acid fragments such as nucleotides.
    - gDNA samples should be carefully collected to make sure that they are free of contaminants.

- **Gel electrophoresis is a powerful means for revealing the condition (including the presence or absence) of DNA in a sample.**
    - Where possible or necessary, a gel should be run to assess the condition of the DNA sample.
        — Impurities, such as detergents or proteins, can be revealed by visible smearing of DNA bands in the gel.
    - RNA, which interferes with 260 nm readings, is often visible at the bottom of a gel.
    - A ladder or smear below a band of interest might indicate nicking or other damage to DNA.