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DNA extraction protocol for genome sequencing v2.0

Forked from DNA extraction protocol for genome sequencing

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1 Works for me dx.doi.org/10.17504/protocols.io.pvgdn3w

Mealybug Team

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ABSTRACT

Protocol to extract DNA from female mealybug (*P.citri*) for genomic sequencing.

This protocol was established by Stevie Bain and Andres Garcia de la Filia in the Ross lab (University of Edinburgh, IEB), which includes a combination of two kits. I modified it slightly based on Dom Laetsch protocol version 1.1 (Blaxter lab, University of Edinburgh, IEB)

Main modifications:
13,000 rpm centrifuge at 4deg all the time instead of 13000 rpm at room temperature
Amounts of reagents kept from Andres's protocol but use Cell lysis buffer and Protein precitpitation solution from Qiagen (Dom's)
Overnight incubation at 56 is in a heated chambre with a rocking platform to allow gentle mixing
All vortexing steps were changed to mixing by inversion and rotation as in Dom's protocol.

Beware:
RNase A concentration should be 100mg/mL the first time and if there is still RNA left, do a second step of RNase A at 20ug/mL as final concentration

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FORK NOTE

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Day 0- Sample preparation for storage at -80

1 **Whole samples:**

- Transfer live sample to a 1.5 Eppendorf tube, label and close lid
- Put the tube in liquid nitrogen and leave until it doesn't bubble anymore
- Store immediately at -80

Dissected tissue (Dom's protocol):

1. Flick sample tubes so that tissue/pellet is NOT located at the bottom of the tube
- pestles will not reach the bottom
- more force can be applied if the tissue/pellet is on the side of the tube
1. Freeze tubes in liquid nitrogen; for each tube do:
2. Open tube carefully without breaking off the lid
- if a lid becomes damaged proceed until step 6, then transfer sample to new tube
1. Crush tissue/pellet using a white/blue pestle through twisting
- stop once tissue/pellet defrosts
- keep pestle in rack afterwards
- do not let the tip of the pestle touch anything besides the sample
1. Freeze tube again (liquid nitrogen) and repeat from 2 **[* add 10% by volume 0.5 M EDTA if storing at this point]**
2.

Tissue dissection in Qiagen buffer:

1. Dissect and move to low quantity buffer (for batches) on ice (not dry ice)
2. Storage?

Day 1- 1st part – lysis and proteinase K digestion

2 Preheat heat block/water bath to 56°C. Or if using the a hot chamber with rocking system, turn it on at 56°C.

3 **From fresh whole sample:**

- Add 3-15 adult females to a 1.5ml Eppendorf tube and add 360µl of Cell Lysis Buffer. Carefully crush females until homogenized.

DNA extraction for Illumina sequencing on B chromosome lines: managed to extract DNA from 1 small female (that started to lay eggs). Also tried on 3 females.

From frozen whole:

- Transfer frozen tubes on dry ice
- with a pestle, crush carefully on dry ice
- Add 360 uL of Cell Lysis Buffer (Qiagen) and crush again with the same pestle to continue homogenizing

From frozen dissected tissue:

- Take out tube from storage and add 360 uL of Cell Lysis Buffer (Qiagen)

4 Add 40µl of Proteinase K (Qiagen: 20mg/mL)

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5 Mix through inversion and rotation, spin down on microfuge (do not mix vigorously)

6 Wrap tube lid with parafilm

7 Incubate overnight at 56 in heated chamber with rocking platform on to allow gentle mixing

Day 2 - RNase digestion and protein precipitation

8 Remove sample from the heated chamber and spin down,

9 Allow sample to cool down to RT. Add 4µl of RNase A (From Qiagen kit: 100 mg/ml), mix by inversion and rotation, spin down and incubate for 10 min at room temperature.

Andres protocol: 2 min at room temperature for 4uL of RNase A in 400 uL of buffer+PK (1 mg/mL).

Dom protocol: 1 hour at 37deg for 4uL RNase A in >610uL of buffer+PK (about 0.65 mg/mL of RNase A)

B chromosome line DNA extraction: I incubated at room temperature for 10 min BUT ended up having RNA left, so had to go for a second round of RNase (incubation at 37 deg for 30 min with RNase A 10mg/ml for a sample that had 40ng/uL and 40 ng/uL of RNA after the first round of RNase.) all other samples had < 15 ng/uL of DNA and no RNA left

10 Add 133µl of Protein Precipitation Solution (Qiagen) to the sample, mix by inversion and rotation, spin down, incubate on ice > 10 min

NB: At this step sample can be stored at -20

Andres protocol: vortex vigorously for 20 seconds and chill the tube on ice for at least 10 minutes.

B chromosome line DNA extraction: incubated for 20 min on ice

11 Centrifuge for 10 minutes at 13,000 rpm at 4°C.

B chromosome line DNA extraction: centrifuged for 15 min

12 Transfer supernatant to new tube, avoid taking the white precipitation, then add 400 uL of isopropanol.

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B chromosome line DNA extraction: managed to transfer on average 450 uL of supernatant

13 Carefully mix the solution by inversion. After this step, DNA mass should be visible.
Spin down.

14 Incubate overnight at -20.
Or more than 10 min on dry ice if cannot do overnight.

B chromosome line DNA extraction: incubated overnight

Day 3 - resuspension

15 Centrifuge for 10 minutes at 13,000 rpm at 4 deg. Discard supernatant by quickly inverting the tube.

16 Add 300µl of 70% ethanol. Detach the DNA pellet from the wall (if the pellet is very big, break it down with a pipette) and carefully invert the tube several times to wash the DNA. Centrifuge for 30 minutes at 13,000 rpm at 4degrees.

Carefully remove ethanol by aspirating using a pipette. Do not discard the ethanol by inverting the tube.

In Andres protocol: centrifuging is for 5 min after wash

Make 70% ethanol fresh and keep on ice to avoid evaporation

Detaching the pellet will make a better wash.

17 Repeat step 6 at least once more until pellet is white.

B chromosome line DNA extraction: pellets were very small or not obvious because of small amount of tissue

18 Invert the tube on tissue paper and air-dry the pellet under the hood for 10-15 minutes or until transparent.

Dom's protocol air dry is for 1 hour. Check first after 15 min.

19 Add 50 µl of TE buffer or Qiagen Elution Buffer, and rehydrate the DNA by incubating overnight at RT or 4°C.

20 Use 1 uL for the Nanodrop to check A260/280 (should be between 1.7 and 1.8, if more than 1.8 there is RNA contamination). Check for 260/230 , if lower than 1, there is other contaminant, so run purification kit.

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Quantify DNA and RNA concentrations using Qubit.

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