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We use this protocol and it's working

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Preparation of Tissue Samples for DNA Extraction and Copy Number Analysis by iDNA Technologies

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[1]Realizing Increased Photosynthetic Efficiency (RIPE)

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Realizing Increased Photosynthetic Efficiency (RIPE)

ABSTRACT

Preparation of leaf tissue for shipment to iDNA Genetics for DNA extraction and copy number analysis.

NOTE: TOBACCO TISSUE IS REGULATED AS DRUGS AND ALCOHOL. IT CANNOT BE SHIPPED TO THE UK. For tobacco samples, extract DNA using Qiagen DNeasy Plant Kit and ship per "Preparation of DNA Samples for Copy Number Analysis by iDNA Technologies".

For all soybean, cowpea, miscanthus, sorghum, maize, either tissue or DNA can be sent if iDNA has established primers for analyzing copy number. However, iDNA prefers to receive tissue when possible.

MATERIALS

Materials

- Cork Borer Sets with Handles (Fisher, S50166A)
- Solid Rubber Stoppers (Fisher, 14-130S)
- Collection Microtubes (racked, 10 x 96) (Qiagen, 19560)
- Collection Microtube Caps, (Qiagen, 1051163)
- Needle
- Parafilm (Fisher, S37440)
- Cardboard box

Equipment

- -80C Freezer
- Freeze dryer, Labcono 2.5L FreeZone or equivalent
- Optional: Bel-Art™ Space Saver Vacuum Desiccators (Fisher, 08-594-16B)

Get a Quote

1 Email iDNA Technologies for a quote. Include the number of samples to be tested, which genes you want copy number analysis on, and which species the samples are from.

Include one sample with a known copy number of 2 as an internal reference standard for each assay (gene).

Note

Minimum of 24 samples is required for analysis. Contact other members of the lab and transformation facility if you do not have the minimum to see if they will be sending samples soon.

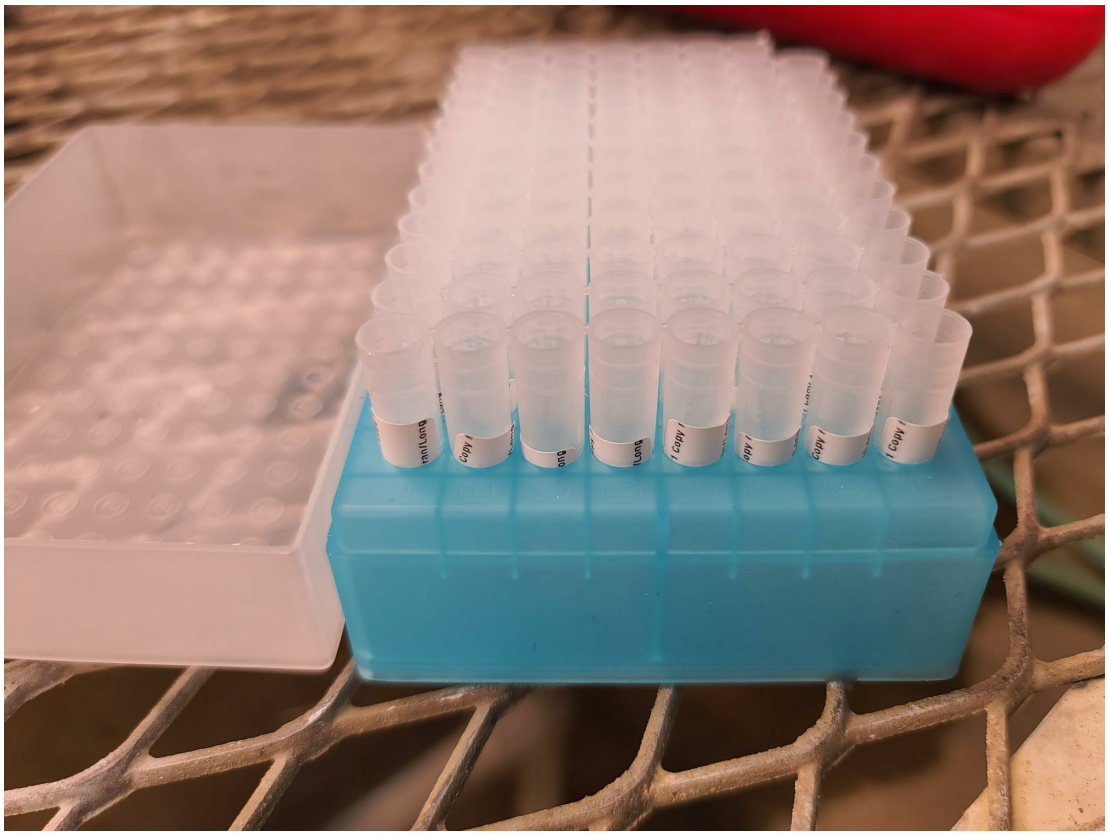
As of 10/3/2023, iDNA Technologies contacts are michala.woodvine@idnagenetics.com and gelli.christodoulou@idnagenetics.com.

2 When you've received the quote, enter it into the appropriate lab group's Quartzy account.

Enter the following information:
Vendor: i-Dna Biotechnology
Catalog #: xxxxxxxxx
Unit size: 1
Qty: 1
Unit Price: From quote (convert from British pound to US dollar before entering)
Upload File: Attach quote from iDNA
Grant ID: Appropriate CFOP for project

Sample Tissue

3 Pre-label the collection tubes.



4 Sample three to four #1 Humboldt cork borer leaf punches (4 mm) from the most recently developed leaf by pressing the cork borer through the leaf onto a rubber stopper and using a twisting motion.

> **Note**
>
> Do not sample more than 4 leaf discs. If too much tissue is sampled, it will interfere with DNA extraction.

5 Push the leaf discs out into the bottom of its pre-labeled collection microtube.

6 Repeat for all desired samples.

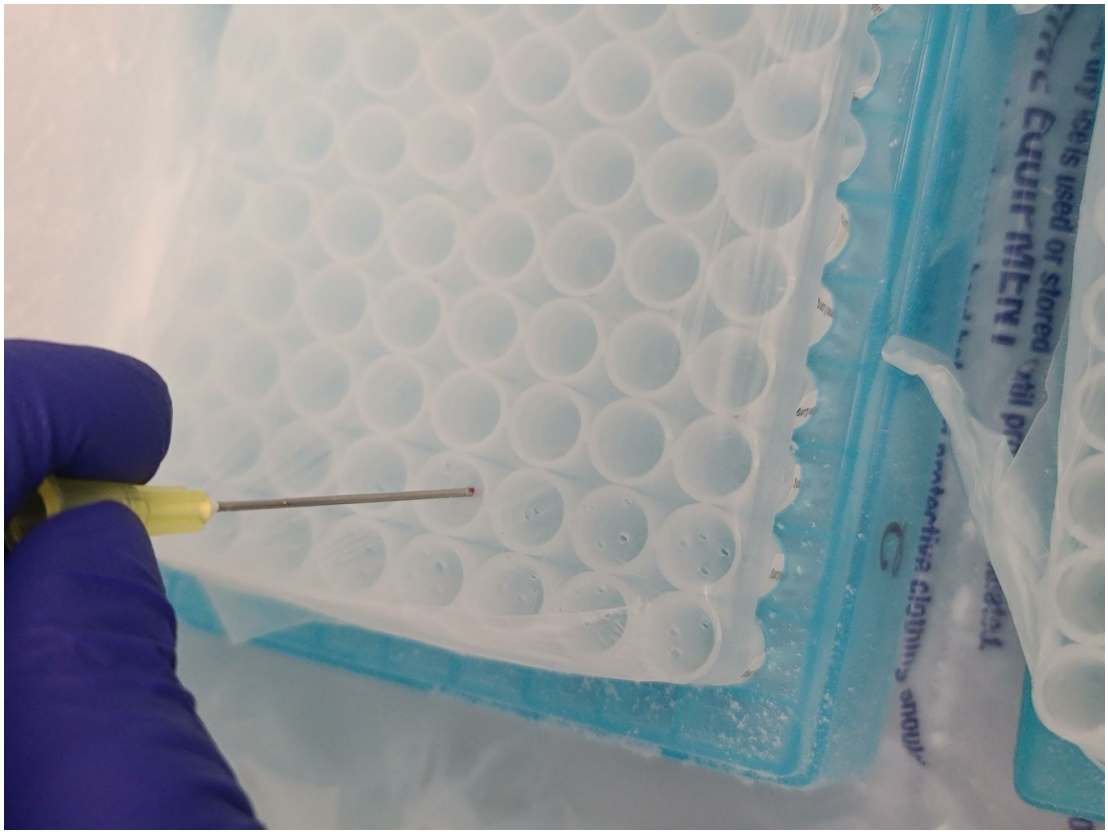
7 Store collection tubes on ice while sampling. Tubes can be at room temperature for short periods of time (i.e. while you are sampling a strip or box of tubes).

Note

It is not necessary to use dry ice or liquid nitrogen at this time. Regular ice is sufficient.

8 Stretch a sheet of parafilm across all tubes in a rack.

9 Poke pin holes using a needle or 10 ul pipette tip in the parafilm over each tube.



10 Cover the tubes in the rack with their lid.

11 Freeze at -80C overnight.

> **Note**
>
> This step is required in preparation for proper freeze drying.

12 Prepare freeze-dryer by pre-cooling and vacuuming to equilibration based on manufacturer's instructions.

13 Transfer tubes from -80C to freeze dryer and lyophilize at >-40C and <0.2 bar overnight.

13.1 **Optional:** If a large number of samples will be prepared or if it is easier to keep samples in the

racks during freeze-drying, a vacuum desiccator can be added to the freeze-dryer to increase capacity up to 4 full collection tube racks.

> **Note**
>
> Connections for tubing and top and bottom of stopcock may need to be sealed with parafilm to create vacuum.

14 Remove samples from freeze dryer, remove parafilm, cap tubes using strip caps.

> **Note**
>
> Remove parafilm slowly as freeze-dried tissue can be staticky and may stick to parafilm or try to jump out of tubes.

15 Replace tube rack lid and tape closed.

16 Clearly label the side of the box, on the box or tape, with the lab name, date, sample tissue type, and gene requested for copy number.

17 Prepare the shipping documents.

18 Weigh the sample box and record the weigh on the commercial invoice form. The number of packages refers to the number of exterior boxes (typically 1). The quantity refers to the number of items in the package, typically we use the number of sample tubes for this value. For unit value, we typically assign each sample tube the value of $1.

19 Give the package and shipping documents to IGB Shipping Department to review. If their advice differs from the above, heed their advice instead.

20 Forward shipping tracking information from IGB Shipping Department on to iDNA Genetics. If you had not previously sent them the completed sample template, "96 well template_iDNA.xlsx", send it with the tracking information.

21 Typically it takes 5-9 days to make it through customs. If tracking indicates a clearance delay for more than a few days, contact the UIUC/IGB purchaser and/or iDNA genetics to help move the package through export/import clearance.