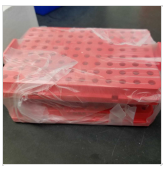

Apr 13, 2021

Preparation of DNA Samples for Copy Number Analysis by iDNA Technologies

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[1]Realizing Increased Photosynthetic Efficiency (RIPE)

1 *Works for me* This protocol is published without a DOI.

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ABSTRACT

Preparation of extracted genomic DNA for copy number analysis by iDNA Genetics.

PROTOCOL CITATION

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KEYWORDS

Copy Number Analysis, DNA, Shipping

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MATERIALS TEXT

96 well PCR plate, non-skirted, Fisher-Scientific 14-230-232.
200 ul tips, USA Scientific Inc 11110700
2-200 ul pipette, Eppendorf, Research Plus
Pcr Plate Heat Seal, foil, pierceable, Bio-rad 1814040.
PCR Plate Sealer, PX1, Bio-rad
8 well strip caps, Fisher-Scientific 14-230-231
Microcentrifuge freezer box, Fisher-Scientific 03-395-455
1-10 ul tip box insert, USA Scientific Inc 11113700
Parafilm, Fisher-Scientific NC9595547
Packing tape, Fisher-Scientific 19-072-082

Quote Preparation

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 1 as an internal reference standard for each assay (gene).

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 4/9/2021, iDNA Technologies contacts are peter.isaac@idnagenetics.com, 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.

2.1 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 Preparation 2s

3 Ensure DNA samples are suspended in an acceptable buffer. Acceptable buffers include EA elution buffer from the Qiagen DNeasy Plant Kit, molecular grade water, or TE (10mM TrisHCl pH8.0, **0.1mM** EDTA).

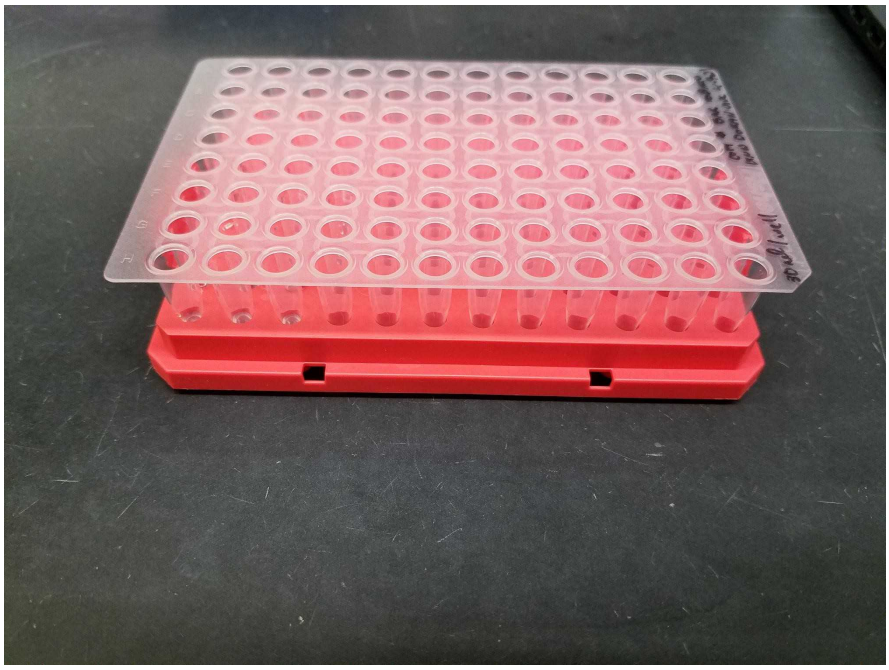
Do not use TE with >0.1 mM EDTA. Too high of a salt concentration can interfere with analysis.

If DNA is in the wrong buffer, perform an ethanol precipitation and wash and resuspend in an acceptable buffer.

4 Ensure that DNA volume and concentration exceeds 30 ul of 4-20 ng/ul (species and gene dependent).

Fluorometric analysis (Qubit or Picogreen e.g.) is preferred. However, if nanodrop is used recommended concentration is >70 ng/ul due to RNA and carbohydrates causing artificially high calculations of DNA in nanodrop readings.

5 Label a non-skirted 96 well PCR plate with your name, date, gene, and species.



6 Fill out the iDNA 96-well template and sample submission form. Fill columns first, before rows. For example if you only have 10 samples, fill A1-8, and B 1-2.

96Well_template_iDNA Technologies.xlsx

For required assay (gene), commonly used assays for RIPE include the following:

- VPZ_Bar is called BarPatF3 in iDNA Technologies assay list
- AP3_Bar is called JICBar in iDNA Technologies assay list
- A mix of genes may be possible for certain combinations.
- For genes other than these, email the gene oglio sequence to the iDNA team to determine the best assay for your needs.

7 Pipette 30 ul of each sample into a unique well.

8 Seal the 96 well plate.

8.1 Heat seal a foil over the 96 well plate using the PCR Plate Sealer at **180 °C** for **00:00:02** . 2s

PCR Plate Sealer
PX1

Bio-Rad **1814000**

8.2 Alternatively, seal the 96 well plate using 8 well strip caps.

9 Place the 96 well plate in a 10 ul tip box insert and place a second 10 ul tip box insert on top. Tightly parafilm all together.

10 Place a folded paper towel in the bottom of a microtube freezer box. Place the reinforced, parafilmed 96 well plate in the box on the paper towel. Add additional paper towels around the edges and over the top.

11 Tape the freezer box closed.

12 Place the microcentrifuge tube freezer box inside a slightly larger, standard cardboard box for shipment.

DNA is stable at room temperature. No ice or cold packs are needed for shipment.

13 Weigh the complete package. Record the weight.

Shipping Document Preparation 2s

14 Prepare the shipping documents.

15 Give the package and shipping documents to IGB Shipping Department to review.

16 Forward shipping tracking information from IGB Shipping Department on to iDNA Genetics.