Macadamia DNA Fingerprinting Service

Precision Tree Breeding Platform

Project booking guidelines:

Projects can be booked using our online form and submitted directly for review and approval. Note that this form has been set up to capture information relevant to both the South African forestry and macadamia industries as well as academic collaborators, therefore some fields may not apply to you.

Below is a list of the fields with some guidelines for completing the form:

Project code and description: We assign a project code to all projects, if you are submitting samples as part of an existing project and you know the code, indicate it here. If no code is indicated, we will assign a code. Please provide a descriptive project name, for example: Identity verification of cultivar 1 in orchard A1.

Company: This could be the name of your farm or research centre, your nursery name or the name of your company.

Contact email: Your email address.

Alternative contact email: If you want to keep anyone else in the loop, add their email address here.

Do you need a sample submission sheet template?: All samples submitted to the platform must be accompanied by a sample submission sheet. This provides a list of all the samples in the shipment as well as relevant information. We have a specific template for this which contains columns relevant to all industries, please see the guidelines (page 5) for completing this sheet. Please do not edit any column headings, or add or remove columns. The format is set for upload into our database and any changes will prevent it from uploading. If there is no relevant column to capture important information, please add it to the comments column.

Proposed submission date: This is the date when you would like to submit the samples to us. We will approve the request if we are able to receive your samples that day and you will receive an automated email informing you of this. If we are unable to accommodate your request at the specified time, we will contact you to make alternative arrangements.

Request type: We allow users to submit requests using this form for several purposes. You can complete this form and select “Advanced warning” if you want to reserve a future date for sample submission, but you can only provide further information closer to the proposed date. We will notify you if we approve the request to book the date, but an official request with the project details will need to be submitted for approval closer to the time to allow us to review and approve the final project request. If you want to make a request for a specific date and can provide information such as the number of samples, objective and required analysis, you can submit an “Official request”.

Contact: melissa.reynolds@fabi.up.ac.za

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Request type continued: If you submitted a request previously and would like to add "Additional information" or edit any information, you can complete only the required fields and the field with new information and submit it, we will then amend the existing request to add the new information. If you would like to request an "Additional analysis" for an existing project request, you can select this option, fill in the required fields and then add the details of the additional request. These options allow users of the Platform to submit all their project request related information to our project management software which automatically makes it available to all members of the team rather than using email which restricts the information to only the recipients of the email. Please make use of these options rather than emailing request related information.

Genus: Select macadamia.

Species: If you are working on a known species of Macadamia, you can indicate this here. If you are not sure what species you are working on please leave this field empty.

Analysis required: We have various different processing pipelines, please select the one that is relevant to you. Macadamia users will most often select either *clonal/cultivar verification* or *parentage analysis.* We also have options to store the leaf tissue in our BioBank for later use or to extract the DNA and store that for later use, if needed.

No. samples: Indicate the number of samples you plan to submit. Note that this is the number of different trees you are sampling, and should not include backup samples. So if you are sampling 10 trees in duplicate, one sample to process and one as a back up sample in case we need to repeat the DNA extraction, you will indicate 10 samples, but you will submit two identical sets of samples consisting of 10 samples each.

Sample type: Select your sample type. If you are submitting multiple different sample types (e.g. leaves and kernels) in one project, select Other.

Is this a bulking project? Or debulking contaminated bulks? As a strategy to reduce costs for large scale screening we bulk samples together. This involves sampling from up to five different trees of the same clone/variety/cultivar into one tube and DNA fingerprinting them as a single sample. If all of the trees bulked together into one reaction match each other, then the resulting DNA fingerprint will not show any indication of contamination. However, if they do not all represent a single cultivar then we will observe evidence of contamination.

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Is this a bulking project? Or debulking contaminated bulks? Continued: In the case of a contaminated bulk sample we can conclude that one or more of the trees included in the bulk does not match the others (and the clonal or cultivar reference DNA fingerprint), however, we cannot say which one/s do not match. To resolve this, each tree included in a contaminated bulk sample can be sampled separately and DNA fingerprinting can be performed to identify the off-type tree/s. This process is called debulking. Bulking should only ever be considered when we are extremely confident in the purity of the samples in a nursery or block in the field. This should only follow extensive DNA fingerprinting to "clean up" the source and is not recommended for first time users of the Platform. Please discuss this with us before considering this option as it may end up being more expensive in the long run if implemented inappropriately.

No. panels: In the case of macadamia only one panel of DNA markers is available, so always select one. This may change in the future.

Objective: Please indicate the objective of the project, this should clearly indicate what question you hope to answer using DNA fingerprinting. Please carefully consider your question and how you will apply the answer. Please provide all relevant information as this will reduce turnaround time.

Additional notes: Anything else that may be relevant to the project.

Other associated projects: If this follows on from another project please indicate the project code and description here.

Reference samples: If you would like us to compare the samples you submit to specific reference samples please indicate this here. Our default will be to compare them to the SAMAC reference set if nothing else is indicated here. If you are submitting samples which will serve as reference samples for other samples in the batch, please indicate it here. For example, if samples 1 – 10 should be used to establish a consensus cultivar reference fingerprint for comparison to samples 11 – 30.

Would you like to request a sampling kit?: We have an established sampling protocol which we recommend to all users of the Platform. If you select this option, we will make up a customised sampling kit for your project based on your request and ship it to you along with sampling guidelines.

Shipping address for the sample kit: If you require a sampling kit, please provide your shipping address here.

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Sample sheet: Please complete our sample submission sheet and upload it here. This should be done after sampling but before samples are shipped. You will likely need to submit an official request first and then use the form again to submit the sample submission sheet. Do this by filling in only the required fields and uploading the sample submission sheet.
Shipping company: If you would like us to follow up on the shipment, please indicate the shipping company.
Waybill number: If you would like us to follow up on the shipment, please provide us with the waybill number.
Waybill: Alternatively, upload a copy of the waybill here and we will extract the information from there.
Invoice to: Some sample submissions are requested under a special project or specific grant request, however, most often the invoice should be sent to the requesting company, this would be the company indicated at the beginning of the form.

Should you have any questions, please contact email Melissa.Reynolds@fabi.up.ac.za.

- Please allow 1-2 working days for the request to be reviewed and accepted.
- Once we have approved your request you will receive an automated email indicating this. Please do not submit any samples without an approved request.
- A sample submission sheet must be submitted when samples are shipped, no samples will be processed without a sample submission sheet.
- Please do not edit/add or remove any column headings in the sample submission sheet as this will prevent it from uploading into our database.

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Filling in your sample submission sheet:

- All samples must be accompanied by a electronic copy of our sample submission sheet.
- The template can be requested here, please download the template, complete it, save it locally and then upload it using our booking system. **Do not complete the template online via google sheets.**
- As with the project booking system, this template has been created for both the forestry industry and other users such as the macadamia industry. As a result, some headings may not seem relevant. Please use the guidelines below to complete the sheet. **Please do not edit/add/remove columns and headings as this will prevent the sheet uploading to our database.**

Guidelines:

FMG sample ID: Leave this blank, we will fill it in with unique sample IDs.
Company sample ID: This is a required column, please fill in your unique sample ID.
Bulk ID: If you have sampled for bulk DNA fingerprinting analysis, please indicate the unique bulk ID here.
No. on bag/tube: This is essential to enable us to match the tube, bag or plate well with the correct information in this sheet. If you are sampling into bags (not recommended, see Sampling Field Guide for proper protocol) please clearly mark the bag with a permanent marker and make a note of the label here. If you are sampling into individual tubes, please mark them clearly with a permanent marker or sticker and note the label contents here. If you are sampling into 96-well blocks, please make a note of both the plate name/number and the well position. The blocks have letters indicating rows and numbers indicating columns in raised profile to the left and above the wells, respectively. Combine these to indicate the well position, for example, well A1 is the first well in row A, column 1. All trees should be sampled in duplicate for routine DNA fingerprinting and in triplicate for special projects. All samples from a specific tree should be labelled with the same label if they are sampled into tubes, indicate only the sample number on the tube for one sample, please do not include duplicates in the sample submission sheet. For plates, each tree should be sampled in duplicate/triplicate in different plates with but keeping to the same sample configuration. For example, tree 1 should be sampled into Plate1-**A1** and Plate2-**A1** in the sample submission sheet indicate Plate1-A1 and clearly label Plate 2 on the side as a duplicate of Plate 1.

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Guidelines:

Date sampled: Indicate the date that the samples were collected in field.

Sample material: Indicate the sample type, e.g. leaves or kernels or pollen etc. Ensure that this is correct for each sample if different sample types are submitted in one project.

Company: This should be the same as that indicated in the project request.

Company contact person: The person responsible for the sampling/project request.

Company project ID: If you have an internal project code you would like to record indicate it here, otherwise leave it blank.

Company trial no.: If you have a trial number or orchard number, indicate it here. Otherwise, leave it blank.

Location: Record the location of the trial or orchard. This may be an area or a farm name, or exact GPS co-ordinates of the trial/orchard or tree.

Source: If you have received this material from a specific nursery or programme and would like to record this, do so here, otherwise, leave it blank.

Species: If you know the specific species or hybrid combination that you are working with indicate this here, otherwise, just fill in macadamia.

Provenance: If you have information regarding the provenance of the tree, such as the region in Australia where it was sourced, you can fill that in here, otherwise leave it blank.

Seed parent provenance: Sometimes breeders only know the provenance of the seed parent, but not the pollen parent. This can be recorded here, otherwise, leave it blank.

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Guidelines:

Family/Seedlot no.: In the case of full/half-sibs you can indicate the family number. If you planted out seed, you can record the seedlot number here, otherwise, leave it blank.
Pedigree/Selection: If you have a common name for the cross or selection in addition to the specific IDs you can add it here, otherwise, leave it blank.
Seed parent: Indicate the exact seed parent ID according to your records, otherwise, leave it blank.
Pollen parent: Indicate the exact pollen parent ID according to your records, otherwise, leave it blank.
Clone ID: This can be used to indicated the name of the cultivar, if unknown, indicate it as unknown. Do not leave this column blank.
Selection ID: If you are making selections for breeding and would like to record unique selection IDs indicate them here, otherwise, leave it blank.
Old clone number: In some cases a cultivar has been renamed and you would like to record the old ID for your records, add this here, otherwise, leave it blank.
Reference: If you have a specific sample in mind that you would like us to use as a reference, please indicate it here. This can also refer to samples submitted in this batch, for example if samples 1 – 3 will first be used to establish a consensus DNA fingerprint and then samples 4-10 will be compared to this new consensus DNA fingerprint. If you would like us to compare your samples to a specific cultivar in our reference set, please indicate this here, otherwise, leave it blank.
Plantation: This can also be used to record the farm or orchard, otherwise, leave it blank.
Compartment: If your orchard or farm is divided up into compartments, record this here, otherwise, leave it blank.

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University of Pretoria

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Guidelines:

Treatment: In some industries the family number is called a treatment, if this is relevant to you, record it here, otherwise, leave it blank.

Rep/column: Depending on the design of your orchard, you may want to record the rep or column in your compartment here. Otherwise, leave it blank.

Plot/block: Depending on the design of your orchard, you may want to record the plot or block number in your compartment here. Otherwise, leave it blank.

Tree/row: Depending on the design of your orchard, you may want to record the tree or row in your compartment here. Otherwise, leave it blank.

Tree/ramet: Depending on the design of your orchard, you may want to record the tree number in your compartment here. Otherwise, leave it blank.

Hedge: This usually applies to propagation hedges in a nursery, if it is not relevant to you, leave it blank.

Healthy/diseased: If you are submitting samples to better understand how different clones/cultivars are responding to disease pressure you may want to record their health status here, if not, leave it blank.

Sowing year/age: If you want to record the age or propagation year of your trees for comparison purposes, record it here, otherwise, leave it blank.

Generation: If you have multiple generations that you want to record, do so here, otherwise, leave it blank.

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Guidelines:

Company phenotype ID (NIRA): If you have submitted samples for phenotyping at any institute/organisation and would like to link this to the genotyping data for future reference, record the phenotyping ID given by the institute/organisation here. Otherwise, leave it blank.

Previous submission ID: If you are resampling a tree that was previously submitted for DNA fingerprinting, please indicate the original submission ID here (either the FMG sample ID or the company sample ID). Otherwise, leave it blank.

FMG field trip sample ID: If the samples were collected as part of an FMG field trip and given a unique ID which does not follow the naming convention of the Precision Tree Breeding Platform, record this here. Otherwise, leave it blank.

Comment: If you have any other information that you would like to record for future reference and there is no suitable existing column, please add it here. Otherwise, leave it blank.

Number of panels requested: If more than one microsatellite marker panel is available for your genera (at present only one is available for macadamia), please indicate your preferred number of panels here. Usually, we have a prescribed panel or number of panels for different types of analysis, however, in some cases, a bigger sample set is submitted but different samples will be used to answer different questions. If this is the case, the number of panels required may differ for groups of samples, the required number of panels for each sample should then be recorded in this column. If you would like us to decide which panels are most suitable, leave this column blank.

If you have any questions, please contact us before uploading the sample submission sheet.

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