

Sampling for genotyping  
using cassavabase and  
Coordinate App v2

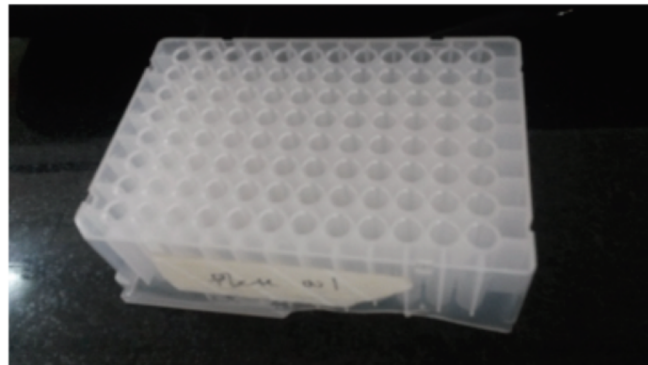
# Required material:

IITA-TMS-IBA121823



**103**

17uyt12ppdIB



Phone with:  
Coordinate App



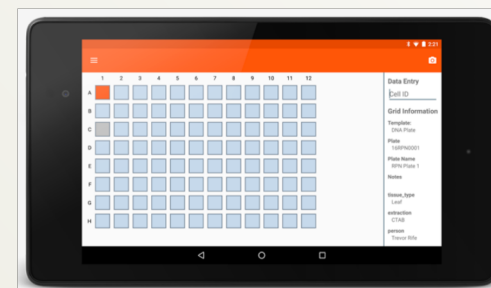
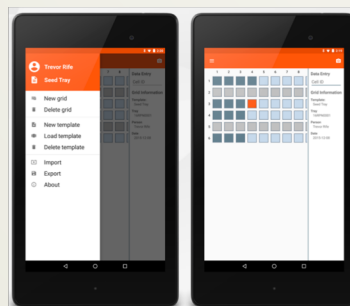
AND  
Barcode  
Scanner App



## Coordinate App



## & Barcodes



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plate labelling

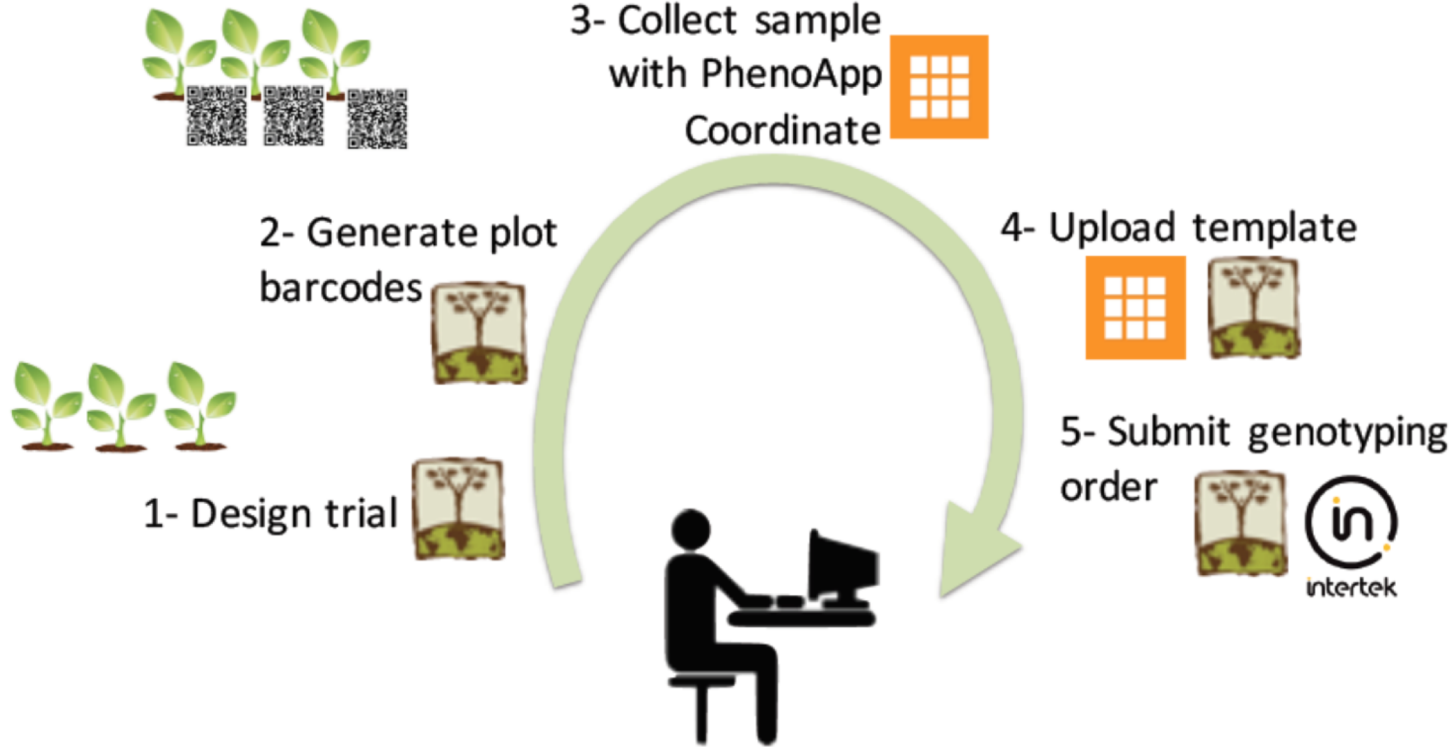
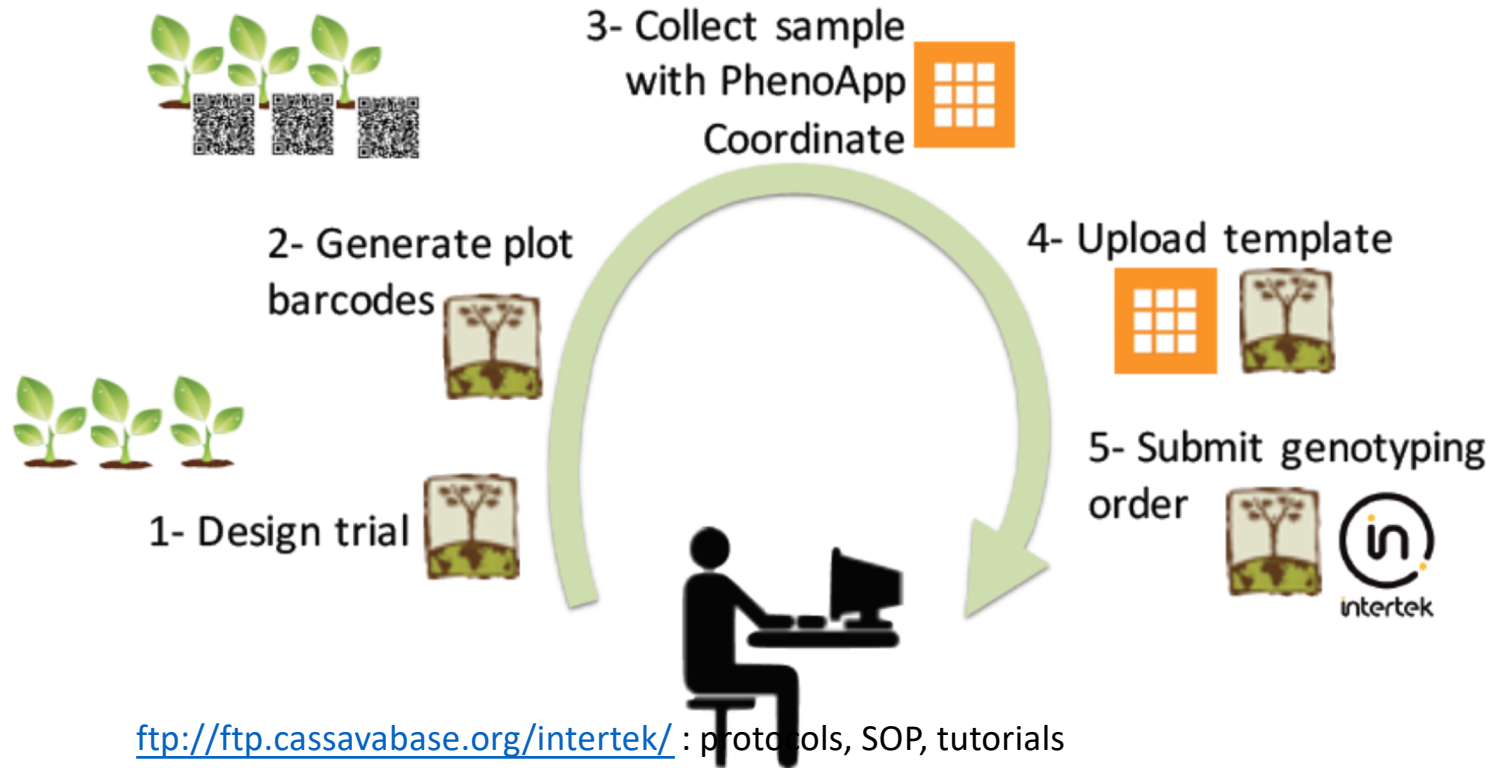


plate labelling



**Embrapa:** 22 plates  
**NRCRI:** 13 plates  
**IITA:** 24 plates (kaspar)  
+ 50 plates (GS, coming up)



<ftp://ftp.cassavabase.org/intertek/> : protocols, SOP, tutorials

<https://cassavabase.org/breeders/genotyping/> : tissue sampling upload interface



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-1- Design trial  
and

-2- Generate barcodes

-1- Design trial: “Manage” -> “Field Trials”

[https://solgenomics.github.io/sgn/03\\_managing\\_breeding\\_data/03\\_07.html#adding-trials](https://solgenomics.github.io/sgn/03_managing_breeding_data/03_07.html#adding-trials)

-2- Generate barcodes and label design

[https://solgenomics.github.io/sgn/03\\_managing\\_breeding\\_data/03\\_11.html](https://solgenomics.github.io/sgn/03_managing_breeding_data/03_11.html)

[https://solgenomics.github.io/sgn/03\\_managing\\_breeding\\_data/03\\_12.html](https://solgenomics.github.io/sgn/03_managing_breeding_data/03_12.html)



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## On trial page

-1- go to barcode section



-2- select “plots barcode”



-3- Ensure Fieldbook option is selected (Wont work otherwise)

Barcode Type:

2D Barcode

Enable 2D Barcode For FieldBook:



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-3- Collect samples  
with PhenoApp Coordinate



**-Step 1-** Create a template for “NextGen” including the  
Required blank wells for DarSeq (G11 and G12)

=> Once created, the template can be used for all plates (grid) to be sampled for genotyping at DartSeq

**-Step 2-** Create a grid and do tissue sampling

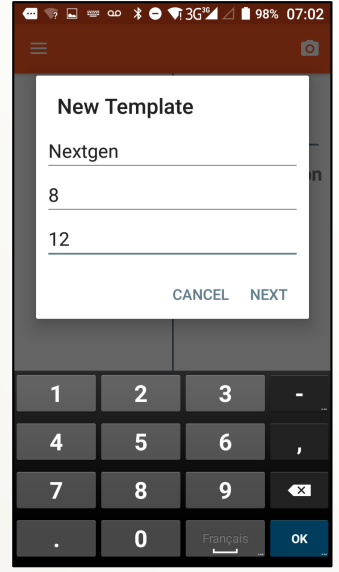
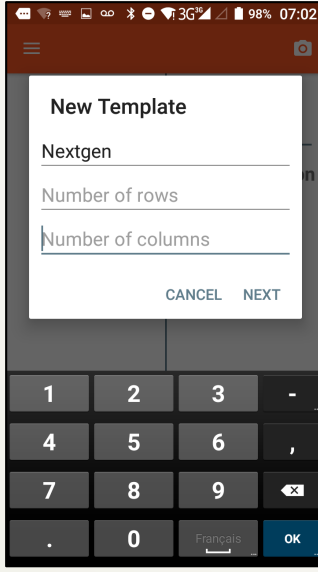
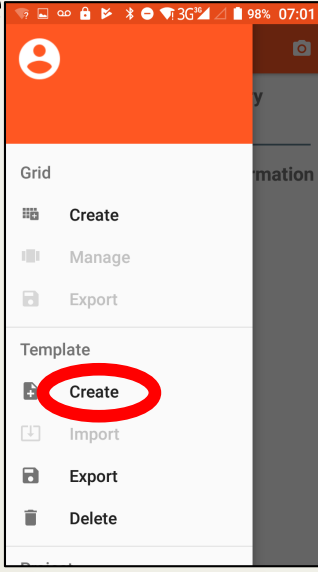
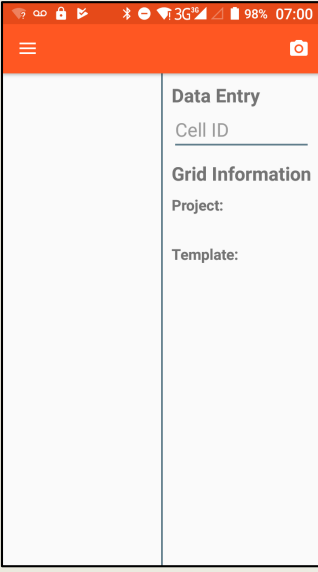
=> For each 96 well plate you need to sample, use the “NextGen” template created in Step 1

**1** -Step1- Create template

**2**

**3**

**4**

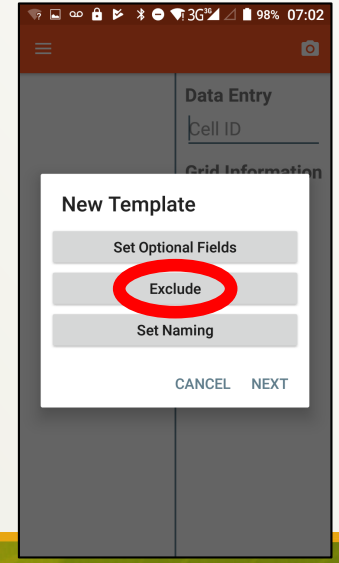
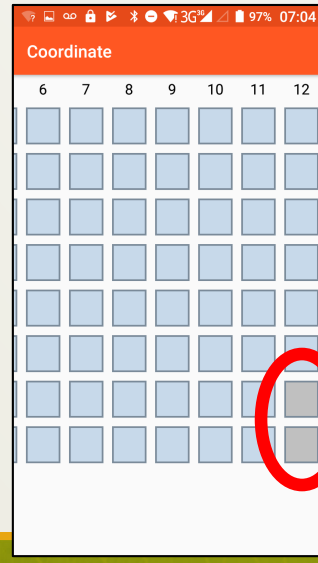
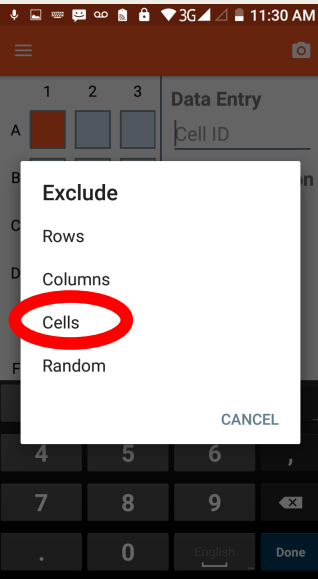
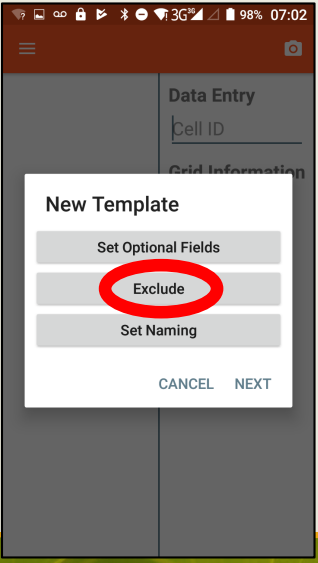


**5** Set blanks

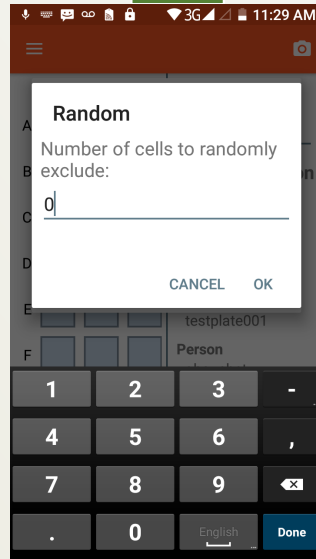
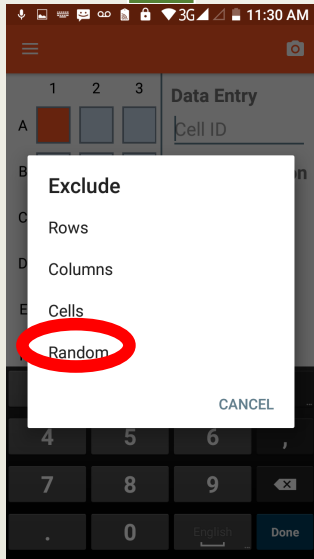
**6**

**7**

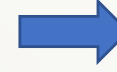
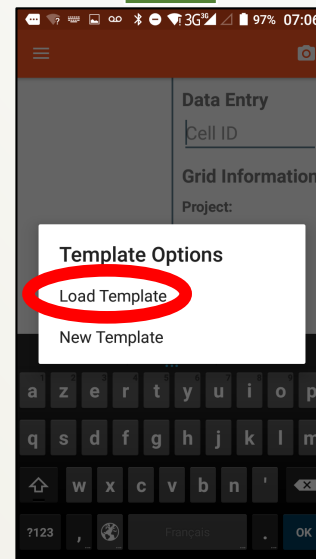
**8**



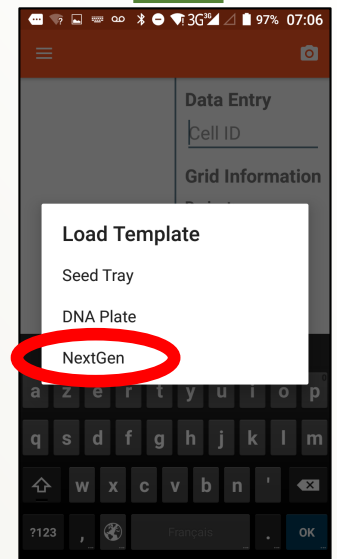
9 Disable random blank 10



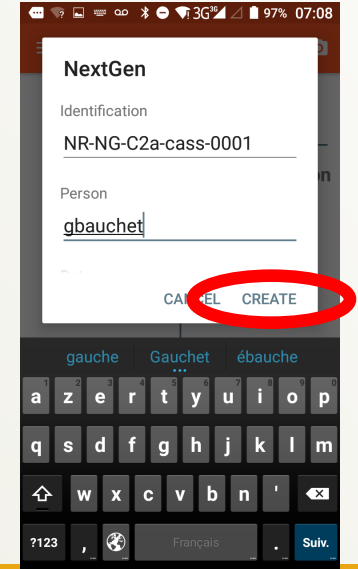
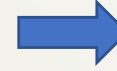
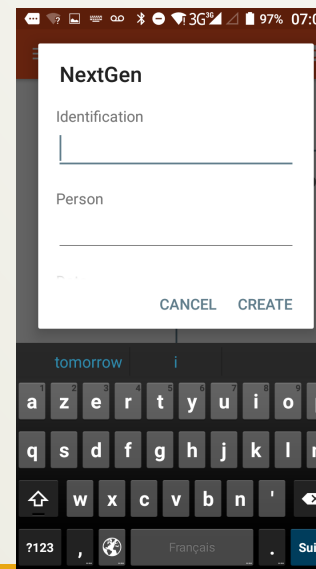
11



12



13 -Step 2- Create Grid 14



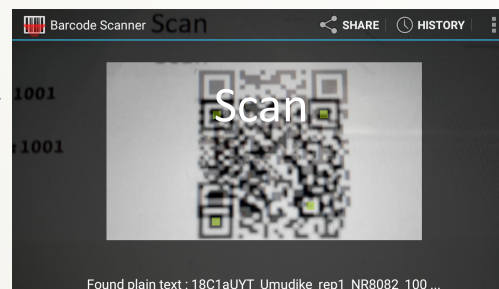
-b create a grid:  
Now you have the “NextGen” template with well **G12** and **H12** set as blanks as required by DartSeq genotyping (confusingly Intertek requires H11 and H12 but we can stick to G12 and H12). You can now create a grid for sample collection. Here the grid is named “NR-NG-C2a-cass-0001”



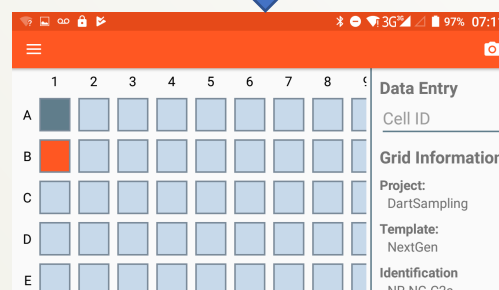
# Scanning-Sampling procedure

Plot1:

18C1aUYT\_Umudike\_rep1\_NR8082\_1001  
pedigree: NA/NA  
stock:NR8082 rep:1 blk:1 plot:1001



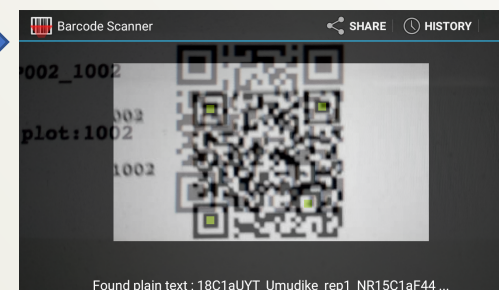
-1- Scan



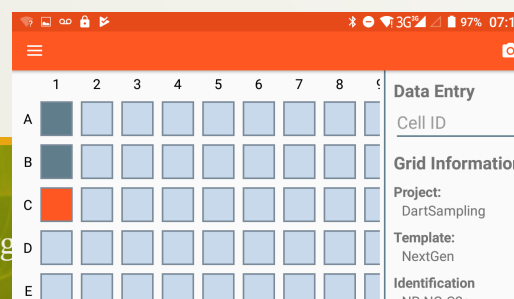
-2- Sample

Plot2:

18C1aUYT\_Umudike\_rep1\_NR15C1aF44P002\_1002  
pedigree: NA/NA  
stock:NR15C1aF44P002 rep:1 blk:1 plot:1002



-1- Scan



-2- Sample



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ssava



Etc....

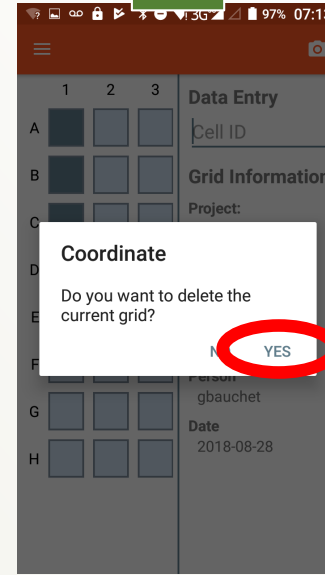
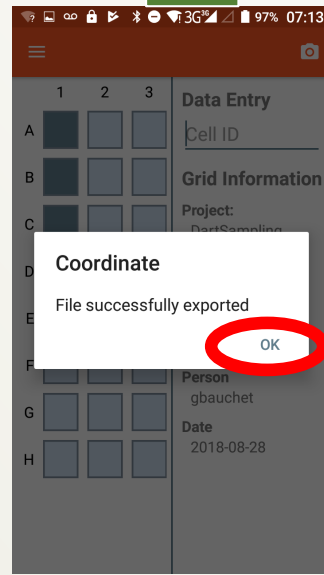
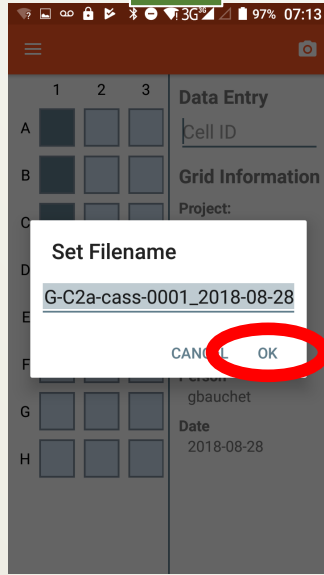
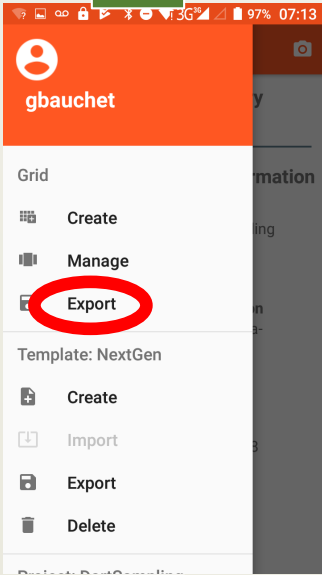
15

Export samples

16

17

18

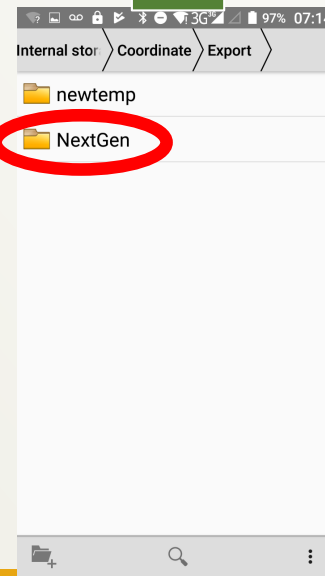
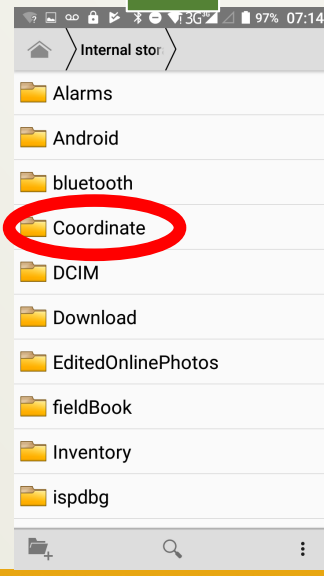
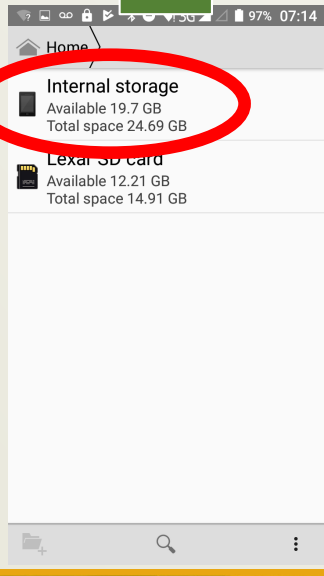
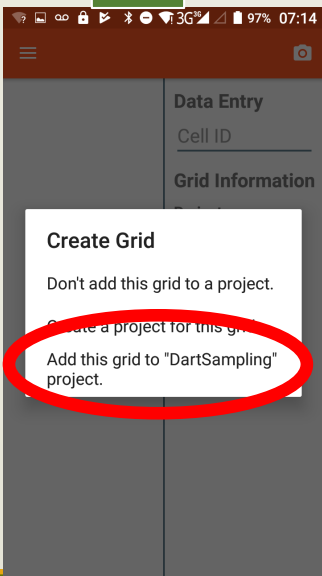


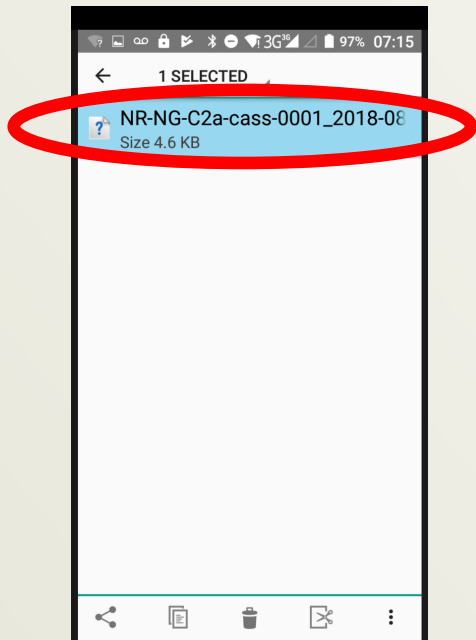
19

20

21

22





Select the file and export it to your laptop



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## Export .csv file example:

NR-NG-C2a-cass-0001\_2018-08-28

Value	Column	Row	Identification	Person	Date
18C1aUYT_Umudike_rep1_NR8082_1001	1	A	NR-NG-C2a-cass-0001	gbauchet	2018-08-28
18C1aUYT_Umudike_rep1_NR15C1aF44P002_1002	1	B	NR-NG-C2a-cass-0001	gbauchet	2018-08-28
18C1aUYT_Umudike_rep1_IITA-TMS-IBA00070_1003	1	C	NR-NG-C2a-cass-0001	gbauchet	2018-08-28
	1	D	NR-NG-C2a-cass-0001	gbauchet	2018-08-28
	1	E	NR-NG-C2a-cass-0001	gbauchet	2018-08-28
	1	F	NR-NG-C2a-cass-0001	gbauchet	2018-08-28
	1	G	NR-NG-C2a-cass-0001	gbauchet	2018-08-28
	1	H	NR-NG-C2a-cass-0001	gbauchet	2018-08-28
	2	A	NR-NG-C2a-cass-0001	gbauchet	2018-08-28
	2	B	NR-NG-C2a-cass-0001	gbauchet	2018-08-28
	2	C	NR-NG-C2a-cass-0001	gbauchet	2018-08-28
	2	D	NR-NG-C2a-cass-0001	gbauchet	2018-08-28
	2	E	NR-NG-C2a-cass-0001	gbauchet	2018-08-28
	2	F	NR-NG-C2a-cass-0001	gbauchet	2018-08-28
	2	G	NR-NG-C2a-cass-0001	gbauchet	2018-08-28
	2	H	NR-NG-C2a-cass-0001	gbauchet	2018-08-28
	3	A	NR-NG-C2a-cass-0001	gbauchet	2018-08-28
	3	B	NR-NG-C2a-cass-0001	gbauchet	2018-08-28
	3	C	NR-NG-C2a-cass-0001	gbauchet	2018-08-28
	3	D	NR-NG-C2a-cass-0001	gbauchet	2018-08-28






-4- Upload template to Cassavabase



<https://cassavabase.org/breeders/genotyping/>

Search **Manage** Analyze Maps About

- User Roles
- Breeding Programs
- Locations
- Accessions
- Seed Lots
- Crosses
- Field Trials
- Genotyping Trials**
- Tissue Samples
- Field Book App
- Phenotyping
- Barcodes
- Label Designer
- Download
- Upload
- ODK Data Collection



Cassava Community Partners

A large group photograph of diverse individuals, including men and women of various ethnicities, standing and sitting together. The photo is overlaid with a semi-transparent white box containing the text 'Cassava Community Partners' in green and black.



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# “Add genotyping trial”

## Manage Genotyping Trials

+ About Genotyping Trials

- Genotyping Trials

Add Genotyping Trial

Upload Genotyping Data

Information

Breeding Programs -- Folders -- Genotyping Trials

Refresh

Search

- + IITA
- + NRCRI

### Add Genotyping Trial

Intro

1

Basic Plate Info

2

Well Info

3

Trial Linkage

4

Confirm

5

### This workflow will guide you through adding a genotyping trial in the database

Genotyping trials represent 96 or 384 well plates.

Each plate has a globally unique Plate ID.

Each well in the plate has a globally unique tissue sample ID.

The "contents" of each well can be either a tissue sample, plant name, plot name, or accession name. This "source" name must be in the database already. This is useful if you provide a field trial entity (such as a plot or plant or tissue sample name), so that phenotypes and genotypes can be directly compared.

If you choose to submit your genotyping trial to a genotyping facility (Cornell IGD, Intertek, BGI, etc) we can generate the files they require for you. Please be aware of their requirements, such as blank well positions and concentrations.

Go to Next Step



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Intro

Basic Plate Info

Well Info

Trial Linkage

Confirm

1

2

3

4

5

### Provide info about your plate

**Genotyping Facility:**

Intertek

**Genotyping Project Name:**

-In Coordinate App this is called "Plate ID"  
-Should match Vendor Project if you have one

NextGenCassava

**Genotyping Plate ID:**

-In Coordinate App this is called "Plate Name"  
-This is the unique name of the plate

NR-NG-C2a-Cass-0001

**Plate Format:**

96 Well

**Sample Type:**

DNA

**Breeding Program:**

NRCRI

**Location:**

Umudike

**Year:**

2018

**Description:**

Test genotyping upload

Fill in plate  
information

Go to Next Step

Intro  
1

Basic Plate Info  
2

Well Info  
3

Trial Linkage  
4


Confirm  
5

### Provide information about the wells in your plate

Select One:

- I am uploading a plate design I made in Excel
- I am uploading a Default Compliant Android Application plate design
- I am uploading a Custom Coordinate Android Application plate design
- I need to design a completely new plate

You want to upload a Custom Android Application file.

 **File format information**  
Spreadsheet format

Select Coordinate CSV File:  No file selected.

Go to Next Step



## Manage Genotyping Trials

+ About Genotyping Trials

- Genotyping Trials

Add Genotyping Trial

Upload Genotyping Data

### Information

#### Search

Double click  
genotyping trial (📊) or folder (📁)  
to view detail page.

Breeding programs (📁)

#### Folders

Create new folder

Move genotyping trial(s) to folder

Move folder

### Breeding Programs -- Folders -- Genotyping Trials

Refresh

- + 📁 IITA
- 📁 NRCRI
  - 📁 2006\_NRCRI
  - 📁 2007\_NRCRI
  - 📁 2008\_NRCRI
  - 📁 2009\_NRCRI
  - 📁 2010\_NRCRI
  - 📁 2011\_NRCRI
  - 📁 2012\_NRCRI
  - 📁 2013\_NRCRI
  - 📁 2014\_NRCRI
  - 📁 2015\_NRCRI
  - 📁 2016\_NRCRI
  - 📁 2017\_NRCRI
  - 📁 2018\_NRCRI\_C2a
    - 📊 NR-NG-C2a-Cass-0001c
    - 📊 NR-NG-C2a-Cass-0002c
    - 📊 NR-NG-C2a-Cass-0004c
    - 📊 NR-NG-C2a-Cass-0005c



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Genotyping trial NR-NG-C2a-Cass-0001c

Generate data formats for Intertek and/or Dartseq

<b>Breeding Program</b>	NRCRI (NRCRI cassava breeding program, Umudike, Nigeria)
<b>Trial Type</b>	Genotyping Trial
<b>Plate Format</b>	96
<b>Plate Sample Type</b>	DNA
<b>Genotyping Facility</b>	intertek
<b>Submitted to Genotyping Facility</b>	no
<b>Genotyping Facility Status</b>	



SGN trial 4040 (NR-NG-C2a-Cass-0001c)

<b>Live Status From Genotyping Facility</b>	
<b>Download PDF</b>	

Exporting formats:

[Download Intertek Formatted File](#) [Download DartSeq Formatted File](#)

(2) DartSeq export excel file format

(1) Intertek export excel file format



## -5- Submit genotyping order

### REMINDER:

For Genomic selection:  
you need to submit to both (1) Intertek and (2) Dart services.

For low density genotyping (ie: Kaspar from HTPG project):  
you solely need to submit to Intertek.



# (1) Intertek: Submit the Intertek export excel file format send excel file by email

[ftp://ftp.cassavabase.org/documents/tutorials/intertek/INTERTEK\\_sample\\_submission\\_form.xls](ftp://ftp.cassavabase.org/documents/tutorials/intertek/INTERTEK_sample_submission_form.xls)

Under the "SampleList" section, paste the Intertek format from cassavabase

Total Quality. Assured.

**Sample ID = Subject ID:** Unique identifier no longer than 30 characters and excluding ", " (comma).

The Sample / Subject ID will be the Sample / Subject ID in the GRID report with SNP results.

**All Sample IDs should be unique**, including the positive (parental) control samples.

**All BLANKS**, e.g. verify plate identity, missing plant in the field, etc. should have an unique ID to be included in the SNP results report.

Wells **H11** and **H12** should be part of this sample list, but should be left empty (contain no text). They are used as lab controls.

**Plate ID:** Unique identifier no longer than 21 characters long and excluding ", " (comma).

**Well location:** location of sample on 96-format tissue plate. Use '**A01**' instead of '**A1**'. There is no restriction in the following order of the samples.

**Comments:** note here any issues which might affect the quality of the samples and/or plates.

Additional columns with sample information, such as pedigree, field locations etc. are welcome, but not required and will **not** be included in the report.

\*The first 3 columns and 'Species / Crop' are Required fields

	Sample ID*	Plate ID*	Well location*	Subject Barcode	Plate Barcode	Comments
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						

Customer details    Sampling&Shipping Instructions    Plate IDs    **Sample List**    Plate layout - Grid    SNP Information    +



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(2) **DartSeq:** Submit the DartSeq export excel file format from cassavabase at <https://ordering.diversityarrays.com/cgi-bin/order/login.pl>  
<https://ordering.diversityarrays.com/cgi-bin/order/order.pl>

The screenshot shows the Diversity Arrays Technology website. At the top, there are navigation buttons: "Get a Free Quote", "Register", and "Login". The IP address "IP: 209.255.227.14" is displayed in the top right. On the left, a sidebar contains several menu items: "Go to DArT Website", "Sample Tracking File Format", "DNA Extraction method", "Australia DNA Shipping Instructions", "Australia Material Shipping Instructions", and "International DNA Shipping Instructions". The main content area is titled "Order Instructions" and provides information for Australian Small Order Quantity (10 Max) Sample Submission for Genetic ID. It includes a list of specimen types: Plant, Animal, and Fungi. A note states "Special conditions apply". Below this, it says "For all other regular service orders please follow these steps:" and provides a table for sample origin and type.

1a. Sample Origin	Within Australia		International	
1b. Sample Type	Animal	Plant	Animal	Plant
1c. Sample				Tissue

On the right side of the page, there is a login section titled "Logon to DArT Order System". It prompts the user to "Please enter your username and password into the fields provided." and includes fields for "Username:" and "Password:". Below these fields are links for "Do not have a username, please register." and "Forgot password?". A "Logon" button is located below the links. A blue box with the text "LOGIN or CREATE your account" has two blue arrows pointing to the "register" and "Logon" elements. Below the login section, there is a "Please note" section stating that the online ordering system uses cookies to maintain a session and is not used to store or persist personal information. It also states that by logging in, the user agrees with the Privacy Policy.



[Create Sample Tracking Template File](#) | [New Order](#) | [List Orders](#) | [Change Details](#) | [Change Password](#)

## Make a New Order

**Step 1:**  
Product Selection & Sample Tracking File

**Step 2:**  
Order Details and Comment

**Step 3:**  
Price & Confirmation

\*: denotes a required field.

### Product Selection & Sample Tracking File

To place an order:  
A Sample Tracking Template File is required. [Click here to create one.](#)  
(For the file format details, [click here.](#))  
Enter your details below and select your Sample Tracking File to upload.

**Organism\*:**   
Please type, the list of matching organisms will appear.

**Product\*:**

**Product description:** A combination of DArT complexity reduction with sequencing on the Next Generation Sequencing platforms. Represents combined marker discovery and genotyping for SNP and SilicoDArTs (Presence/Absence of DNA fragments in genomic representations). Approximately 10,000 DNA fragments from DArT representations are assayed for polymorphism.

**Sample file\*:**  DArT-extract-Cassava-t-2019-03-16\_test.csv

Cassava

Cassava  
DartSeqLD 1.0

DartSeq format file from  
Cassavabase genotyping  
trial page (See slide 23)

[Download Intertek Formatted File](#)

[Download DartSeq Formatted File](#)



# Make a New Order

**Step 1:** Product Selection & Sample Tracking File  
**Step 2:** Order Details and Comment  
**Step 3:** Price & Confirmation

\*: denotes a required field.

## Order Details and Comment

**Provide a brief description of the service material and aims for service:**  
  
*This information will help us aim to extract maximum value from the data.*

**Are you sending tissues for extraction rather than purified DNA?:**  
  
*Please observe very strict Australian quarantine requirements if shipping from outside Australia. Please follow strict shipping instructions in order to avoid delays or loss of your shipment.*

**Do you agree to apply our special DNA purification step in case your samples fail our QC?:**  
  
*Please note the price of this extra service is equivalent to US \$200 per plate.*

**Which marker density (volume of sequencing) would you like to apply?:**  
  
*We may contact you with additional recommendation in case of apparent discrepancy between your material/aims and selected assay format.*

**Please enter number(s) of your previous service(s) to be co-analysed with this service at no additional charge. You can also request here running TWO analyses on subsets of samples in the order. Otherwise leave blank:**  
  
*Please note: Requests for co-analysis or performing additional analysis on the subset of samples after the service is performed will attract an additional charge.*

**Provide your preferred format for the SNP data:**  
  
*As a standard, in addition to SilicoDart markers, we report two formats for SNP markers: 2-row binary scoring and 1-row (0,1,2).*

**Do you require raw (fastq) data?:**  
  
*Selecting YES will attract a small additional fee equivalent to US \$200 for data preparation and shipment. This fee may be higher if you request raw data after service is completed, but we do keep all raw data from the start of our service. Note that the disk with data will be delivered to your address registered in our system, unless instructed otherwise.*

**Which reference genome(s) would you like to use for aligning marker sequences to be reported for this service?:**  
  
*Please provide a link to publicly accessible reference sequence or contact us to incorporate your proprietary sequence into DArTdb*

**If you have been provided with a discounted price please indicate the quoted price and reference from our quotation service or DArT officer name and date of email:**  
  
*Please note that without this information you will be invoiced our standard price!*

**If you already paid for this service please provide service number or any other valid proof of your prepayment:**

**Provide any additional information to appear on the invoice like purchase order, tax number, contact details (if different from stored in the system) etc.:**  
  
*Please add also a preferred date if you want invoice backdated, otherwise the invoice is likely to be dated when service is completed/report provided.*

**Please, choose a currency for your invoice:**  
  
*Please add also a preferred date if you want invoice backdated, otherwise the invoice is likely to be dated when service is completed/report provided.*

NextGen Cassava-Cornell University project genotyping service

NO

YES

Medium

A Variant Call format 4.1 or beyond. Aligned to ref. genome V7. Should include read count (AD) addition to genotype-call (GT)

YES

Cassava Reference genomes V6 AND V7

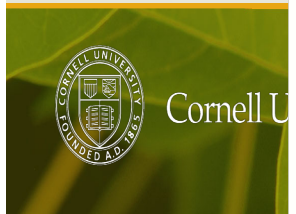
DArTSeq assay, including ApeK1 enzyme. Co analyze with previous NextGen samples. A \$9 per sample price was agreed

Bill to Cornell services

Add the following data recipients:  
[gjb99@cornell.edu](mailto:gjb99@cornell.edu)  
[mw489@cornell.edu](mailto:mw489@cornell.edu)

USD

In case you have any additional requests (i.e. fast report delivery, downstream data analysis etc) or comments please [contact us](#) and mention service number in the subject.



[Create Sample Tracking Template File](#)[New Order](#)[List Orders](#)[Change Details](#)[Change Password](#)

## Make a New Order

**Step 1:**

Product Selection &amp; Sample Tracking File

**Step 2:**

Order Details and Comment

**Step 3:**

Price &amp; Confirmation

### Confirmation

**Number of Samples:** 94**Plate Details:**

PlateID	Number of Samples
1	94

**Price:** AUD 700.00

- Would you like to confirm this order? Once an order is confirmed, we will create an order specification for you. You need to download this PDF file, print it, answer the relevant questions, sign it and send it back to us with your extract plate(s). Your order will not be accepted until your DNA plate(s) arrive with all the relevant documents and pass our DNA quality check.
- If you have made a mistake, uploading an incorrect Sample Tracking File for this order, please do not confirm this order and make another order with the correct file.
- If, AFTER confirming the order, you realise that the Sample Tracking File is slightly inaccurate, you will have the opportunity to update the file, provided the number of plates and the number of samples in every plate do not change. You cannot change the file once the DNA samples have arrived at our facility.
- When you are ready to pack and ship the DNA or non-DNA samples, please follow one of the following instructions which is applicable to you:
  1. DNA shipping instructions for Australian customers
  2. Material shipping instructions for Australian customers
  3. International DNA shipping instructions and documentation required by the Australian quarantine system
  4. International material shipping instructions and documentation required by the Australian quarantine system

 I read and understood DNA quality requirements and shipping instructions.[Confirm Order](#)

Cornell University

nextgencassava.org • cassavabase.org

@nextgencassava



# Thank you!

All documentation available here:

<ftp://ftp.cassavabase.org/intertek/>



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