



CIP
INTERNATIONAL
POTATO CENTER



Excellence in
Breeding
Platform

Genomic tools to accelerate potato breeding

Hannele Lindqvist Kreuze
and potato breeding team

International Potato Center (CIP)

ALAP Virtual Meeting

10 November 2022



Screening for biotic stress resistance

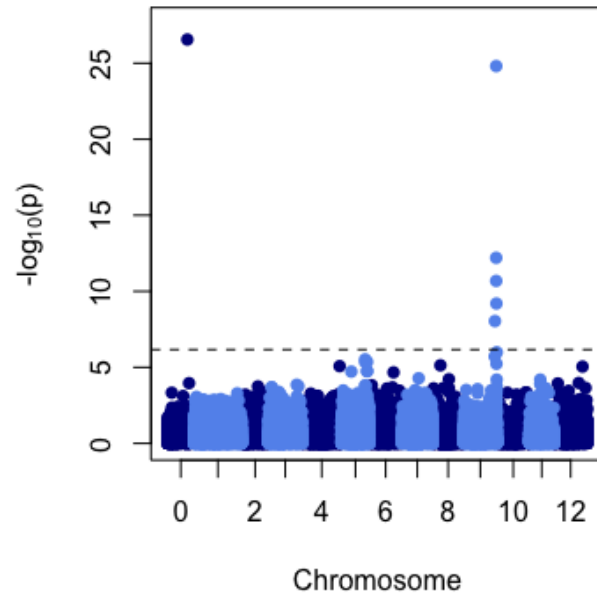
	PLRV - potato	PVY - potato	Late Blight - potato
Resistance evaluation	Field or Green house	Field or Green house	Field, replicated trials
Assay duration	>2 seasons	1 season (?)	> 2 seasons
Inoculation method	Aphid, infector plants	mechanical and graft inoculation	Infector plants
Phenotypic evaluation	ELISA test	ELISA test	Visual estimate
Number of pathogen strains	few	few	several
Type of resistance	monogenic & polygenic	Monogenic & polygenic	monogenic & polygenic
Resistance sources	Rare, RI adg	Ry adg, Ry sto + few others	Various major genes and QTL

PLRV inoculation: aphids and grafting



How about using molecular markers?

Late blight resistance QTL in chromosome 9 in CIP potato breeding lines



GBS Marker	Ref	Alt	Trial	$-\log_{10}p$	Effect
ST4.03ch09 60067335	A	G	China 2015	22.38	-0.28
			China 2016	23.23	-0.25
			Peru 2014	15.46	-0.22
Solcap_c2_56 418	A	C	Peru 2001-2006		

Li et al., 2012. Theor. Appl. Genet. Conditional QTL underlying resistance to late blight in a diploid potato population.

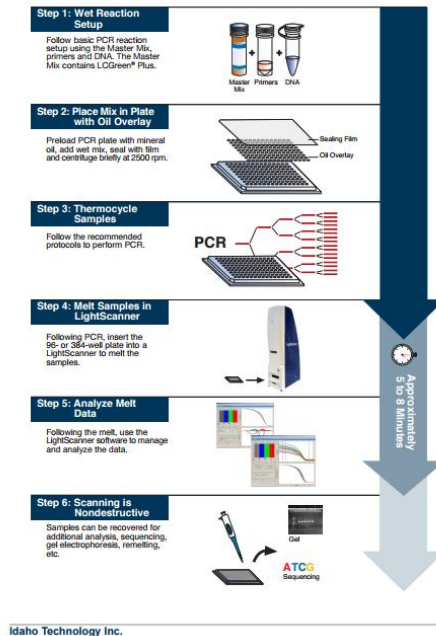
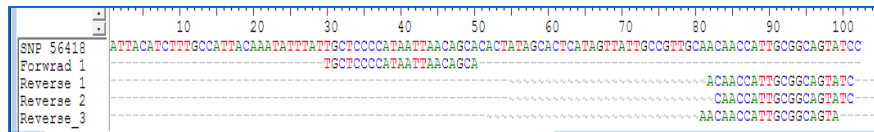
Lindqvist-Kreuze et al., 2014. Phytopathology. Phenotypic stability and genome-wide association study of late blight resistance in potato genotypes adapted to the highland tropics.

Lindqvist-Kreuze et al., 2021. Global multi-environment resistance QTL for foliar late blight resistance in tetraploid potato with tropical adaptation. **G3, 11(11), p.jkab251.**

HRM marker for late blight resistance based on Solcap snp_c2_56418

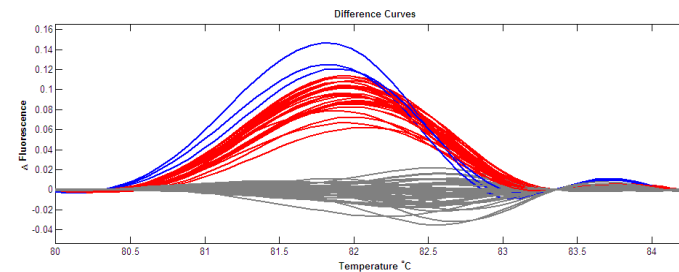
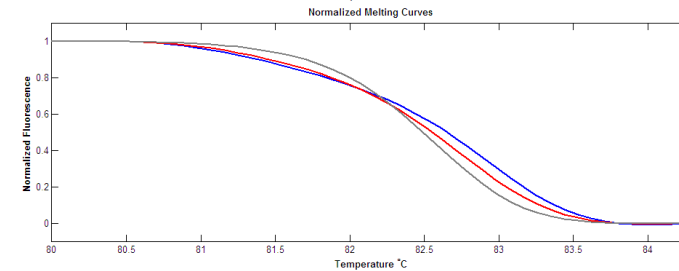
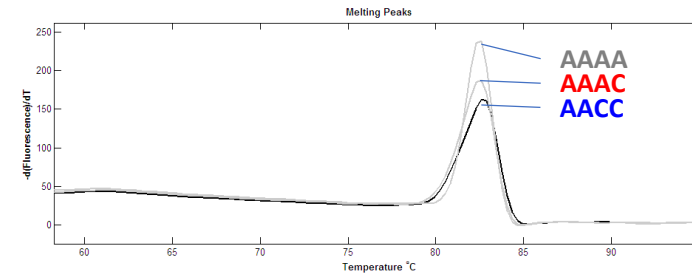
cost: \$4 / sample

solcap_snp_c2_56418 PGSC0003DMB000000280 354041
 GGATACTGCCGCAATGGTTGTTGCAACGGCAATACTATGAGTGCTATAG[T/G]TGCTGTTAATTATGGGGAGCAATAAATATTTGTAATGGCAAAGATGTAAT



PCR

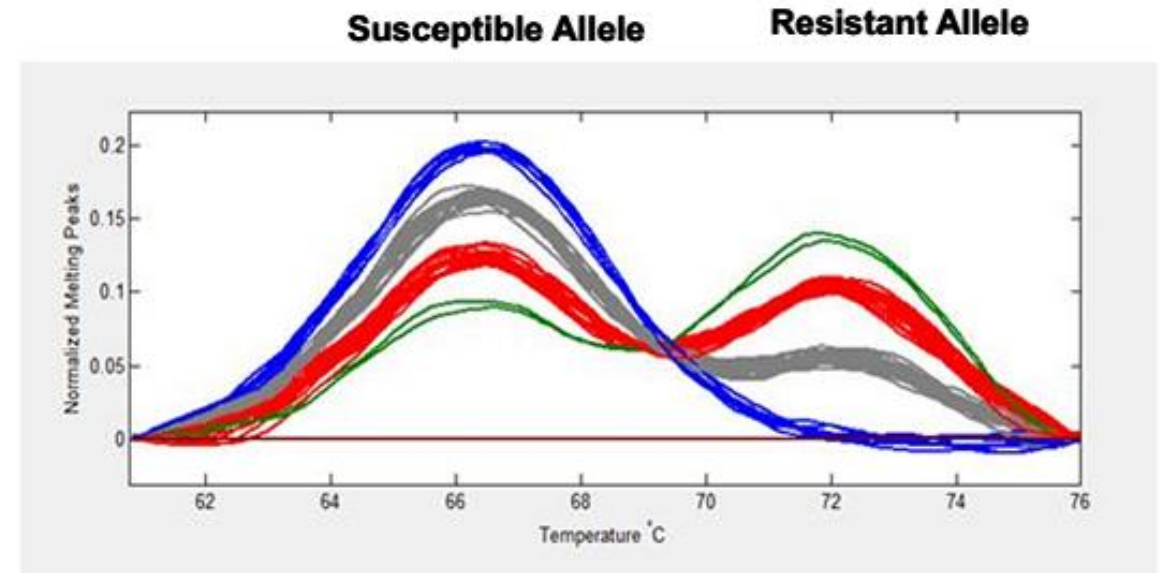
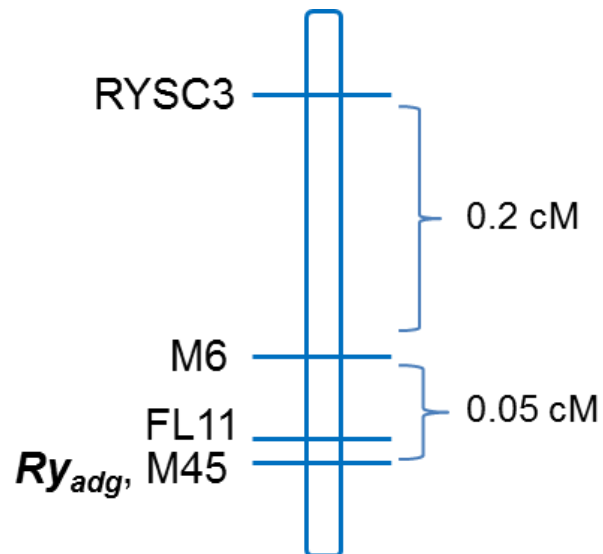
HRM



PVY resistance in CIP breeding populations mainly in chromosome 11 based on Ry_{adg}

cost: \$4 / sample

- RYSC3 marker tightly linked to Ry_{adg} locus on chromosome 11 (Kasai et al., 2000)
- HRM marker based on M6 developed at CIP (Herrera et al., 2018. TAG)



HRM analysis on the F1 population CxT:

aaaa (LBR-43) = 30

Aaaa (Costanera) = 101

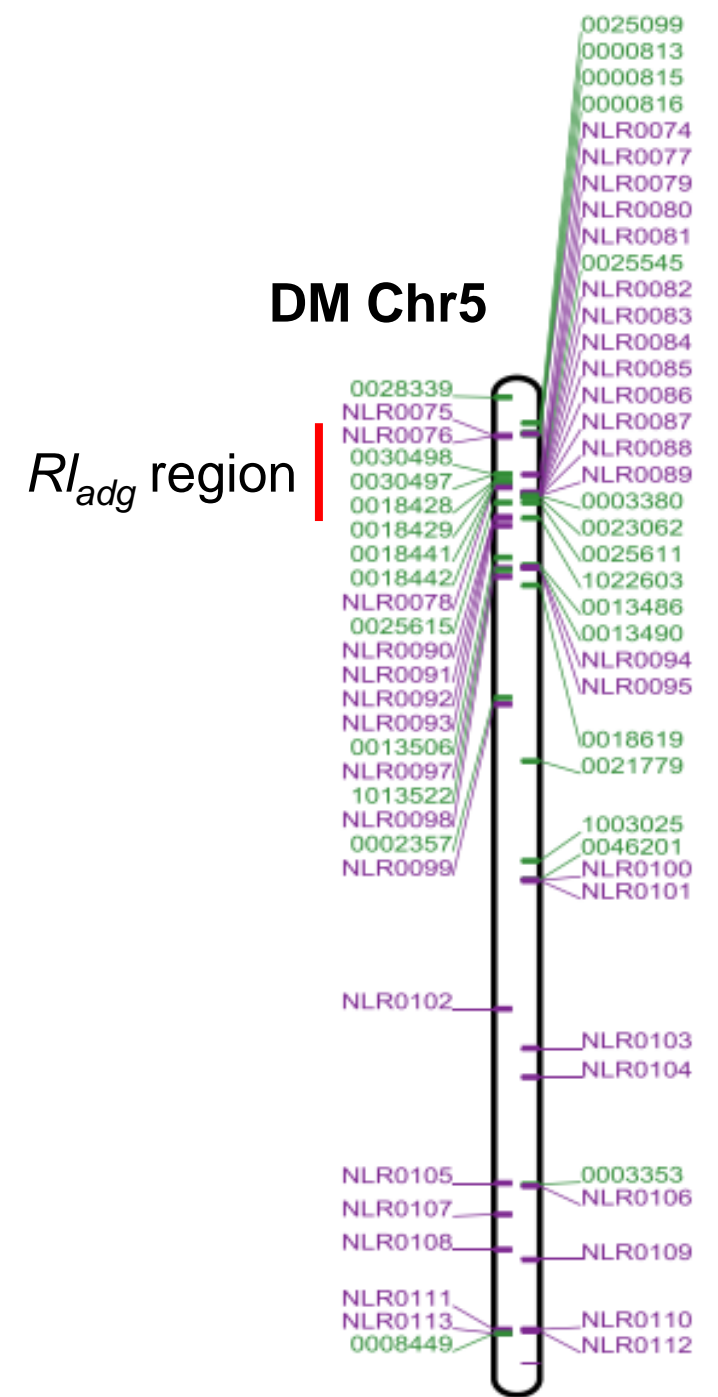
AAaa (Txy2) = 84

AAAa = 2

Rl_{adg} confers PLRV resistance and maps to Chr5

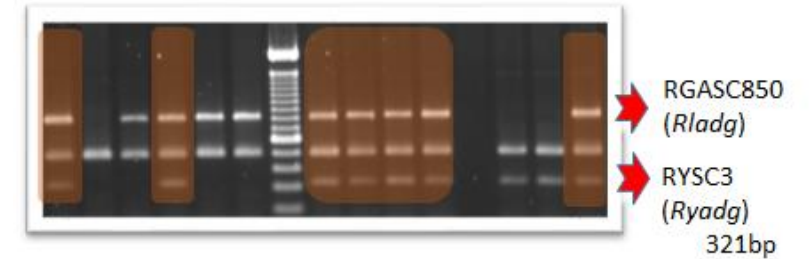
- PLRV resistance gene Rl_{adg} in *S. tuberosum ssp. andigena* accession LOP-868 mapped in Chr5 at CIP (Velazquez et al. 2009)
- Rl_{adg} is a *Bs4* homologue maps to 5 Mb interval of Chr5 with many NLR encoding genes (R. Heal & J. Jones, Sainsbury labs, 2022)

← Telomere chromosome 5 in potato



Multiplex assay combining PLRV and PVY resistance assays

- PLRV SCAR: tightly linked to Rl_{adg} locus on chromosome 5 (Mihovilovich et al., 2014)
- PVY SCAR: tightly linked to Ry_{adg} locus on chromosome 11 (Kasai et al., 2000)

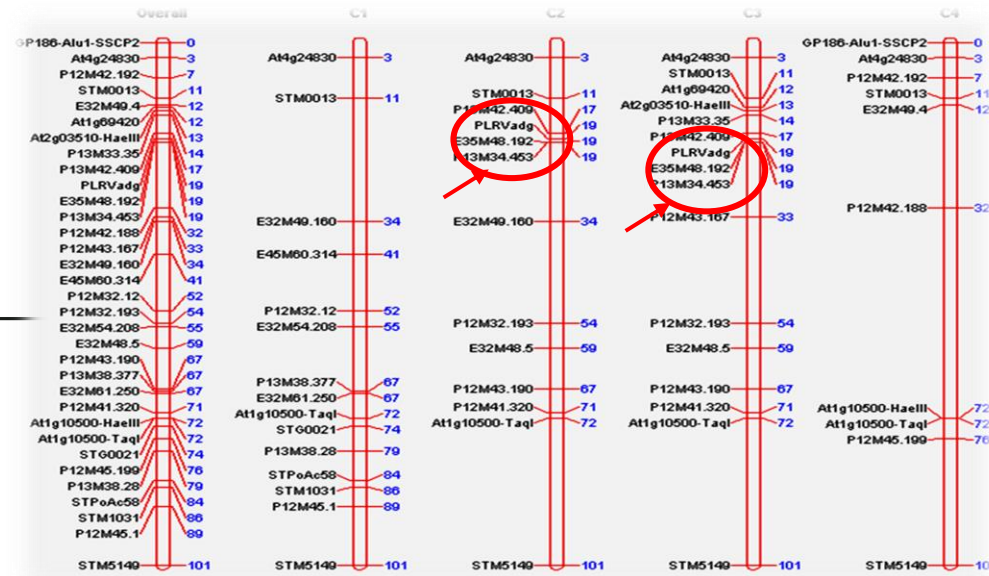


Plant Mol Biol Rep (2014) 32:117–128
DOI 10.1007/s11105-013-0629-5


ORIGINAL PAPER

An RGA-Derived SCAR Marker Linked to PLRV Resistance from *Solanum tuberosum* ssp. *andigena*

Elisa Mihovilovich • Mariela Aponte •
Hannele Lindqvist-Kreuz • Merideth Bonierbale



cost: \$5 / sample



Why are the breeders not using the markers?

Disconnection between breeders and molecular breeders?

Long turnaround time?

Too high cost?

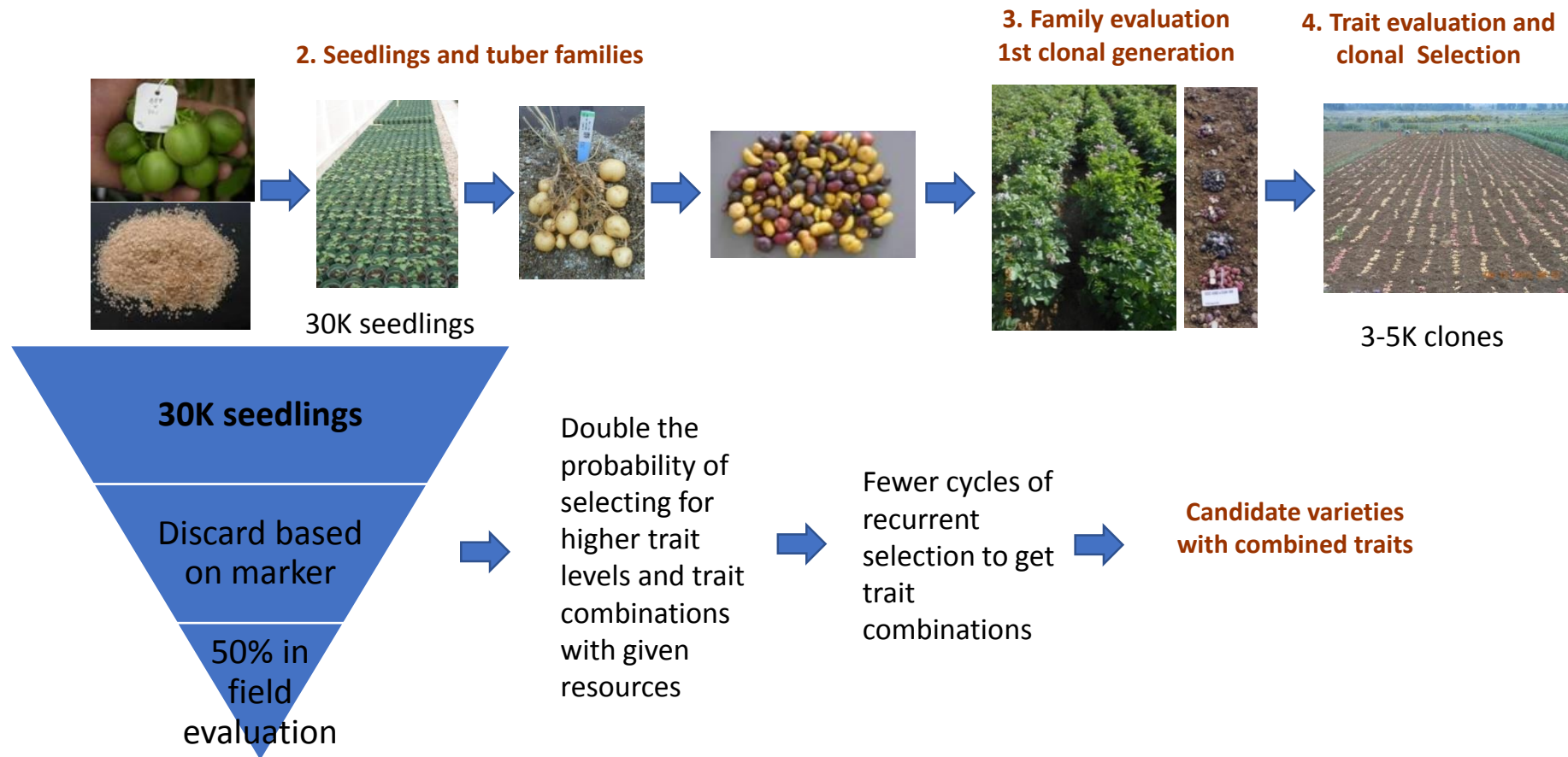
How to interpret results?

Do these markers work?

Cost comparison 96 samples (in house 2016)

component	HRM	SCAR
DNA extraction	\$192	\$192
Service fee		
PCR reagents + plastics	\$25	\$21
LCGreen dye	\$8.6	-
HotStart Taq	-	\$74
Staff time	\$300	\$300
Bench fee	\$168	\$168
Total cost USD	\$694	\$755
Turnaround time	4 days	4 days

CIP-potato-HQ forward selection initial plan 2017



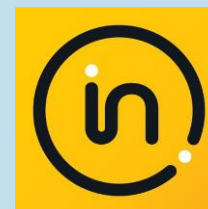
Challenge: to optimize and scale up existing genotyping technologies for accelerated breeding while maintaining low cost

Tasks:

- Convert existing diagnostic SNP markers to KASP
- Set up a protocol for sampling and processing
- Set up a data management system and process to return the results to breeders

Solution: How does it work?

- Shared service agreement for CGIAR centers and their partners to use KASP genotyping platform at Intertek
- Large sample volume = attractive prize/datapoint
- Service includes DNA extraction and genotyping
- Quick turnaround time of 10-15 days to receive results

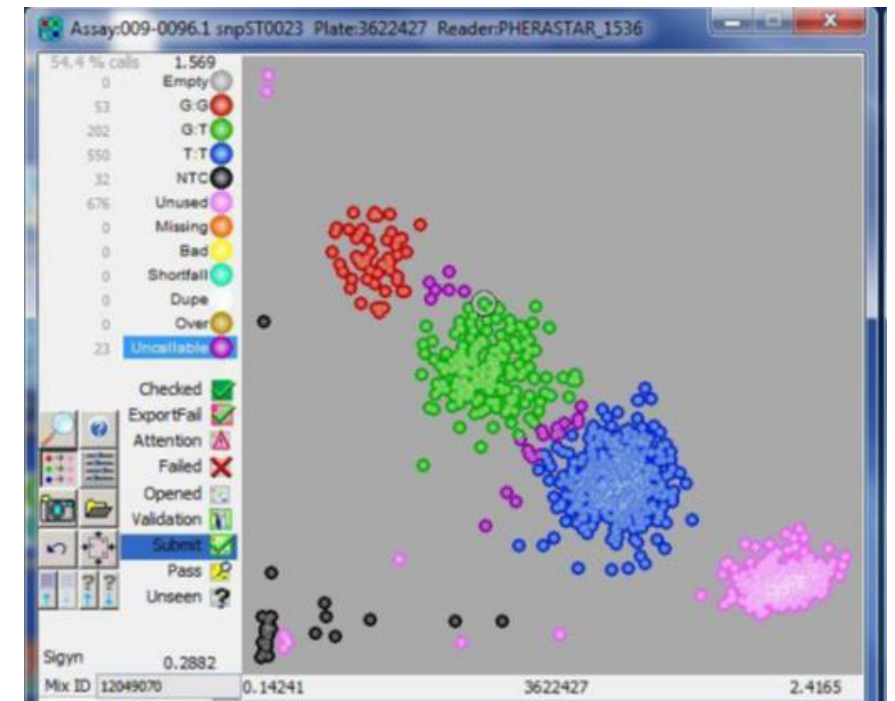


Trait markers

Table. CIP trait markers available at Intertek

Trait	SNP id	Intertek id	Gene/ QTL	SNP	Chr
Late blight resistance	S9_6121167	snpST00020	R8- QTL	a/c	9
	Solcap_snp_c2_56418	snpST00023	R8- QTL	t/g	9
PVY resistance	M6F1R4_711	snpST00052	Ryadg	g/a	11
	M6F1_R4_817	snpST00073	Ryadg	a/g	11

Figure. Illustration of the results for snpST00023 in tetraploid CIP breeding lines



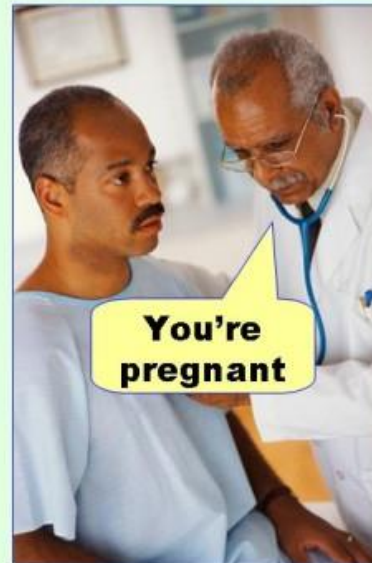
Kante et al. 2021, Kompetitive Allele Specific PCR (KASP) Markers for Potato: An Effective Tool for Increased Genetic Gains. *Agronomy*, 11(11), p.2315.

Trait marker assay verification

Table. Assay verification of the PVY *Ryadg* (snpST00052, snpST00073) and late blight (snpST00020, snpST00023) KASP markers

Marker ID	Number of Clones	α	β	s
snpST00052	78	0.0	0.16	0.83
snpST00073	78	0.0	0.16	0.83
snpST00020	73	0.02	0.13	0.88
snpST00023	77	0.22	0.35	0.65

α **Type I error**
(false positive)



β **Type II error**
(false negative)



Kante et al. 2021, Kompetitive Allele Specific PCR (KASP) Markers for Potato: An Effective Tool for Increased Genetic Gains. *Agronomy*, 11(11), p.2315.

Search Toolbox

Keywords / authors

Type

- Any -

Topic

- Any -

Search

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Quick links

KASP low density genotyping Platform

Tools

Genotyping / sequencing tools and services

Important: Notice on Intertek service availability from December 2021 January 2022

A DNA-based molecular marker is a genomic DNA (gDNA) fragment located at a specific position that may or may not be linked to a specific agricultural interest. Trait linked DNA based markers allow us to easily breed materials for favorable alleles associated with traits of interest.

The EiB low-density genotyping service is based on KASP markers. Kom Allele Specific PCR (KASP) is a simplified fluorescence-based method to genotype specific polymorphisms or INDELS. This approach is cost effective



Banana



Cassava



Chickpea



Cowpea



Fish



Groundnut



Maize



Pearl Millet



Pigeonpea



Potato



Rice



Sorghum



Soybean



Sweetpotato



Wheat

<https://excellenceinbreeding.org/module3>

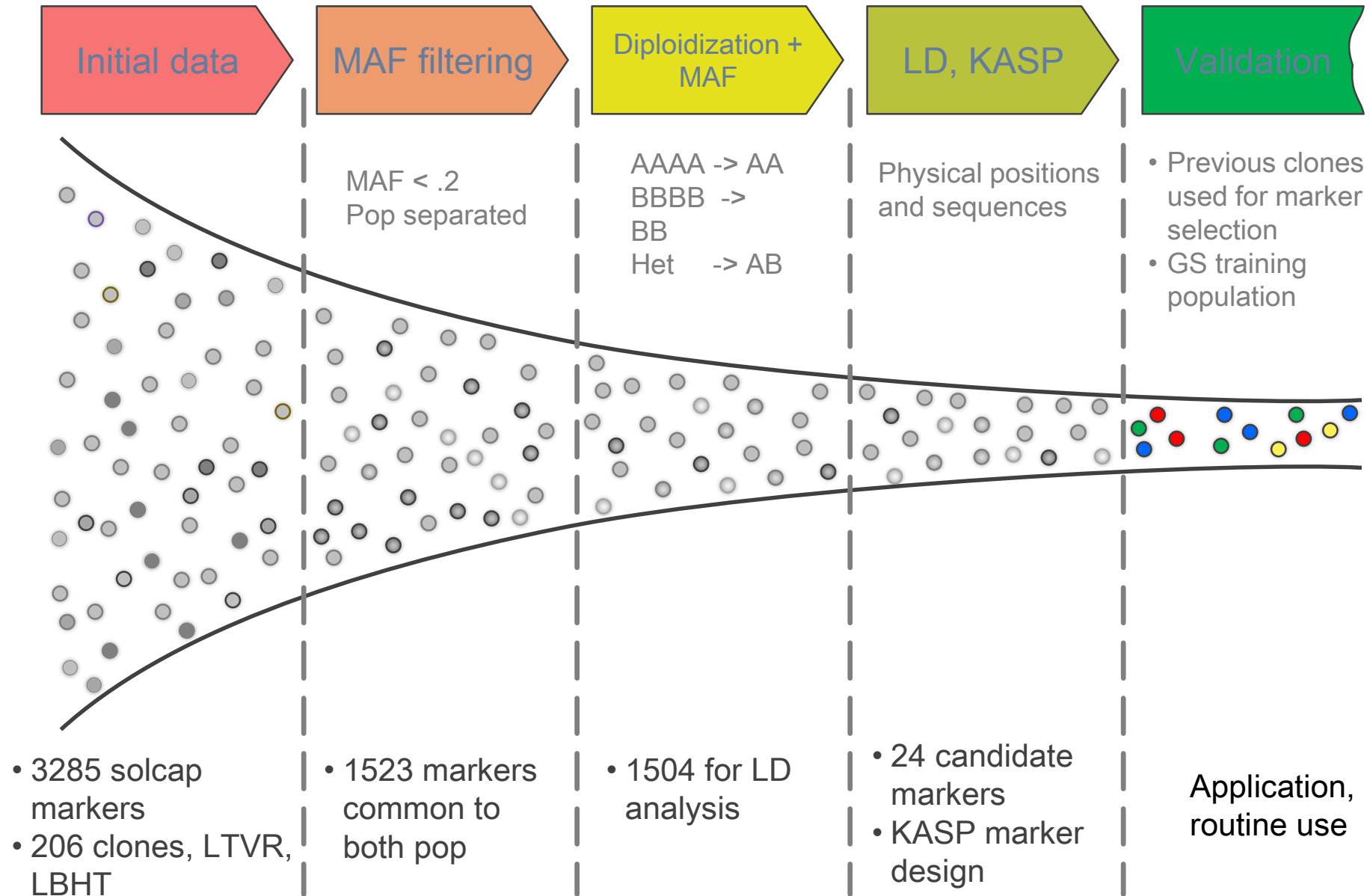
Potato KASP trait markers

S/N	Intertek ID	SNP_ID	Trait	Description	Crop	Species	Position (ref. Stv4.03)		Favourable allele	Unfavorable allele	SNP	Gene / QTL
							Chr	Stv4.03)				
1	snpST00052	M6F1R4_711	PVY	Potato Virus Y, Ryadg	Potato	<i>S. tuberosum</i>	11	2499608	A	G	A/G	Ryadg
2	snpST00073	M6F1R4_817	PVY	Potato Virus Y, Ryadg	Potato	<i>S. tuberosum</i>	11	2499502	G	A	G/A	Ryadg
3	snpST00082	YES3_a	PVY	Potato Virus Y, Rysto	Potato	<i>S. tuberosum</i>	12	2352742	T	G	T/G	Rysto
4	snpST00083	YES3_b (backup)	PVY	Potato Virus Y, Rysto	Potato	<i>S. tuberosum</i>	12	2352616	DEL	INS	DEL/INS	Rysto
5	snpST00020	S9_61261167	Late Blight	late blight resistance	Potato	<i>S. tuberosum</i>	9	67379976	C	A	C/A	R8-QTL
6	snpST00023	solcap_snp_c2_56418	Late Blight	late blight resistance R2 gene	Potato	<i>S. tuberosum</i>	9	66379167	G	T	G/T	R8-QTL
7	snpST00106	CPRISNP13	LRB	late blight resistance R2 gene	Potato	<i>S. tuberosum</i>	4	5522862	CGAAA	TGATT	CGAAA/TGA TT	R2
8	snpST00107	CPRISNP14	PVY	Potato Virus Y, Rysto	Potato	<i>S. tuberosum</i>	11	284162	CAATTCC	TAATTCT	CAATTCC/TA ATTCT	<i>Ny(o,n)sto</i>
9	snpST00297	ST4_03ch12_58961580	SC	Self-compatibility	Potato	<i>S. tuberosum</i>	12	58961580	A	G	A/G	<i>Sli</i>
10	snpST00298	ST4_03ch12_58962561	SC	Self-compatibility	Potato	<i>S. tuberosum</i>	12	58962561	A	G	A/G	<i>Sli</i>
11	snpST00299	ST4_03ch12_58974932	SC	Self-compatibility	Potato	<i>S. tuberosum</i>	12	58974932	A	T	A/T	<i>Sli</i>
12	snpST00300	ST4_03ch12_59002442	SC	Self-compatibility	Potato	<i>S. tuberosum</i>	12	59002442	T	C	T/C	<i>Sli</i>
13	snpST00301	ST4_03ch12_59019319	SC	Self-compatibility	Potato	<i>S. tuberosum</i>	12	59019319	A	T	A/T	<i>Sli</i>
14	snpST00302	ST4_03ch12_59023684	SC	Self-compatibility	Potato	<i>S. tuberosum</i>	12	59023684	T	C	T/C	<i>Sli</i>
15	snpST00303	ST4_03ch12_59155291	SC	Self-compatibility	Potato	<i>S. tuberosum</i>	12	59155291	C	A	C/A	<i>Sli</i>
16	snpST00304	ST4_03ch12_59184424	SC	Self-compatibility	Potato	<i>S. tuberosum</i>	12	59184424	C	T	C/T	<i>Sli</i>
17	snpST00305	ST4_03ch12_59211572	SC	Self-compatibility	Potato	<i>S. tuberosum</i>	12	59211572	A	G	A/G	<i>Sli</i>
18	snpST00306	ST4_03ch12_59271443	SC	Self-compatibility	Potato	<i>S. tuberosum</i>	12	59271443	T	C	T/C	<i>Sli</i>

Potato QC KASP markers

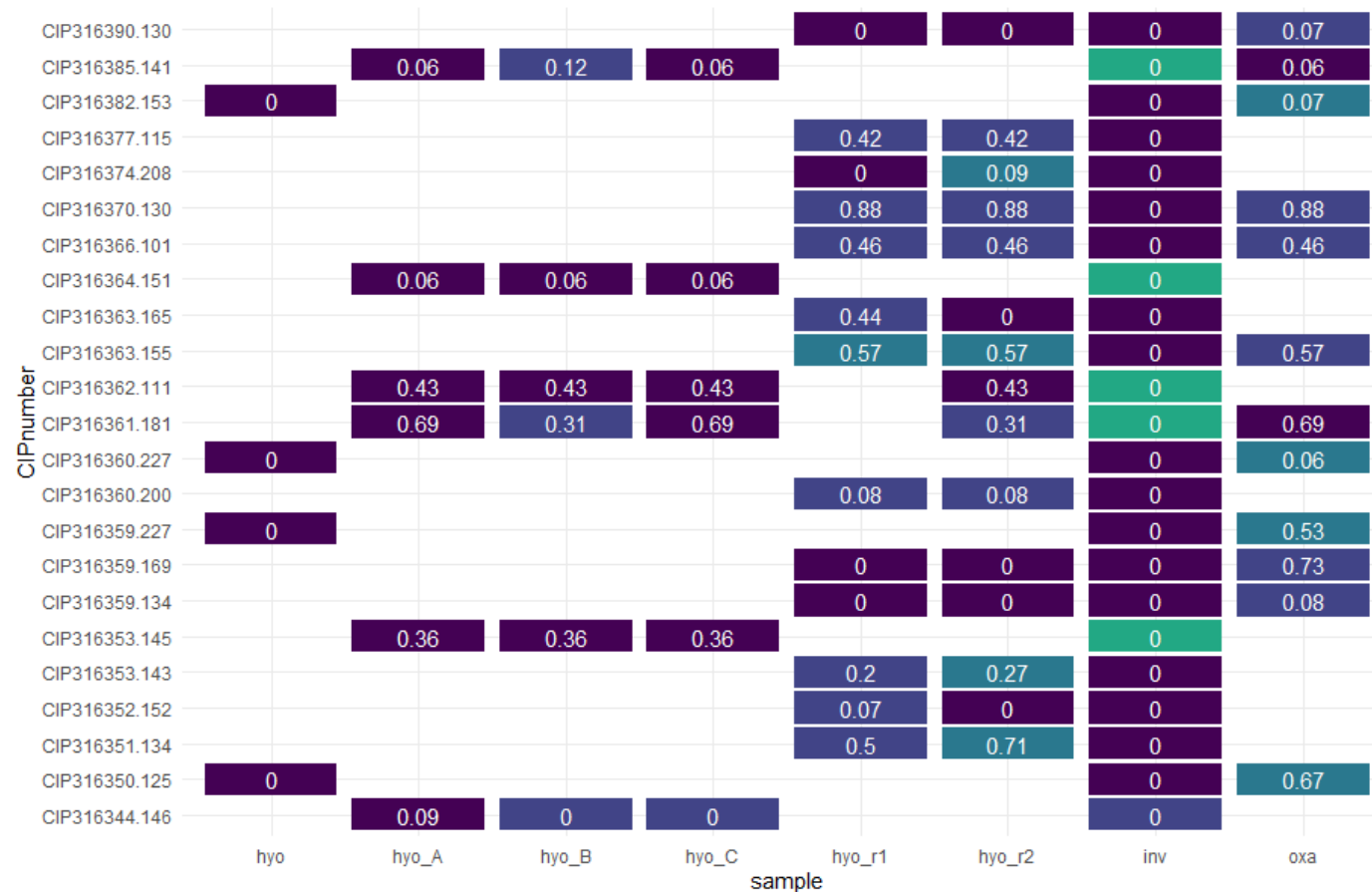
S/N	Intertek ID	Customer SNP ID	Trait	Description	n	Chr	Position	Reference genome	Allele 1	Allele 2	SNP Use	Region	Source
1	snpST00118	4	Quality Control	solcap_snp_c1_460	QC	1	112323	potato_69011_map_context_DM_v3	T	C	T/C QC	Peru	CIP
2	snpST00119	0	Quality Control	solcap_snp_c1_480	QC	1	566236	potato_69011_map_context_DM_v3	T	C	T/C QC	Peru	CIP
3	snpST00121	1	Quality Control	solcap_snp_c2_763	QC	2	623110	potato_69011_map_context_DM_v3	G	A	G/A QC	Peru	CIP
4	snpST00122	63	Quality Control	solcap_snp_c2_483	QC	3	105444	potato_69011_map_context_DM_v3	C	G	C/G QC	Peru	CIP
5	snpST00125	42	Quality Control	solcap_snp_c1_150	QC	4	3085410	potato_69011_map_context_DM_v3	A	G	A/G QC	Peru	CIP
6	snpST00127	3	Quality Control	solcap_snp_c2_825	QC	5	129363	potato_69011_map_context_DM_v3	A	G	A/G QC	Peru	CIP
7	snpST00132	44	Quality Control	solcap_snp_c2_290	QC	8	403319	potato_69011_map_context_DM_v3	T	C	T/C QC	Peru	CIP
8	snpST00135	17	Quality Control	solcap_snp_c1_115	QC	9	63530	potato_69011_map_context_DM_v3	A	G	A/G QC	Peru	CIP
9	snpST00136	solcap_snp_c1_305	Quality Control	solcap_snp_c1_305	QC	10	1098620	potato_69011_map_context_DM_v3	T	C	T/C QC	Peru	CIP
10	snpST00137	17	Quality Control	solcap_snp_c2_389	QC	10	239328	potato_69011_map_context_DM_v3	C	T	C/T QC	Peru	CIP
11	snpST00138	12	Quality Control	solcap_snp_c2_571	QC	11	400006	potato_69011_map_context_DM_v3	A	G	A/G QC	Peru	CIP
12	* snpST00067	2	Quality Control	solcap_snp_c1_221	QC	11	232784	potato_69011_map_context_DM_v3	T	C	T/C QC	Peru	CIP
13	snpST00174	solcap_snp_c1_456	Quality Control	solcap_snp_c1_456	QC	3	1151967	potato_69011_map_context_DM_v3	C	T	C/T QC	Peru	CIP

Selection of quality control markers



Application of QC markers

	Greenhouse (LM)	Huancayo (hyo)	Oxapampa (oxa)	Total samples
Number of samples	114	224	38	376
Within plot, 3 samples		17		
Across replications		77		



Genotype	Diploid			Tetraploid		
	M1	M2	M3	M1	M2	M3
CIP316344_106	T:T		T:T	T:T:T:T		T:T:T:T
CIP316344_154	T:T	C:T	C:T	T:T:T:T	C:C:C:T	C:C:T:T
CIP316345_168	T:T	C:T	C:T	T:T:T:T	C:C:C:T	C:C:T:T
CIP316345_237	C:T	C:C	T:T	C:T:T:T	C:C:C:C	T:T:T:T
CIP316345_245	C:T	C:T	C:T	C:T:T:T	C:C:C:T	
CIP316348_165	T:T	C:T	T:T	T:T:T:T	C:C:C:T	T:T:T:T
CIP316348_172	T:T	C:T	T:T	T:T:T:T	C:C:T:T	T:T:T:T

Low-density genotyping service (LDSG)

Platform – KASP genotyping method

Primary Application – QC/QA at F1 stage & trait specific genotyping

Data available for usage– **KASP markers available for 18 crops & fish**

Target users – CGIAR, NARS & NARIs breeding institutions

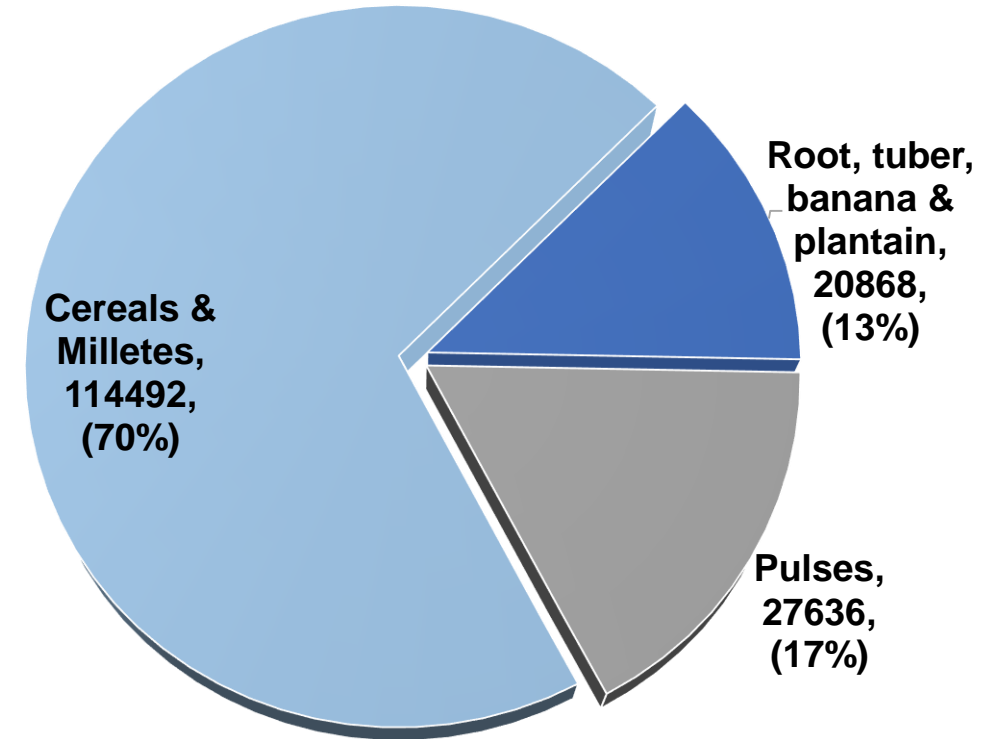
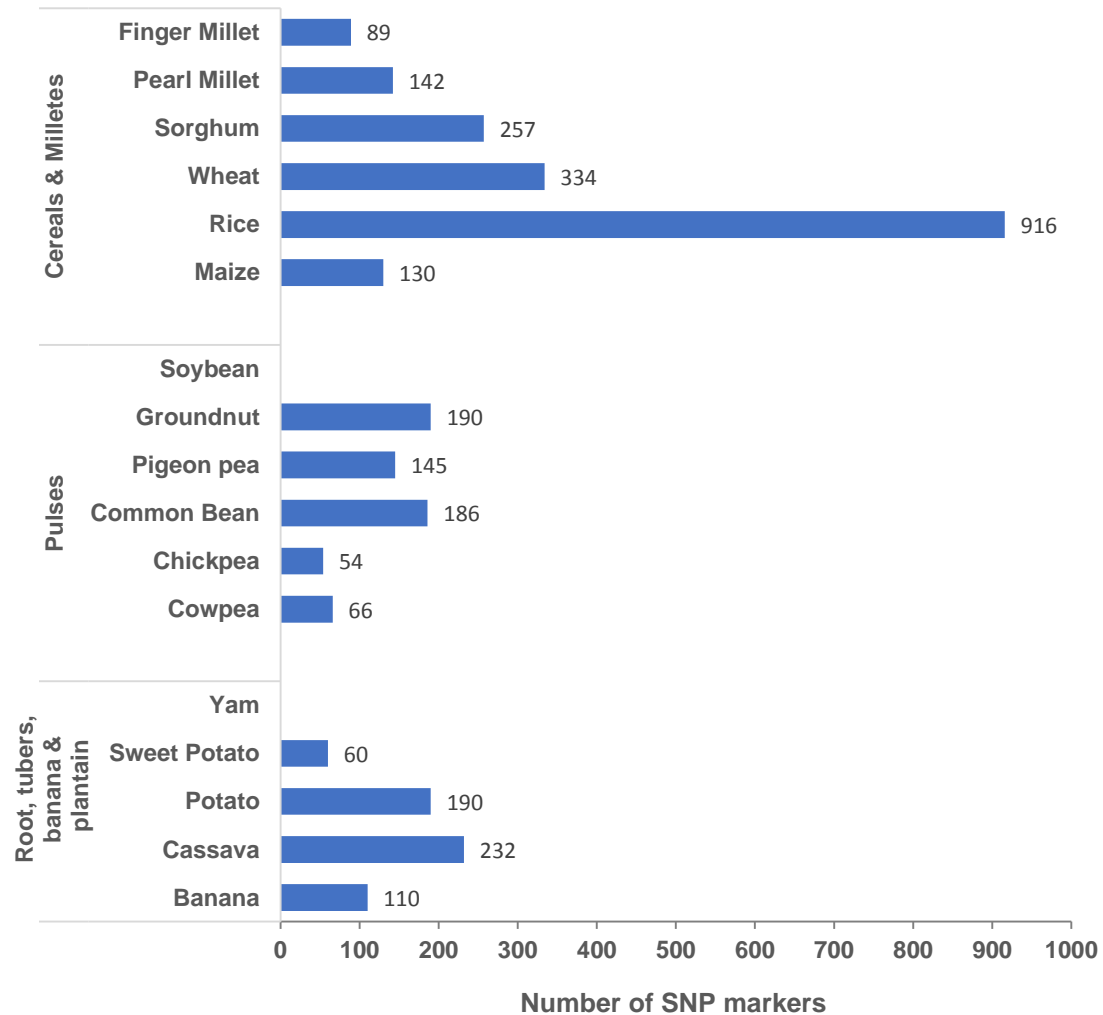
Cost – Depends on number of markers to use

Turnaround time – 10–15 days

Integration of SNP markers in CGIAR & NARS BPs in Africa- 2020

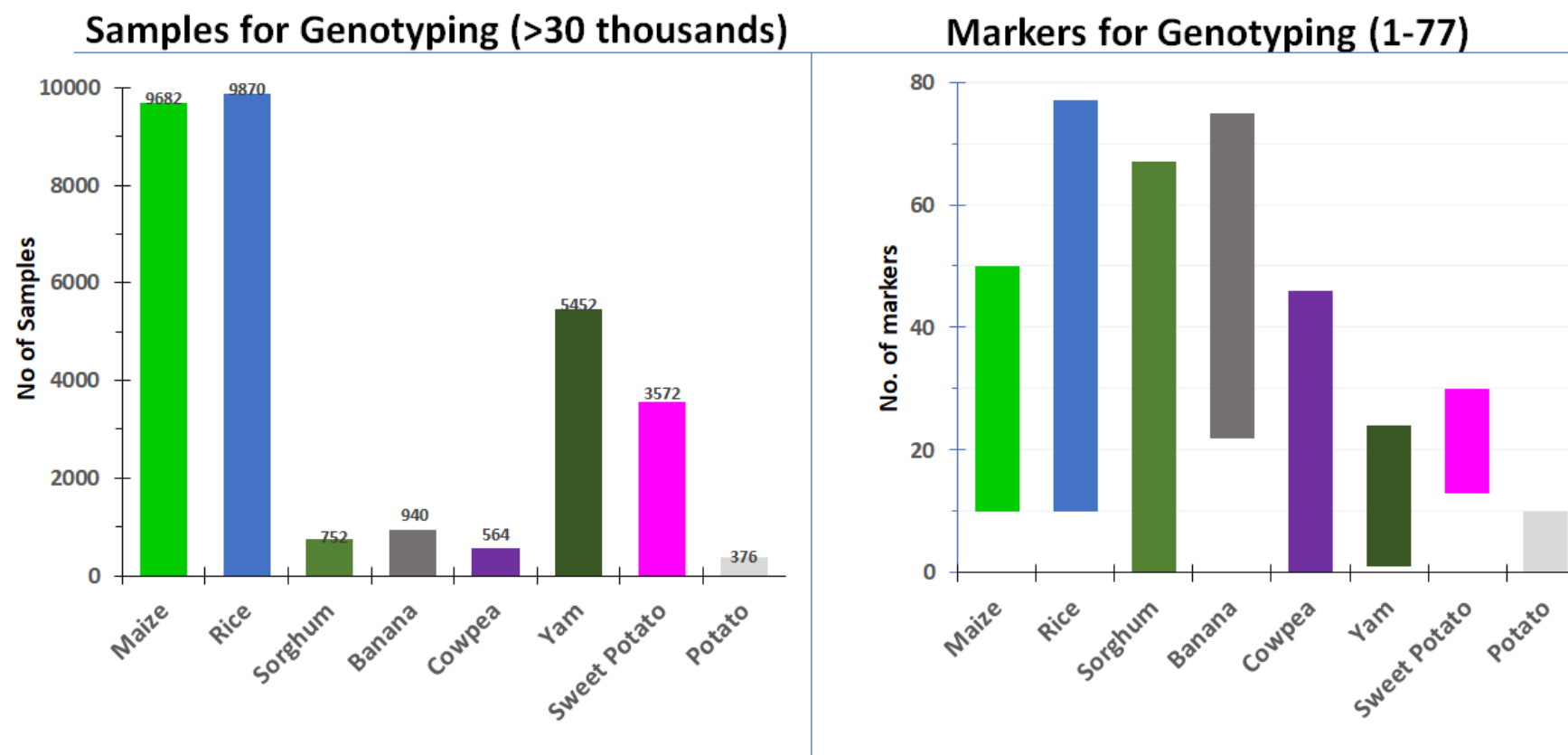


INITIATIVE ON
Breeding Resources



Genotyping of different field crops
(sample size approx. 163,000)

Integration of SNP markers in CGIAR & NARS BPs in Africa - 2021

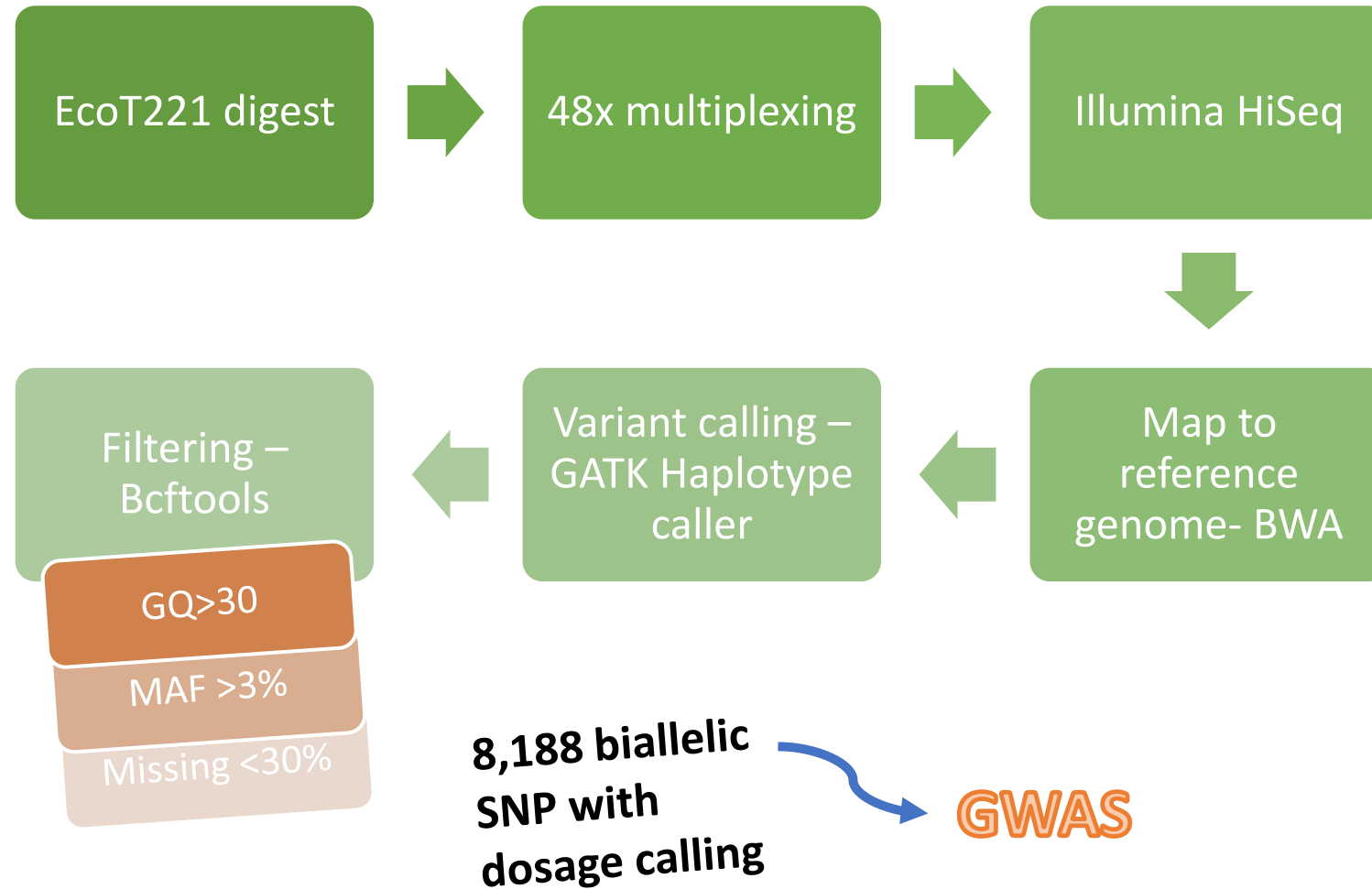


Past molecular markers at CIP potato Breeding program

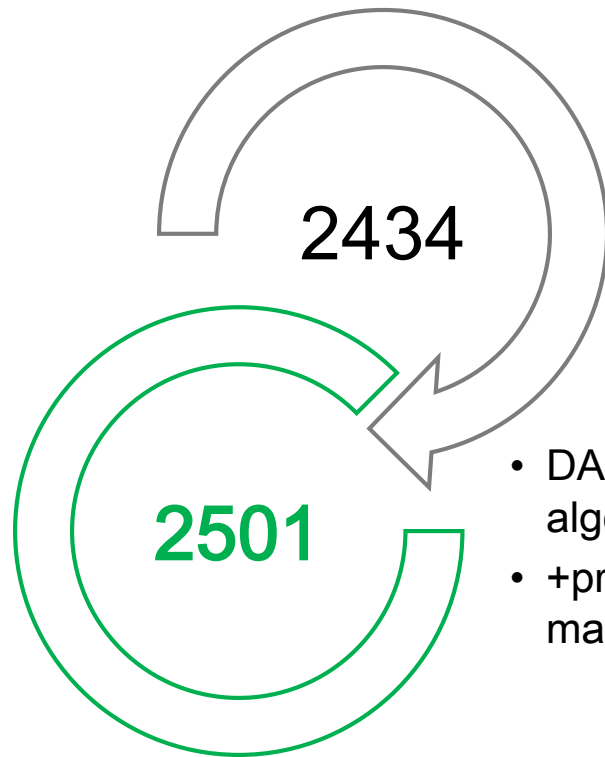
method	purpose	Where?	Technical effort	Data processing effort	TAT	Cost/sample /datapoint
SSR, COS	Fingerprint, genetic mapping	CIP	+++	+++	++	
Illumina SoICAP SNP	Genetic mapping, GWAS	Service provider	+	++	+++	
GBS		Service provider + CIP	+	+++++	++++	

Sample size 4 plates (4*96=384)

Genotyping of the tetraploid diversity panel using GBS (Genotyping By Sequencing)

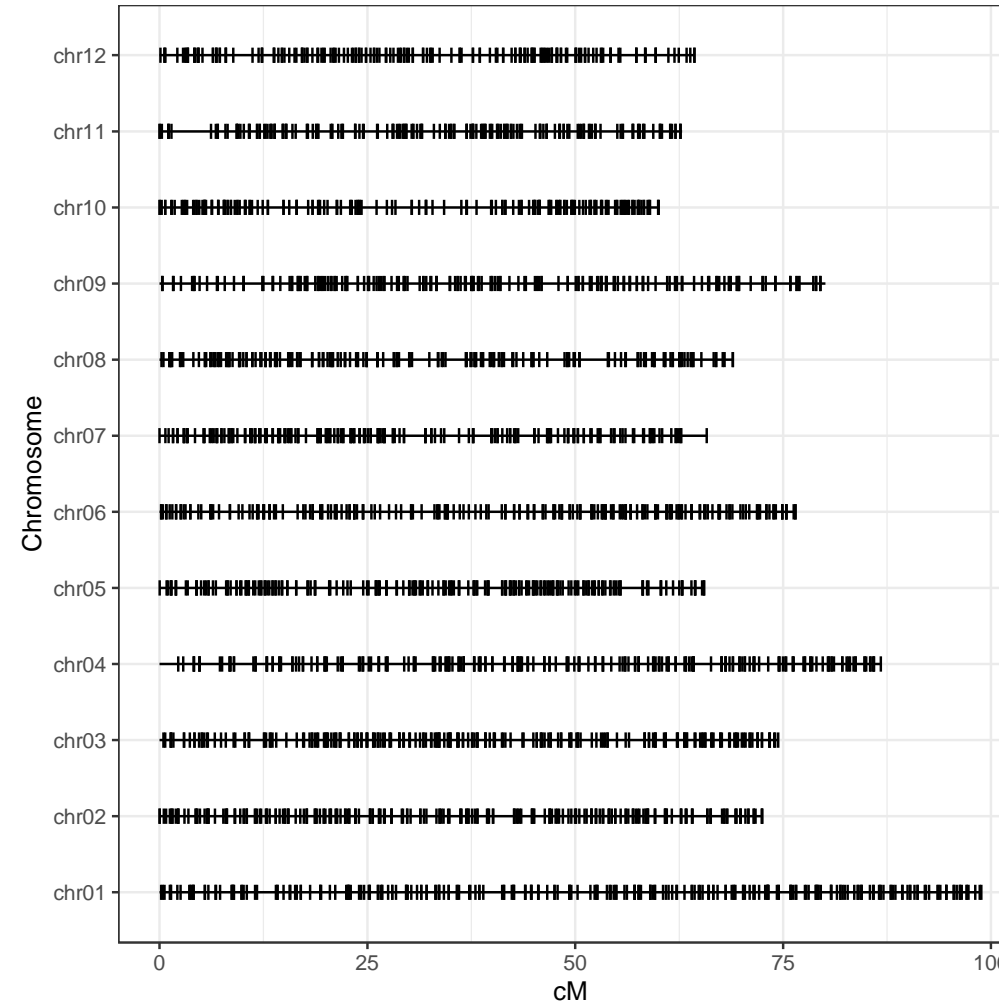


Mid-density platform



- 1cM bins / chr
- 3 SNP with the highest MAF and 1kb apart

- DArT primer design algorithm
- +previously selected markers



Distribution of 2501 genomic markers

Simple bioinformatics

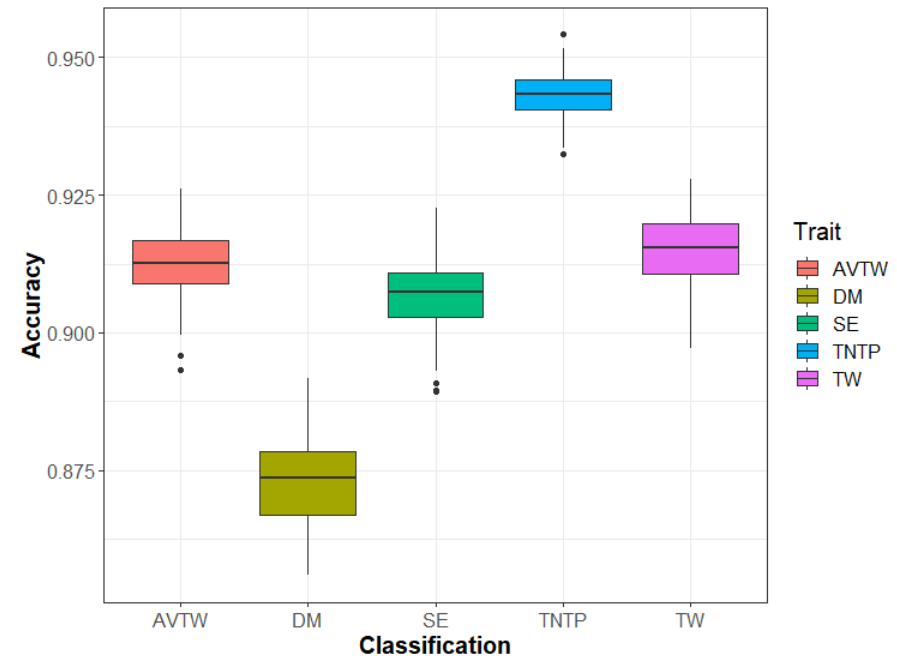
Relatively low cost

Possible inclusion of any mapped marker

High cross validation prediction accuracy using DArTag marker panel designed for potato

- 3 full-sib families genotyped with DArTag markers
- Phenotypic data from 3 environments
 - DM= dry matter (tubers)
 - SE= senescence (foliage maturity)
 - AVTW= tuber weight
 - TNTP=tuber number

PhD thesis: Joao Nomura



Field trial in Majes, Peru, 2018.



Comparison of DArTag and DArTSeq marker systems

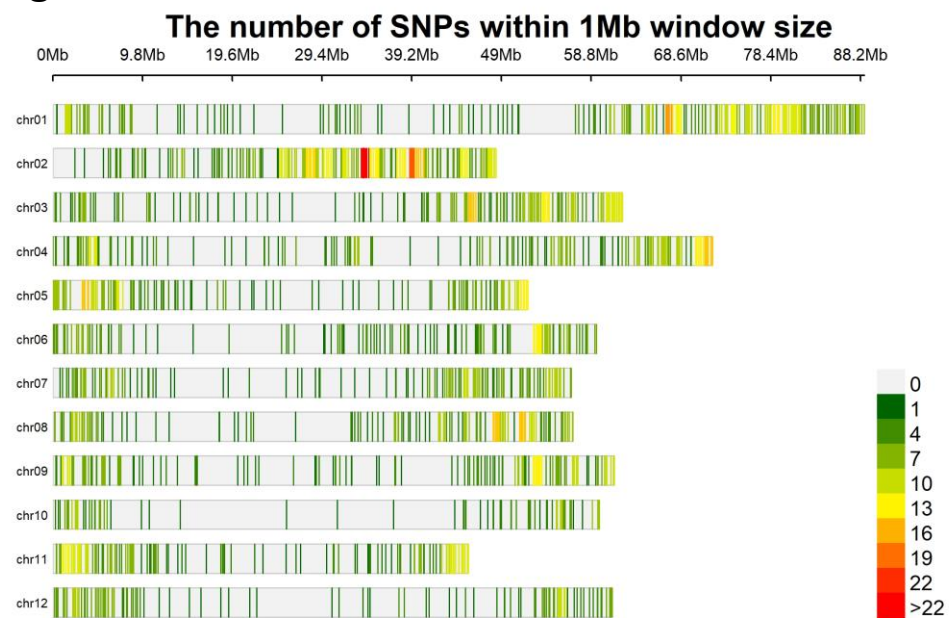
PhD thesis: Joao Nomura

Number of markers

	DArTag				LD	HD
	DArTag 10	DArTag 25	DArTag 61	DArTag Dipl		
Initial number	2503				3328	23905
Mean mis. prop.	0.037	0.032	0.022	0.011	0.011	0.008
Computational Time	~ 1h 30 min				~ 12h	~ 2 days
Final number of markers MAF 0.1	1474	1472	1318	1819	2187	5579
Final number of markers MAF 0.05	1599	1584	1406	1852	2293	5754

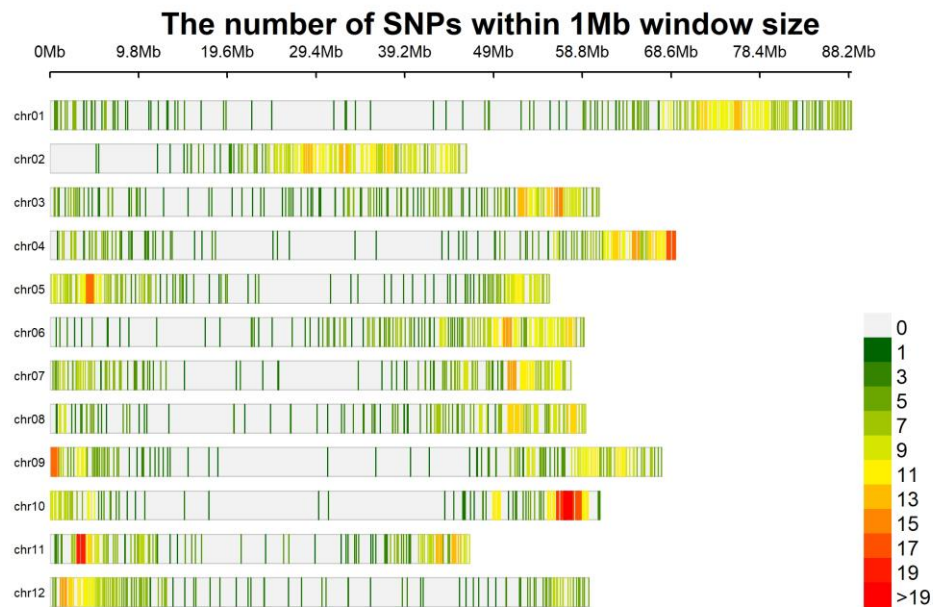
Markers position

DARtag

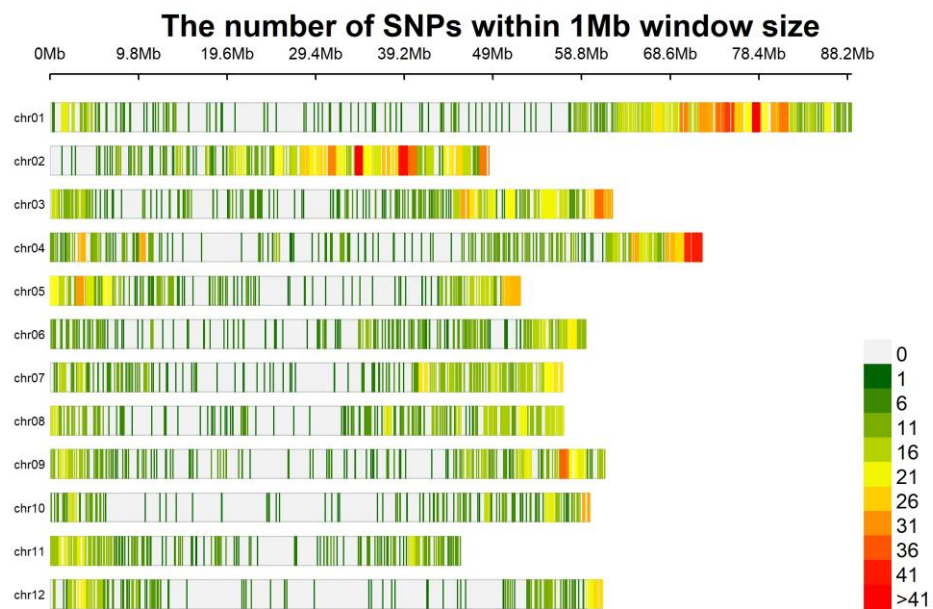


PhD thesis: Joao Nomura

LD



HD



Genomic prediction: Trait = tuber weight

PhD thesis: Joao Nomura

	DArTag 10	DArTag 25	DArTag 61	DArTag dipl	LD	HD
	TW					
H ²	0.111	0.110	0.111	0.110	0.113	0.110
Pheno rgg	0.231					
Pedigree rgg	0.414					
GV rgg	0.550	0.549	0.549	0.441	0.454	0.454
r 8-2	0.912 Bb	0.912 ABb	0.915 Aa	0.865 Ca	0.862 CDab	0.859 Db

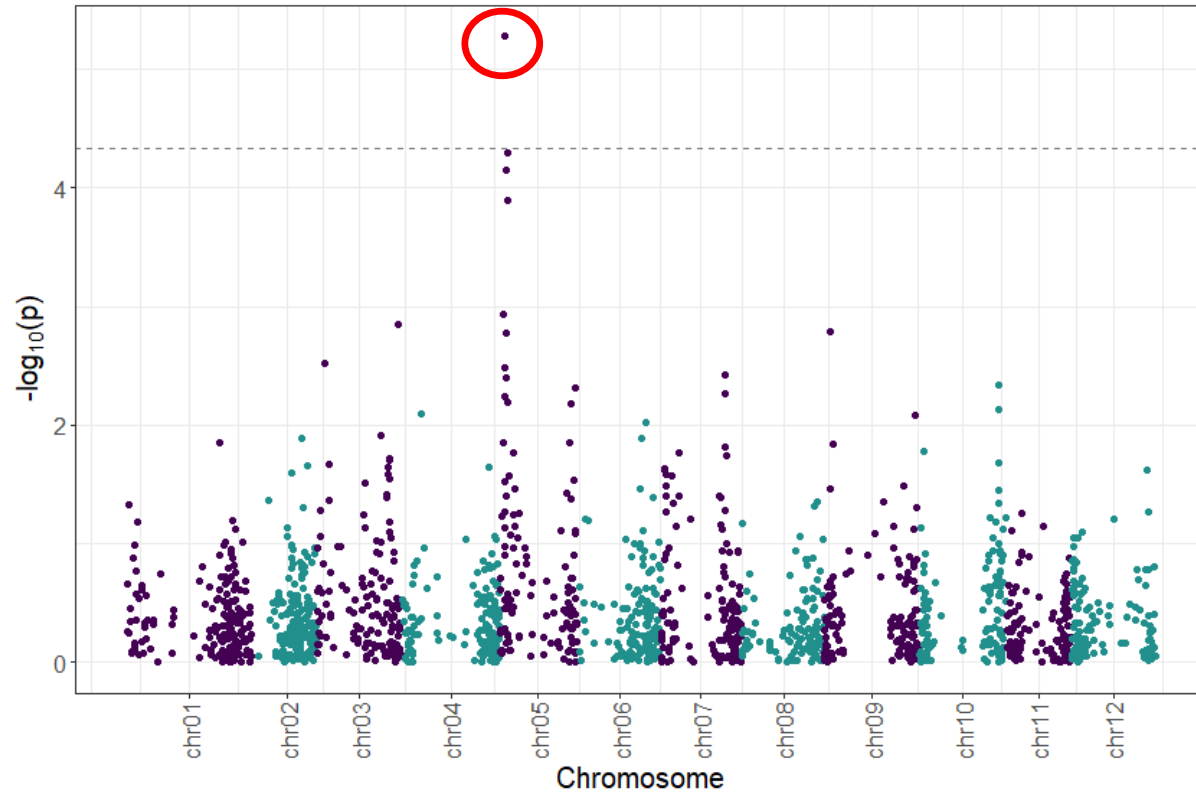
Genomic prediction: Trait= dry matter content

PhD thesis: Joao Nomura

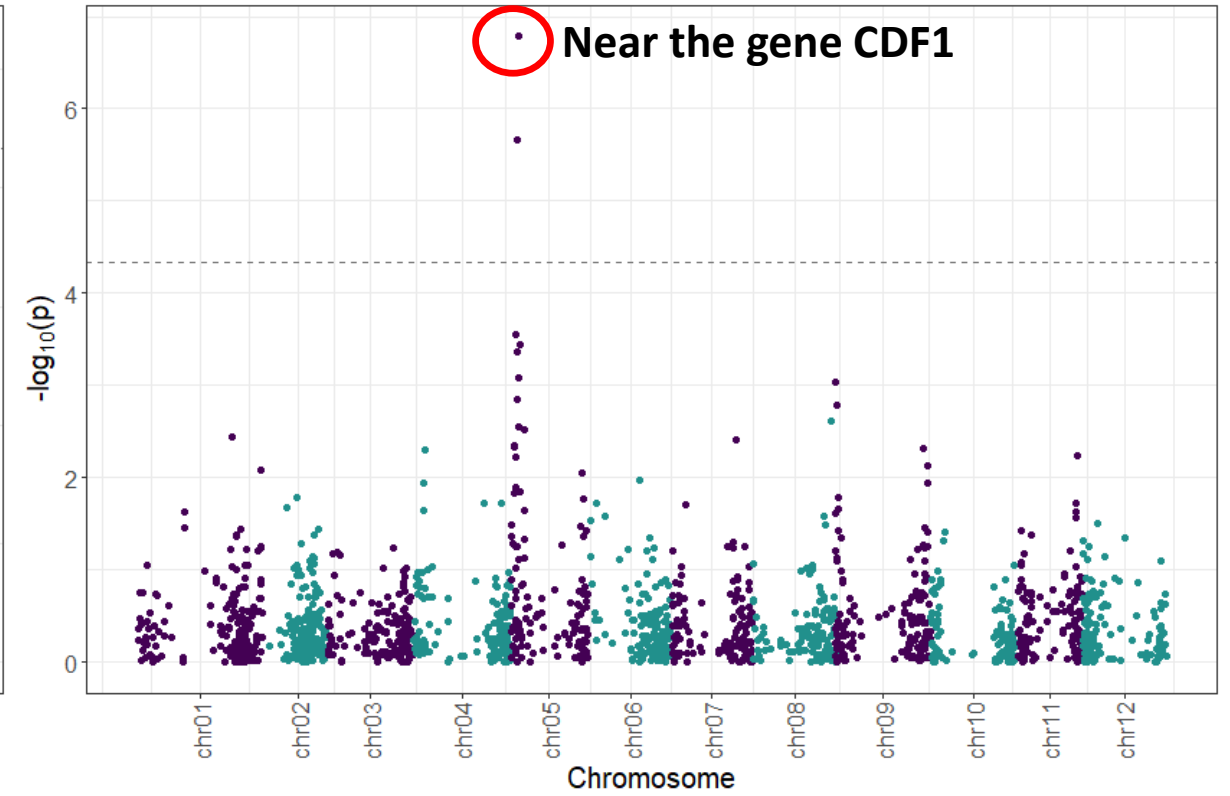
	DARtag 10	DARtag 25	DARtag 61	DARtag dipl	LD	HD
	DM					
H ²	0.486	0.488	0.488	0.449	0.462	0.456
Pheno rgg	0.617					
Pedigree rgg	0.667					
GV rgg	0.760	0.762	0.763	0.665	0.712	0.719
r 8-2	0.871 Aa	0.872 Aa	0.873 Aa	0.817 Ba	0.803 Cb	0.799 Cc

GWAS

- Senescence



- Yield Drought Treatment



Mid-density genotyping service (MDSG)

Platform – DArTAg genotyping method

Primary Application – GS,

Secondary Application – Diversity studies, DNA-Fingerprinting,

Panels available: **9 panels validated and in continuous use**

Target users – CGIAR, NARS & NARIs breeding institutions

Cost – US \$11 per sample

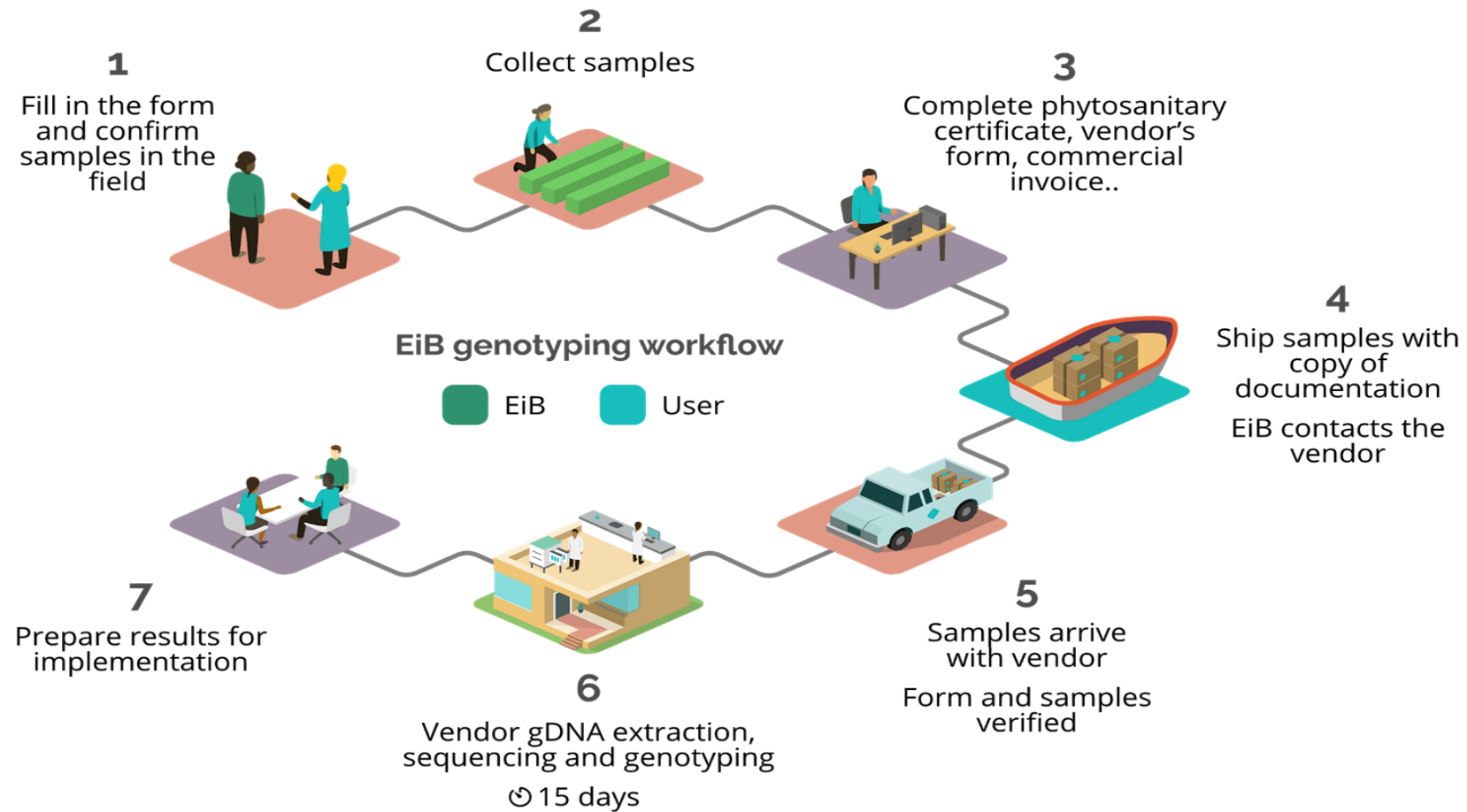
Turnaround time – 10–15 days.

Mid-density DArTtag panels



Crop/Status	Panel name	Vendor	Marker Density	Planning	Design	Validation	Implementation
Rice	1K RiCA (V4)	Agriplex/DArT	1K	✓	✓	✓	✓
Potato	Potato DArTag EiB (1.0)	INT/DArT	2.1K	✓	✓	✓	✓
Wheat	Wheat DArTag EiB 3.9K (2.0)	INT/DArT	3.9K	✓	✓	✓	✓
Maize	Maize DArTag EiB (2.0)	INT/DArT	3.5K	✓	✓	✓	✓
Sorghum	Sorghum DArTag EiB (1.0)	INT/DArT	3.5K	✓	✓	✓	✓
Common Bean	Common bean DArTag EiB (1.0)	INT/DArT	1.9K	✓	✓	✓	✓
Cowpea	Cowpea DArTag EiB (1.0)	INT/DArT	2.6K	✓	✓	✓	✓
Groundnut	Groundnut DArTag EiB (1.0)	INT/DArT	2.5K	✓	✓	✓	✓
Pigeon Pea	Pigeonpea DArTag EiB (1.0)	INT/DArT	2K	✓	✓	✓	✓
Finger Millet		INT/DArT	2K	✓	✓		
Cassava		INT/DArT	3-4K	✓	✓	✓	

Genotyping Workflow

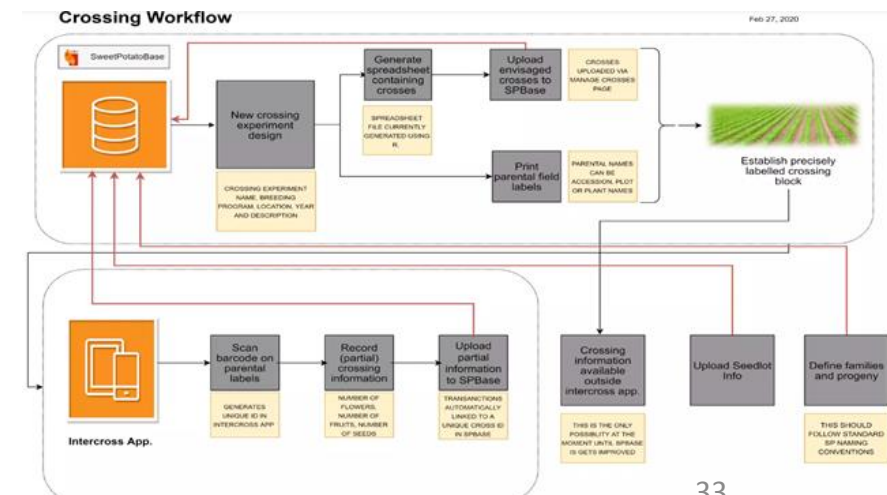
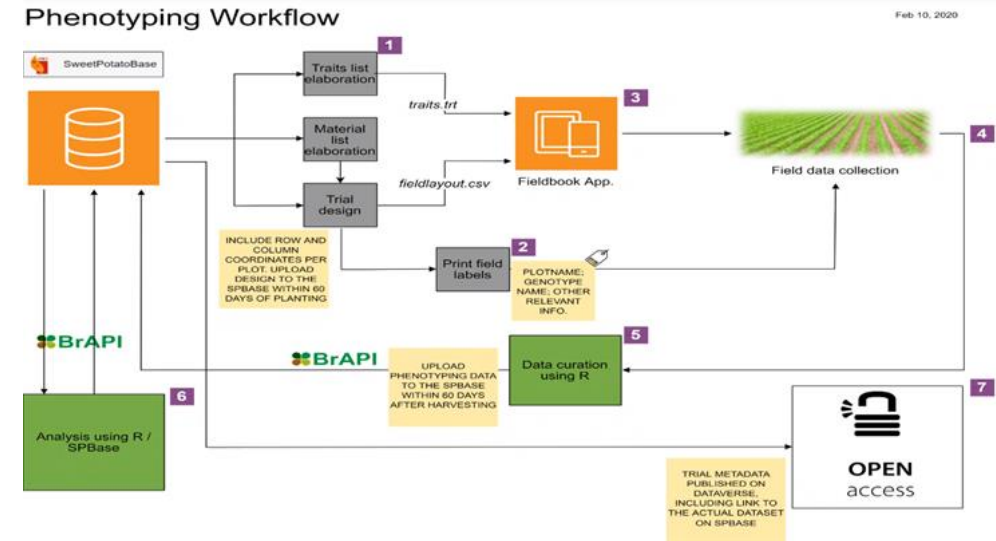


It all starts with high quality data...

- Standardization of the central integrated database system

(BreedBase)

- Sweetpotatobase: <https://sweetpotatobase.org/>
- Potatobase: <https://potatobase.org/>
- **SOPs** for breeding data management established for sweetpotato; ongoing for potato:
 - clear data workflows built around BreedBase
 - digital data collection with the use of barcode labels
 - naming convention across different breeding platforms
 - data recording and curation rules (e.g. NA/0)
 - recording row and column coordinates in the field



Lessons for others



Teamwork

- Molecular team in isolation is worth very little; need buy-in from the field breeders and management
- Consider offering “free” genotyping so field breeders can confirm tentative results in their program
- Clear roles and responsibilities within and among the teams for accountability



Operations

- Sampling strategy in place (protocols available in multiple languages)
- SOP developed
- Scripts for data analysis in R to support result interpretation

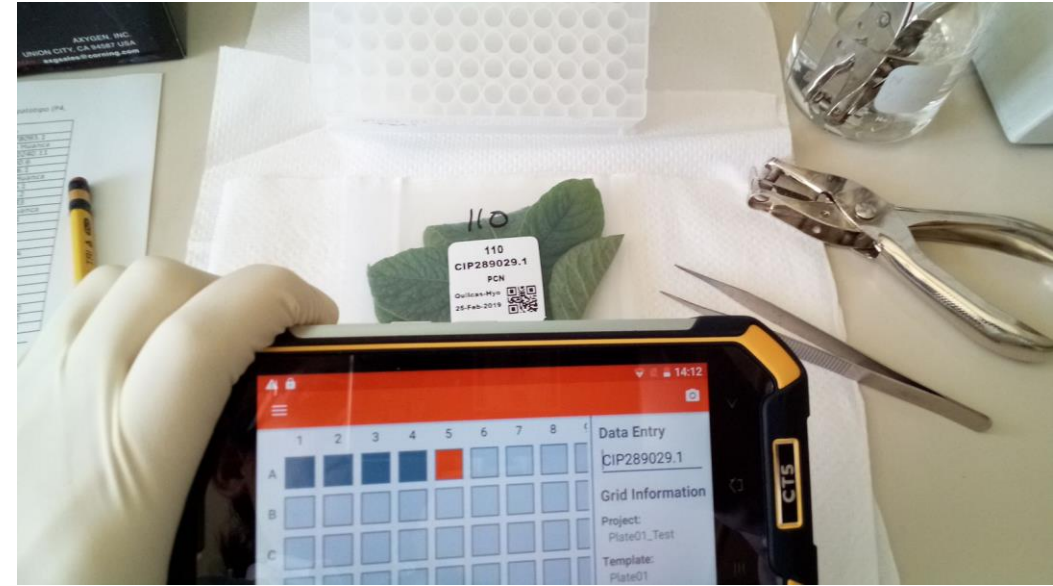


Data management

- In the absence of a database, organize all files: genotyping order forms, original results files from vendor, processed files
- Consistent sample names, trial names, etc.
- Database is required for the next steps

What's next?

- PotatoBase fully operational and with genotypic data
- Data from vendor directly to PotatoBase
- Generating order forms from PotatoBase
- Hybrid verification timing in the breeding process?
- Genome assembly for better marker development (currently available potato markers were developed using North American genepool)
- Add all markers in the mid-density marker set (Trait, QC)



Resources

- Kante et al, 2021. Agronomy 2021, 11(11), 2315;
<https://doi.org/10.3390/agronomy11112315>
- <https://excellenceinbreeding.org/toolbox/services/potato-mid-density-genotyping-services>
- <https://excellenceinbreeding.org/toolbox/tools/kasp-low-density-genotyping-platform>

Genomic tools to accelerate potato breeding

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