Marker-Assisted Selection

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Peanut Breeder's Tools Workshop
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Marker-Assisted Selection Applications

- Genetic purity
- Confirm crosses
- Whole genome mapping
- Trait association –
  - High oleic/linoleic acid ratio
  - Nematode resistance
  - Leaf spots
  - Seed size

Marker-Assisted Selection
Genetic Purity; Confirm Crosses

C1805: Tifguard x Florida07 — 04/08

↓

38 of 46 F1 seeds are hybrids

self ↙ ↘ BC1

1140 F_2 plants

↓

Phenotyping for marker validation in F_3 plants

↓

TifNV-HiOL

Tifguard x F1 — 09/08

↓ BC2

Tifguard x BC1 — 02/09

↓ BC3

Tifguard x BC2 — 08/09

↓ Self BC3

High O/L Tifguard — 06/10

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Holbrook, C.C., P. Ozias-Akins, Y. Chu, A.K. Culbreath, C.K. Kvien, T.B. Brenneman. 2017. Registration of 'TifNV-High O/L' Peanut. J. Plant Registr

Marker-Assisted Selection Applications

- Genetic purity
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  - High oleic/linoleic acid ratio
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Marker-Assisted Selection
Trait Association

Parent	Common or unique parent	Market class	Oleic acid[a]	TSWV[bc]	Early leaf spot[bd]	Late leaf spot[be]	White mold[bf]	Sclerotinia[bg]	CBR[bh]
Tifrunner	Common	Runner	L	R	MR	MR	S	U	U
Florida-07	Common	Runner	H	R	S	S	MR	U	U
N08082olJCT	Unique	Virginia	H	MR	MS	U	U	MR	MR
C76-16	Unique	Runner	L	MR	U	U	U	U	U
NC 3033	Unique	Virginia	L	HS	MR	HS	R	U	HR
NM Valencia A	Unique	Valencia	L	S	S	S	HS	HS	U
OLin	Unique	Spanish	H	MS	S	S	U	R	U
SSD 6	Unique	Exotic[i]	L	HR	U	U	U	U	U
SPT 06-06	Unique	Exotic[i]	L	U	HR	HR	U	U	U
Florunner	Unique	Runner	L	HS	S	S	S	S	S

White Mold

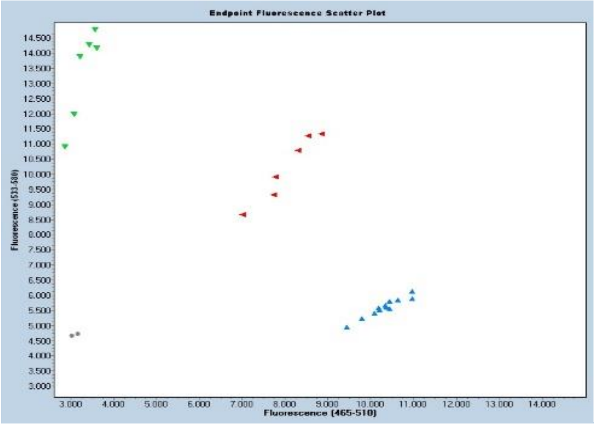
Early and Late Leaf Spots

Tomato Spotted Wilt Virus

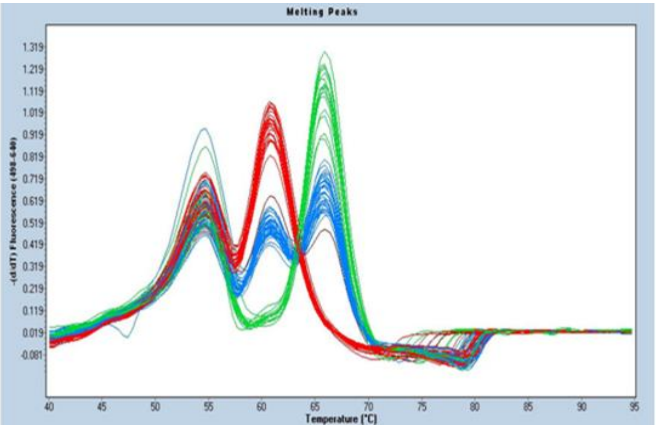
Holbrook, C.C., et al. 2013. …Recombinant Inbred Line (RIL) Populations for Peanut. Peanut Sci. 40:89-94

Marker-Assisted Selection
Single Nucleotide Polymorphism (SNP)

KASP Assay



Melting Curve Assay



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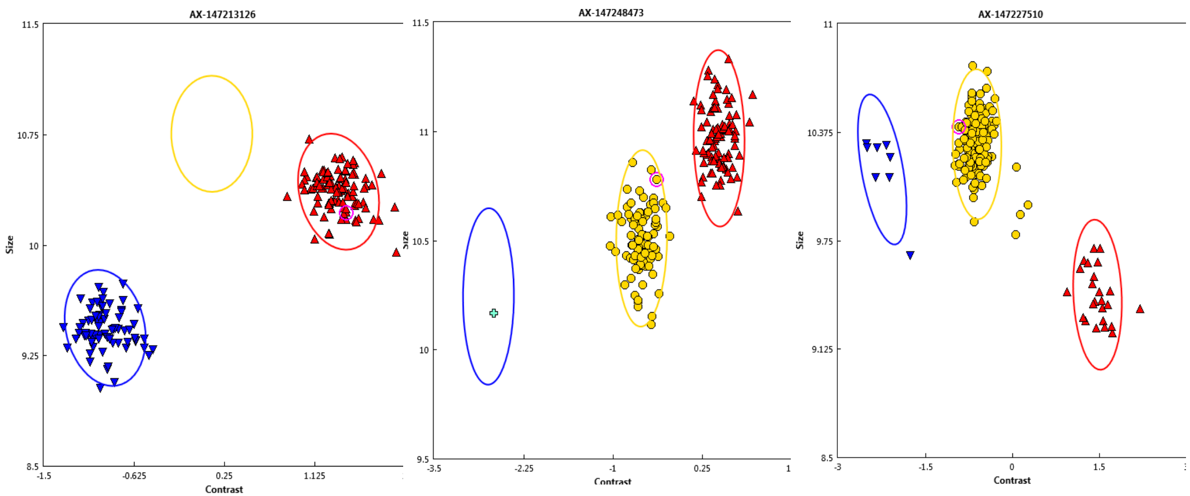
Marker-Assisted Selection
Genome-wide SNP Assay

Simple SNP
Alternate allele clusters

'Heterozygote' clusters reflect detection of homeologous regions;
additional clusters evidence of tetrasomy



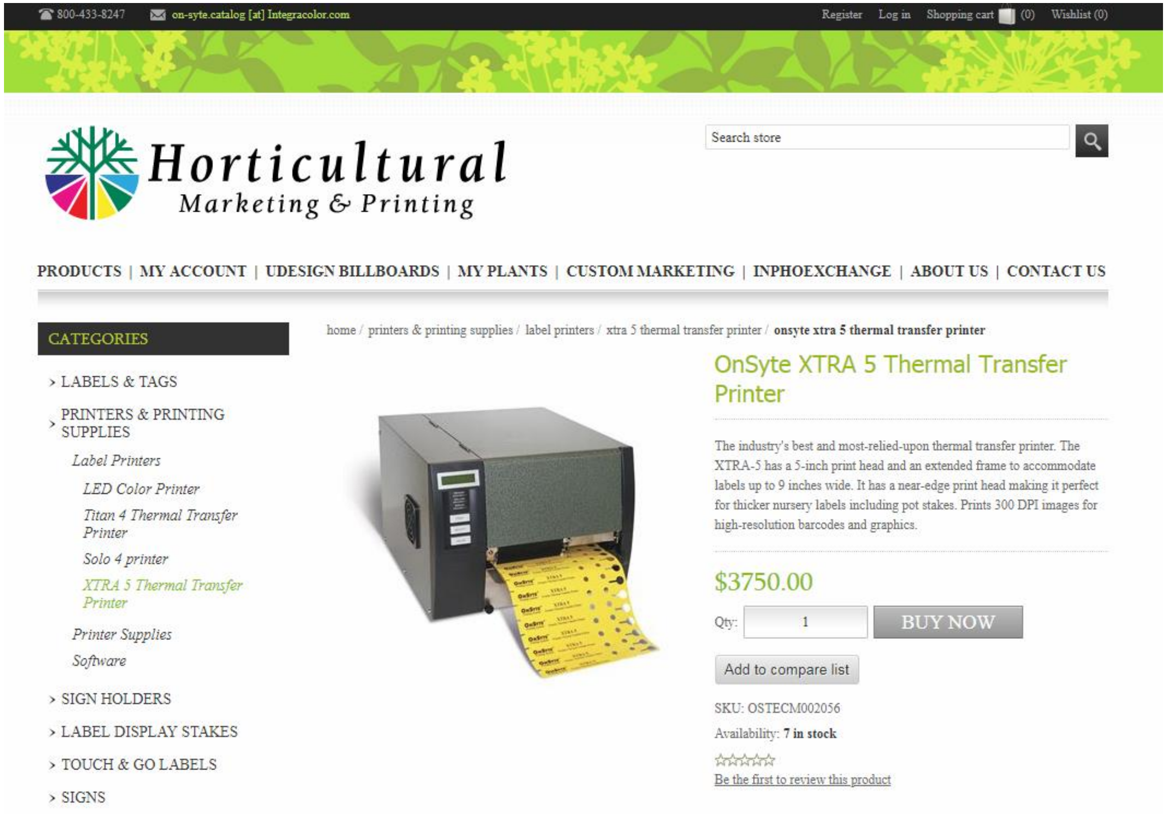
Affymetrix (Thermo-Fisher) Axiom_Arachis and _Arachis2 Arrays

Marker-Assisted Selection Protocol
Tissue Collection

Tagging a field plant with a printed label

Marker-Assisted Selection Protocol
Tissue Collection

Option for tag printing



Marker-Assisted Selection Protocol
Tissue Collection

Collect a folded leaf into an eppendorf tube labeled with coordinating number

Marker-Assisted Selection Protocol
Tissue Collection

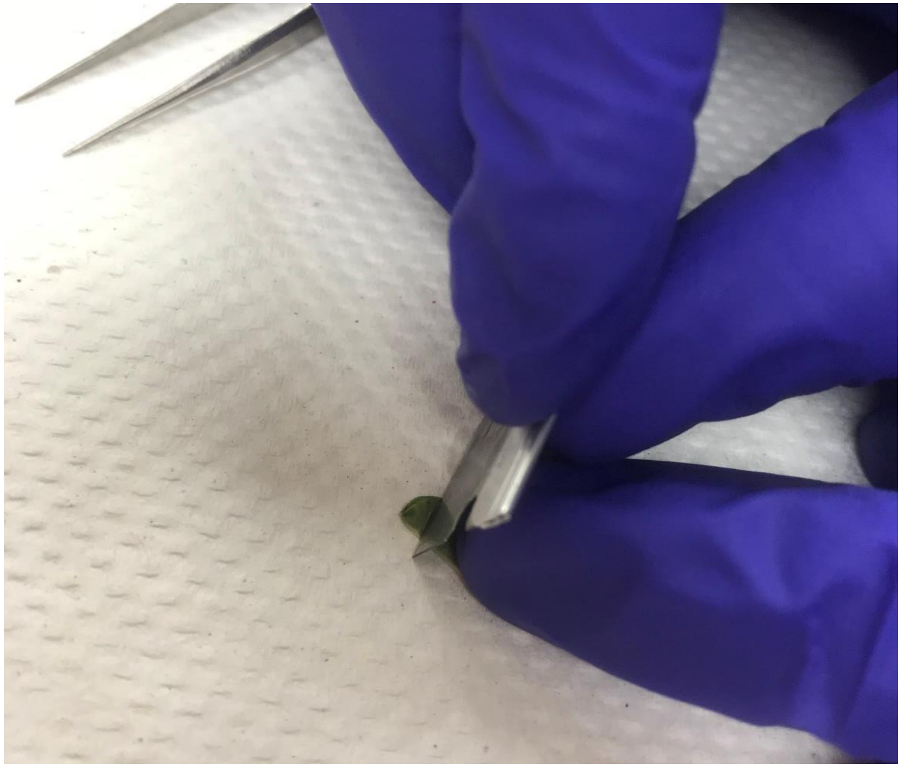
Develop a 96-well-plate arrayed with field samples

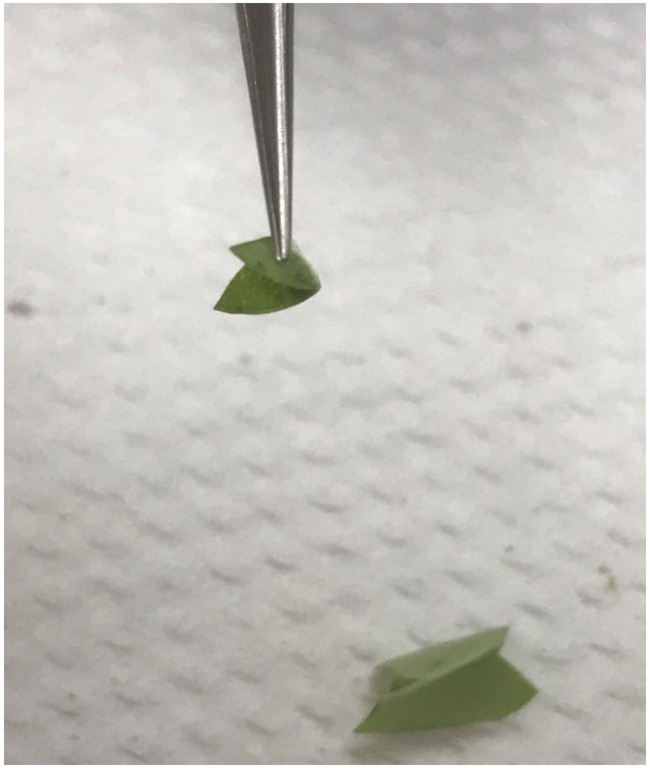
	1	2	3	4	5	6	7	8	9	10	11	12
A	C2686_S17	C2686_S25	C2690_S06	C2688_S03	C2692_S01	C2692_S09	C2692_S17	C2694_S06	C2696_S05	C2696_S13	C2696_S21	C2696_S29
B	C2686_S18	C2686_S26	C2690_S07	C2688_S04	C2692_S02	C2692_S10	C2692_S18	C2694_S07	C2696_S06	C2696_S14	C2696_S22	C2696_S30
C	C2686_S19	C2686_S27	C2690_S08	C2688_S05	C2692_S03	C2692_S11	C2692_S19	C2694_S08	C2696_S07	C2696_S15	C2696_S23	C2696_S31
D	C2686_S20	C2690_S01	C2690_S09	C2688_S06	C2692_S04	C2692_S12	C2694_S01	C2694_S09	C2696_S08	C2696_S16	C2696_S24	C2696_S32
E	C2686_S21	C2690_S02	C2690_S10	C2688_S07	C2692_S05	C2692_S13	C2694_S02	C2696_S01	C2696_S09	C2696_S17	C2696_S25	C2696_S33
F	C2686_S22	C2690_S03	C2690_S11	C2688_S08	C2692_S06	C2692_S14	C2694_S03	C2696_S02	C2696_S10	C2696_S18	C2696_S26	C2696_S34
G	C2686_S23	C2690_S04	C2688_S01	C2688_S09	C2692_S07	C2692_S15	C2694_S04	C2696_S03	C2696_S11	C2696_S19	C2696_S27	C2696_S35
H	C2686_S24	C2690_S05	C2688_S02	C2688_S10	C2692_S08	C2692_S16	C2694_S05	C2696_S04	C2696_S12	C2696_S20	C2696_S28	

Leave a blank here as control for contamination in PCR

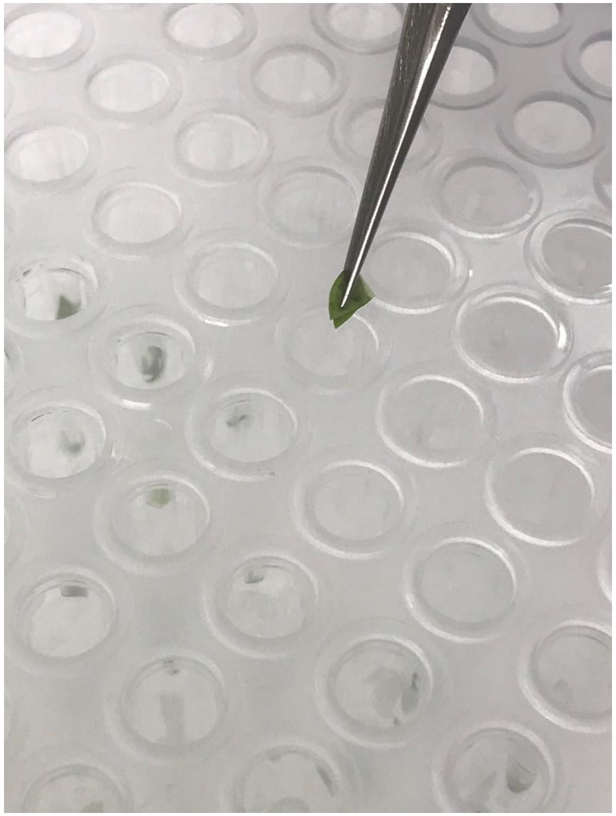
Marker-Assisted Selection Protocol
Tissue Collection

Slice the leaf tissue (clean blade in between on cotton saturated with 70% ethanol)



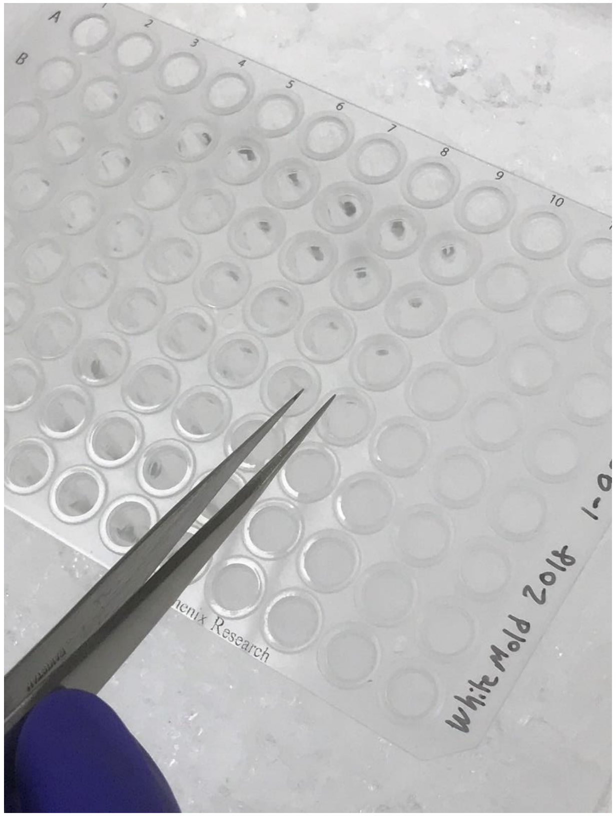


Marker-Assisted Selection Protocol
Tissue Collection



- Make sure the leaf tissue is placed close to the bottom of the plate

- Keep the plate on ice during the process

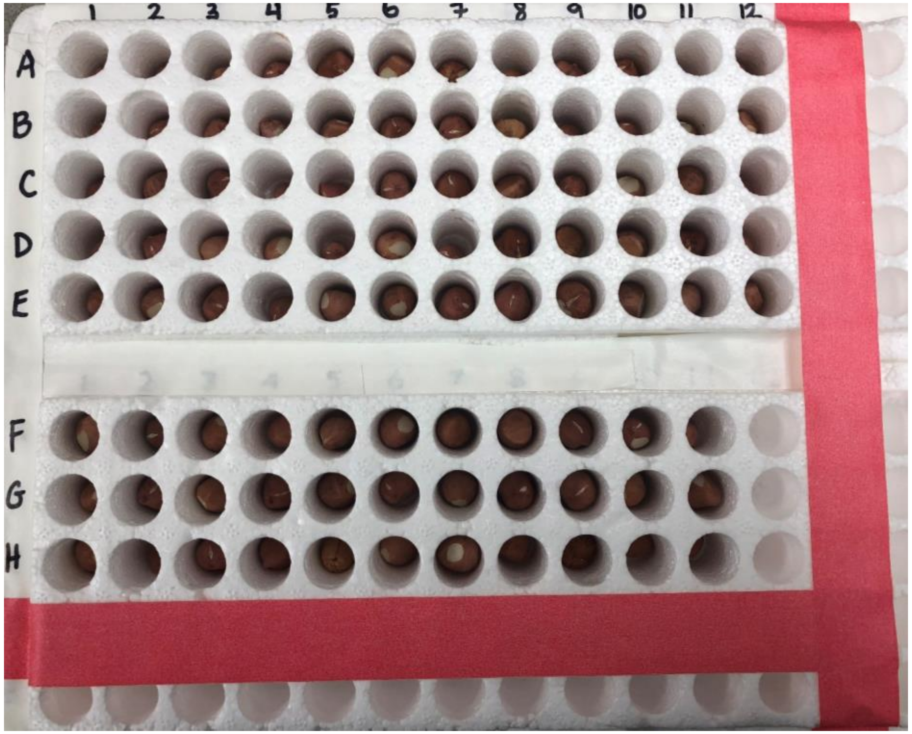


Marker-Assisted Selection Protocol
Tissue Collection

Seed chip sample collection (slice thickness ~1mm)

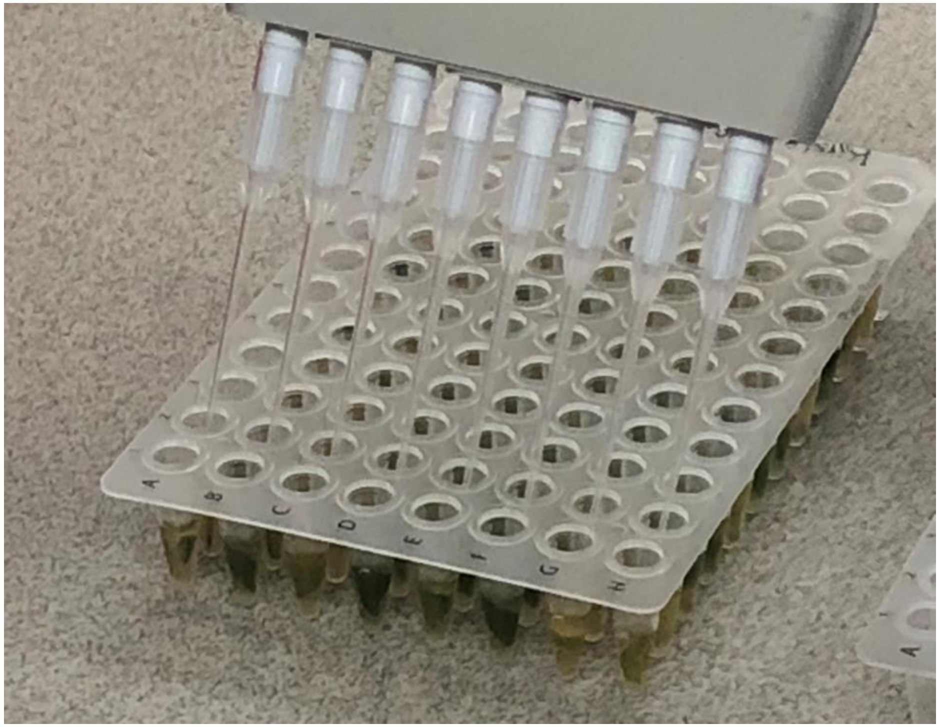
Marker-Assisted Selection Protocol
Tissue Collection

Use Styrofoam microcentrifuge tube storage boxes to make an array parallel to a 96-well-plate



Marker-Assisted Selection
DNA Extraction

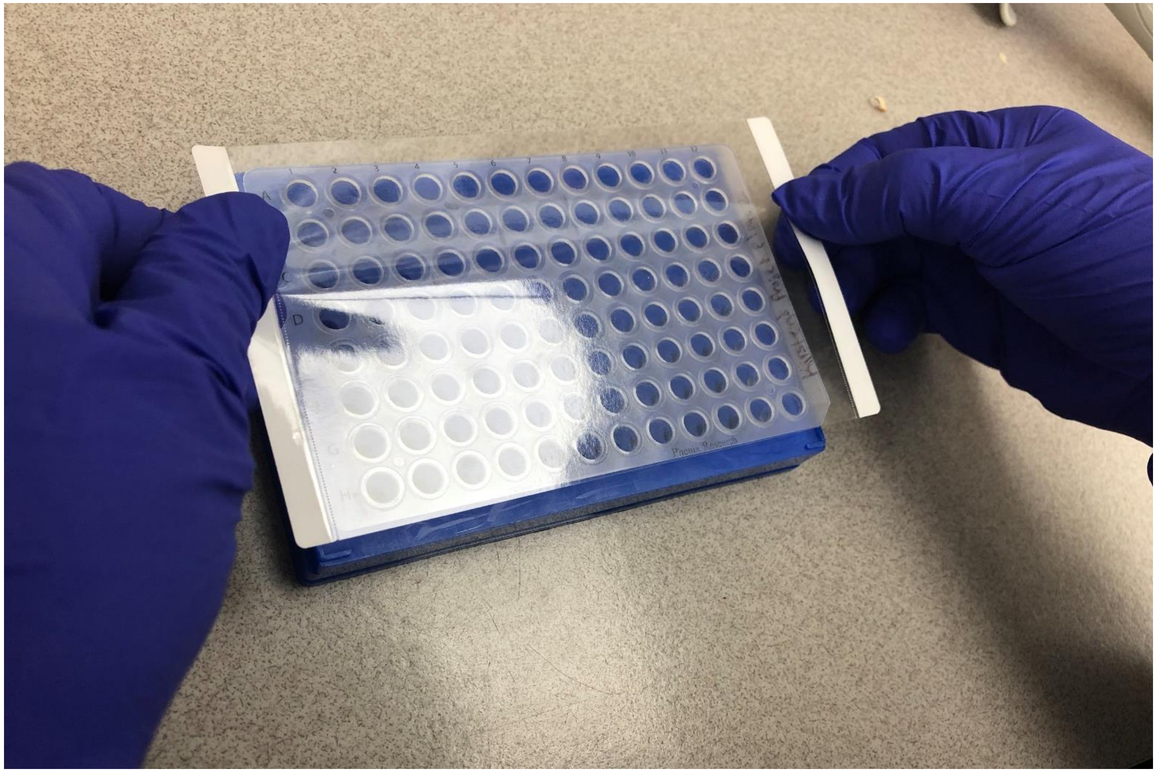
Proceed immediately to DNA extraction by adding 50 ul of buffer A to each well of the sample plate



Buffer A
100 mM NaOH
2% Tween 20

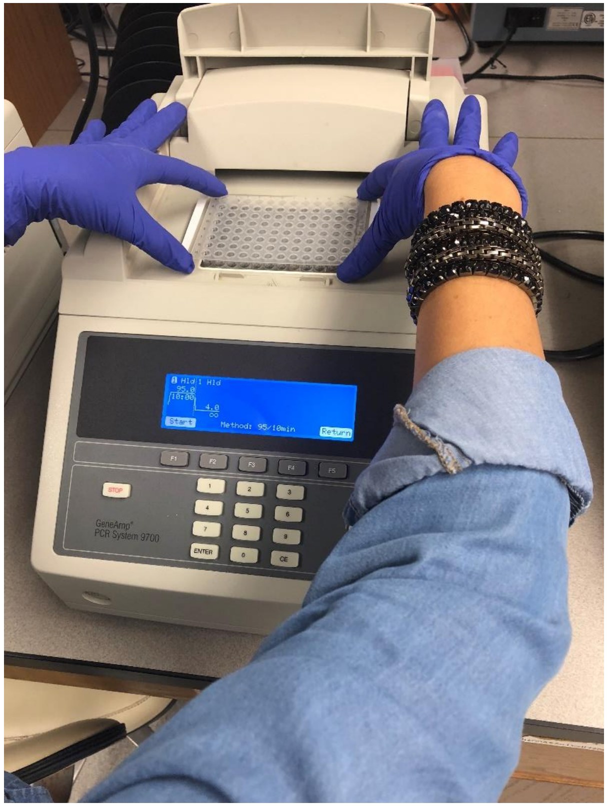
Marker-Assisted Selection
DNA Extraction

Seal the plate



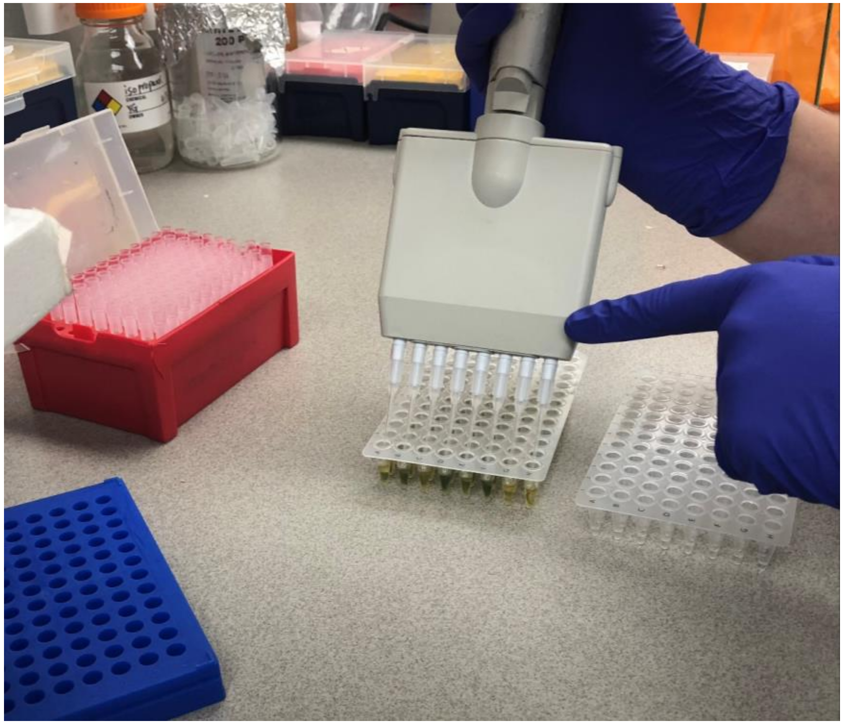
Marker-Assisted Selection
DNA Extraction

Incubate plate at 95 C for 10 min using a Thermocycler



Marker-Assisted Selection
DNA Extraction

Add 50 ul of buffer B to each well

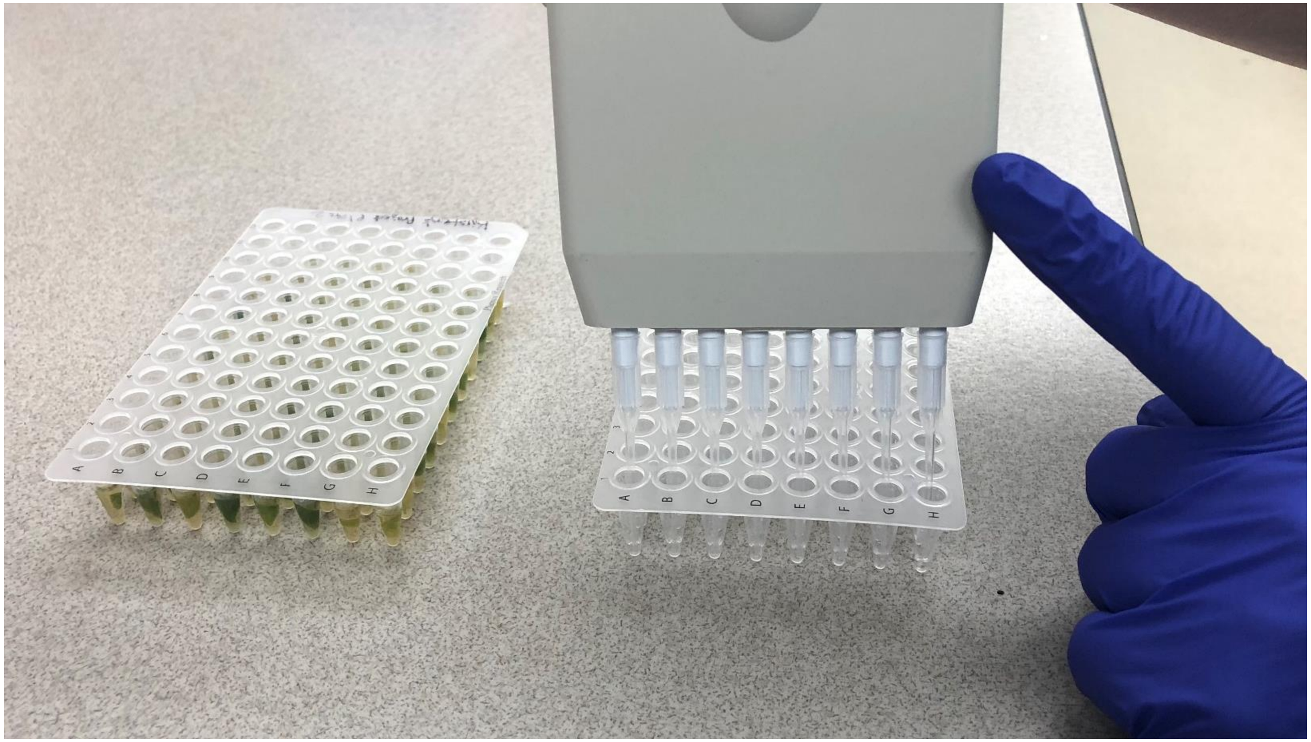


Buffer B
100 mM Tris-HCl
2 mM Na_2EDTA

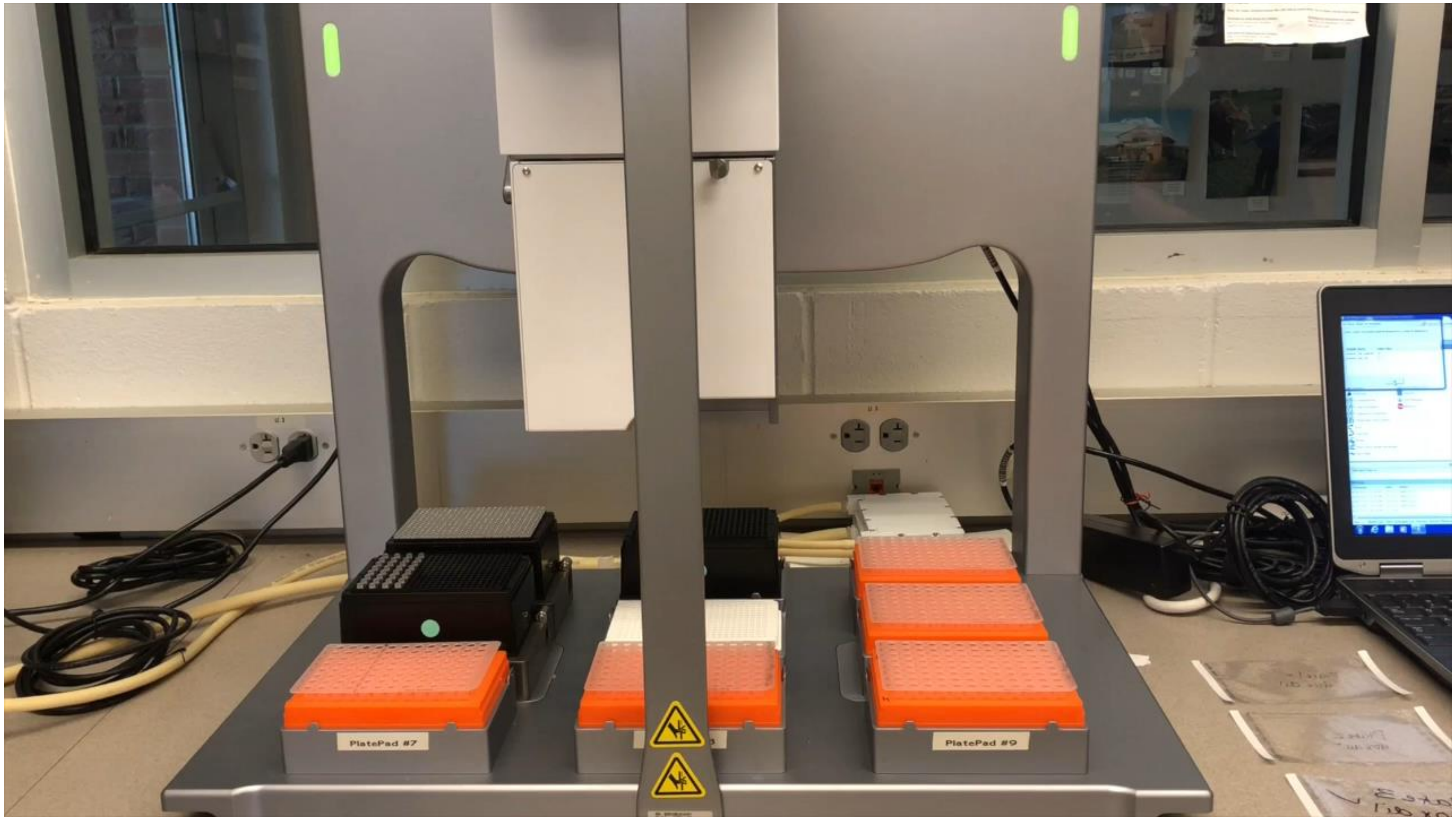
Marker-Assisted Selection
DNA Dilution

Dilute the samples 40x to 60x with TE buffer

Take 2 ul of original extract and transfer to 78 ul or 118 ul of TE buffer filled in a new plate



Marker-Assisted Selection
PCR Reaction Setup with BRAVO liquid handler



Marker-Assisted Selection
Run SNP Assay with Lightcycler 480

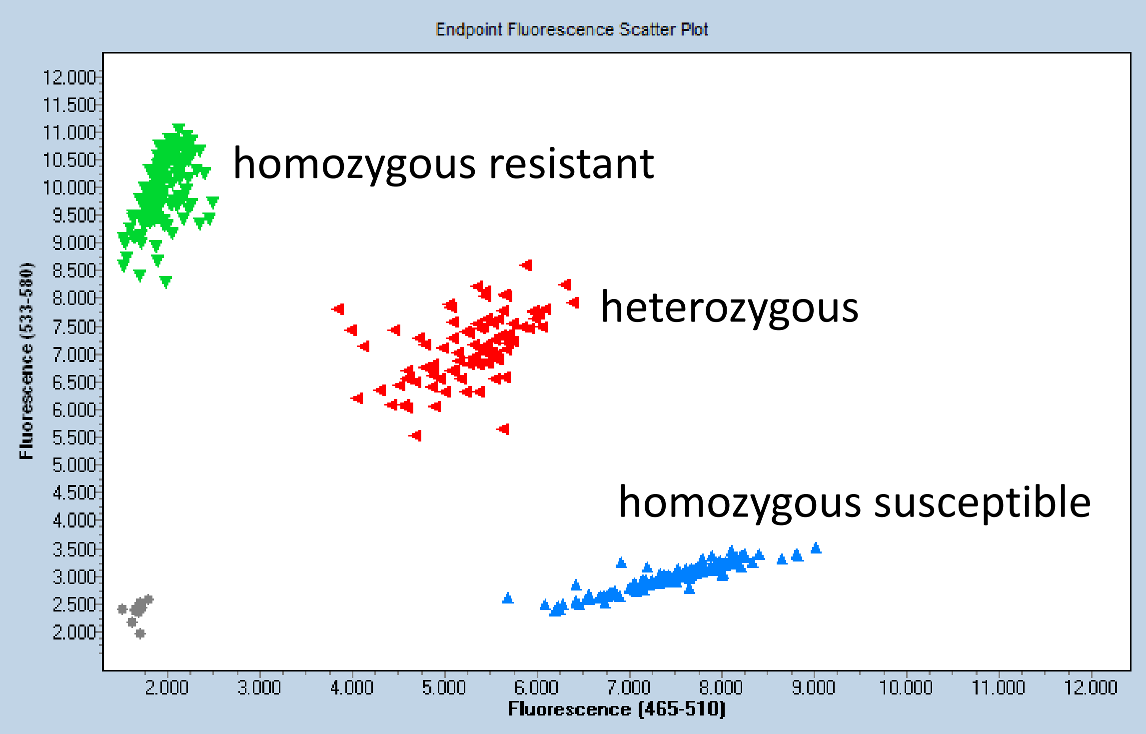
LightCycler® 480 II Real-Time PCR Cycler (Roche)

Item No.: 4162-13
Details: LightCycler 480 II Real-Time PCR Cycler
Mfr. Item No.: D100 03
Your Cost: $62,000.00/EA

Sign in to Buy

Marker-Assisted Selection
KASP Assay Results

Can be exported to Excel



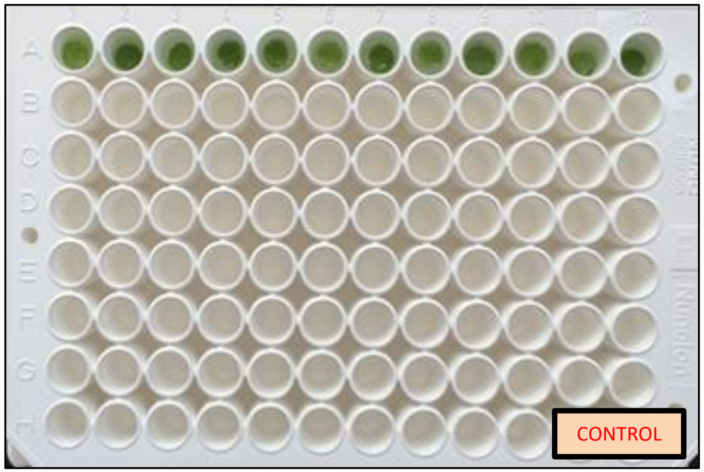
Cost per sample for DNA extraction ($0.21) and SNP assay – (0.45/marker)

Marker-Assisted Selection Protocol
Tissue Collection

Intertek SNP Genotyping Service

agritech.sweden@intertek.com

Punch leaf discs with hole punch – 2 discs per sample for peanut



Collect leaf discs in sequential order (H11 and H12 should be left blank)

oven dry (50-60 C) overnight or lyophilize

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Tissue Collection

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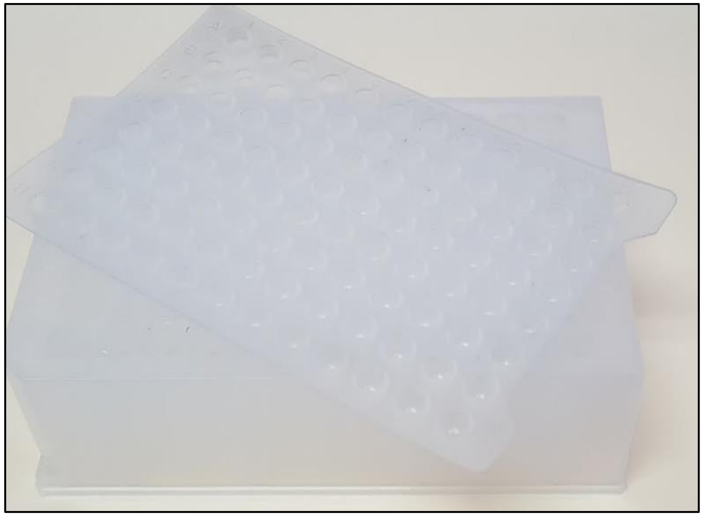

Seal 96 well plate with silicon sealing mat during transportation

Ship to:

INTERTEK SWEDEN LAB

Send the shipment to:
Intertek ScanBi Diagnostics,
Elevenborgsvägen 2,
230 53 Alnarp
Sweden

Phone: +46 40 69 28 001

Our UPS cost for 2 x 96-well plates was ~$64 ($0.33/sample)
Per sample assay cost is $2
10 SNPs per panel

Marker-Assisted Selection
Ozias-Akins Lab vs. Intertek

Tifton cost
$2/sample
4 markers

		Tifton FAD2B	Intertek snpAH0002
leaf	1	heterozygote	A:-
seed	1	heterozygote	A:-
leaf	2	heterozygote	A:-
seed	2	heterozygote	A:-
leaf	3	wildtype	-:-
seed	3	wildtype	-:-
leaf	4	heterozygote	A:-
seed	4	heterozygote	A:-
leaf	5	heterozygote	A:-
seed	5	heterozygote	A:-
leaf	6	heterozygote	A:-
seed	6	heterozygote	A:-
leaf	7	wildtype	-:-
seed	7	wildtype	-:-
leaf	8	heterozygote	A:-
seed	8	heterozygote	A:-
leaf	9	heterozygote	A:-
seed	9	heterozygote	A:-
leaf	10	heterozygote	A:-
seed	10	heterozygote	A:-
leaf	11	mutant	A:A
seed	11	mutant	A:A
leaf	12	heterozygote	A:-
seed	12	heterozygote	A:-

		Tifton FAD2B	Intertek snpAH0002
leaf	13	heterozygote	A:-
seed	13	Unknown	A:-
leaf	14	wildtype	-:-
seed	14	wildtype	-:-
leaf	15	wildtype	-:-
seed	15	wildtype	-:-
leaf	16	heterozygote	A:-
seed	16	heterozygote	A:-
leaf	17	wildtype	-:-
seed	17	wildtype	-:-
leaf	18	mutant	A:A
seed	18	Unknown	A:A
leaf	19	heterozygote	A:-
seed	19	Unknown	A:-
leaf	20	wildtype	-:-
seed	20	Unknown	-:-
leaf	21	mutant	A:A
seed	21	mutant	A:A
leaf	22	heterozygote	A:-
seed	22	Unknown	A:-
leaf	23	wildtype	-:-
seed	23	Unknown	-:-
leaf	24	heterozygote	A:-
seed	24	Unknown	A:-

Intertek cost
$2/sample
10 markers
But not all markers in panel may be useful

Marker-Assisted Selection Summary

- Marker discovery is upstream and dependent on populations and phenotyping
- Trait-associated markers can save a breeding program time and resources
- Some programs may have equipment and skilled personnel to conduct MAS in-house
- For those who don’t, out-sourcing option now exists for peanut

Marker-Assisted Selection Acknowledgements

NIFA

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USDA Agricultural Research Service

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FROM THE AMERICAN PEOPLE