Bonafide BDI ™ WildSUN: A Novel Molecular Quality Assurance Tool to detect Wild Sunflower Contamination in Cultivated Sunflower

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Introduction

Wild sunflower (*Helianthus annus var.annus*) is a native North American species & commonly grows near areas where commercial cultivated sunflower is produced in United States.

http://www.unl.edu/dpilson/sunflower.html

- Wild germplasm serves as a excellent repository of useful genes for traits like, disease resistance, cytoplasm male sterility& etc.
- USDA estimates that the economic value of traits already bred into cultivated sunflower from wild species is an estimated $267 million to $384million annually (NSA).

Resistant and Tolerant Sclerotinia Hybrids

http://www.sunflowernsa.com/growers/diseases/sclerotinia/

Key concerns

- Wild pollen maintains viability for long distances & successfully hybridizes with cultivated sunflower to yield a fertile hybrid
- Wild sunflower contamination can lead to the introduction of agronomical less desirable traits:

— Multiple branches & heads

—Self-In compatible

—Seed Shattering & dormancy

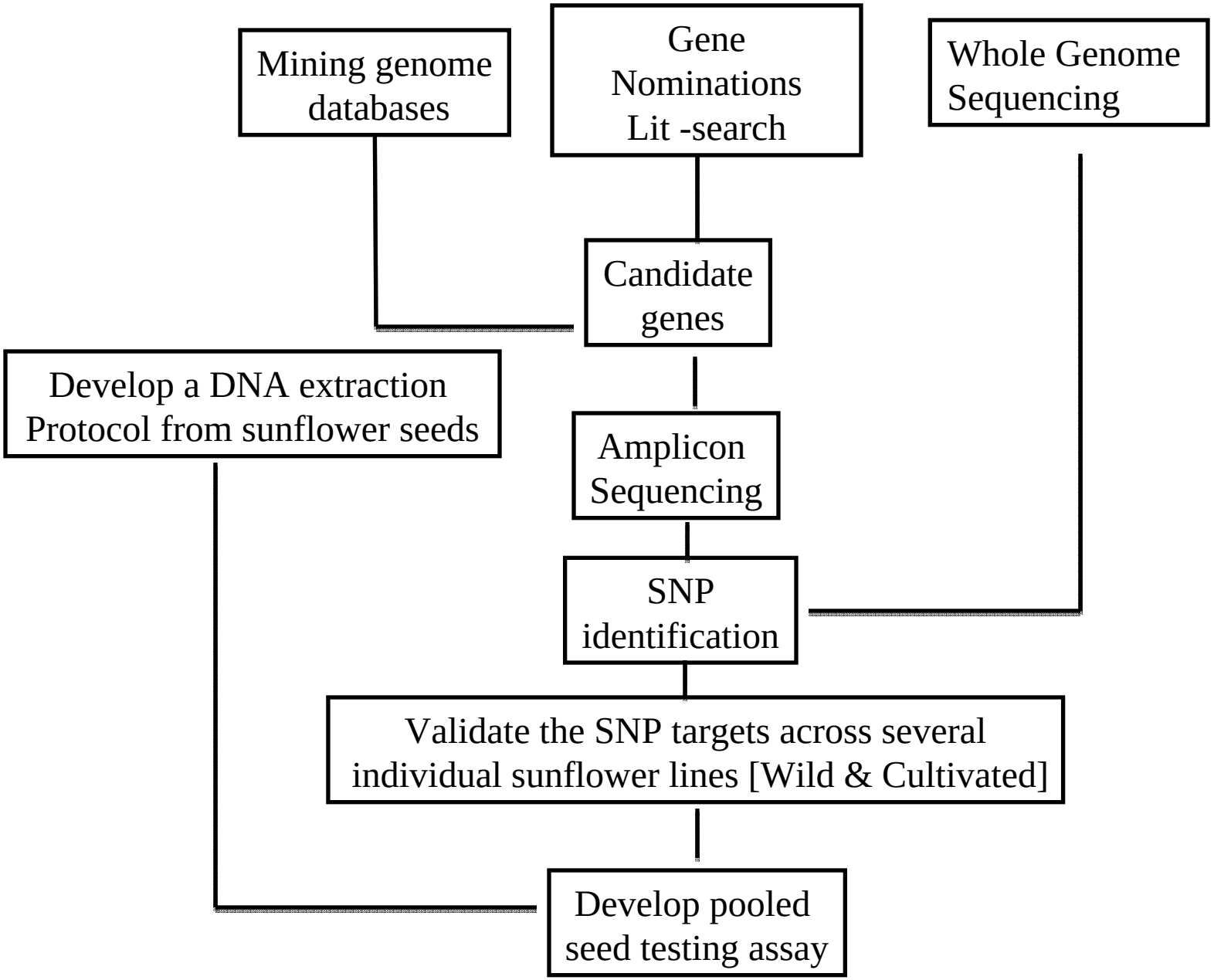
—Vigorous growth habits

- Currently available procedures to detect wild sunflower contamination are tedious and time consuming and less accurate.

DNA-based markers and potential tools for diagnostics

- DNA based assays can be employed during any stage of plant development.
- Not influenced by external environment, unlike phenotypic markers.
- Cost effective & less time consuming.

Development of WildSUN AP test



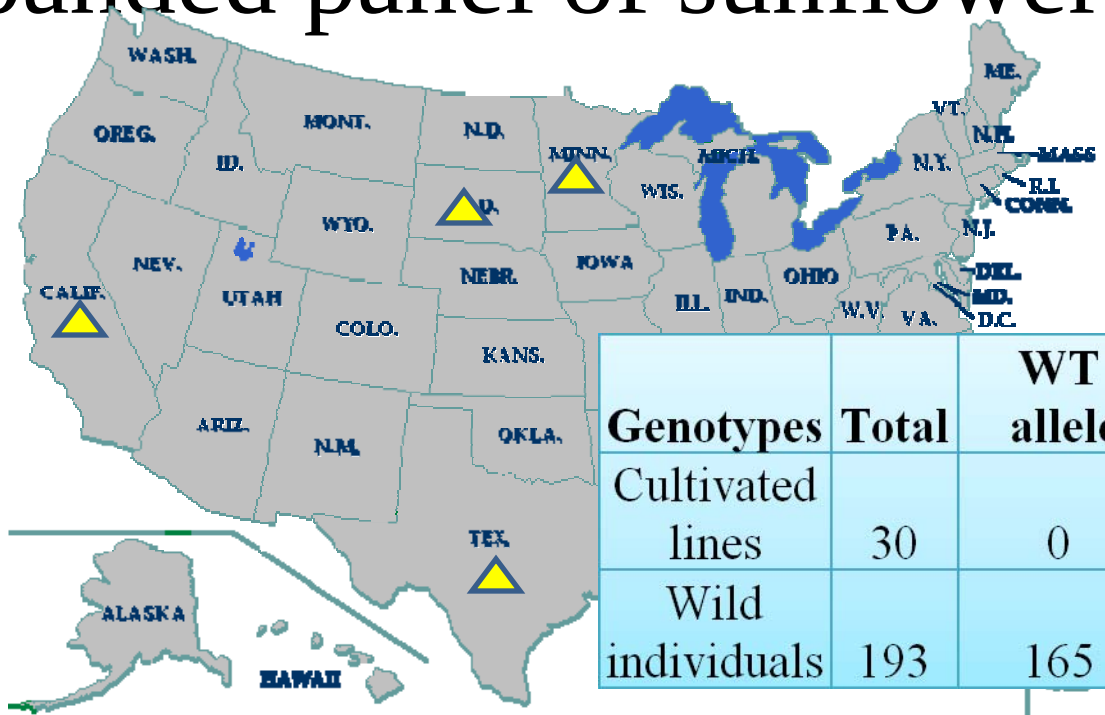
Identifying the target SNP for assay Development

Candidate marker	# Cul offtypes	# wild offtypes	Und
BDI001	1	2	6
BDI002	14	14	10
BDI003	0	0	65
BDI004	0	5	41
BDI005	15	13	1
BDI006	0	34	4
BDI007	0	26	15
BDI008	0	19	9
BDI009	0	5	16
BDI010	0	29	3
BDI011	N/A	N/A	0
BDI012	2	3	6
BDI013	N/A	N/A	60
BDI014	0	2	14
BDI015	0	28	13
BDI016	0	28	12
BDI017	2	6	53
BDI018	0	22	8
BDI019	0	22	2
BDI020	27	5	3
BDI021	0	0	9
BDI022	0	36	0
BDI023	0	35	1
BDI024	0	13	0
BDI025	0	0	3
BDI026	0	24	2

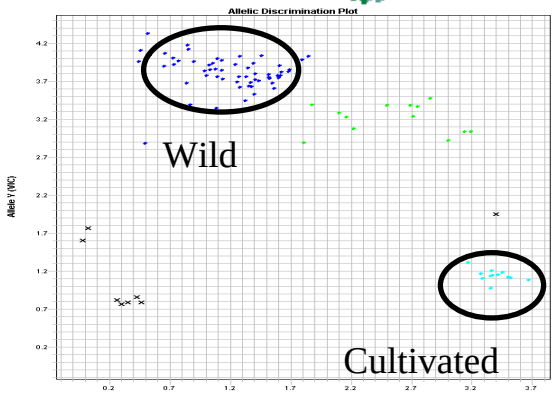
30 cultivated

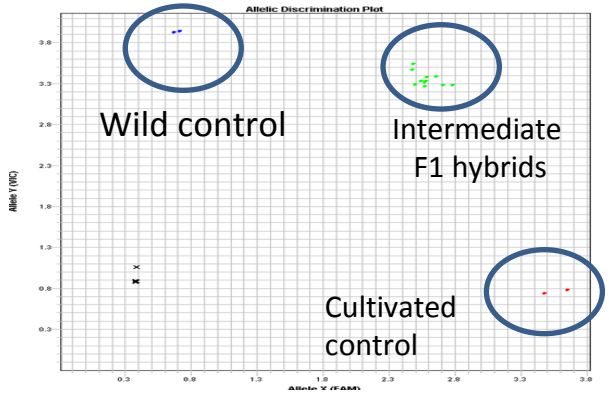
36 wild

Screening the target SNP on a expanded panel of sunflower lines

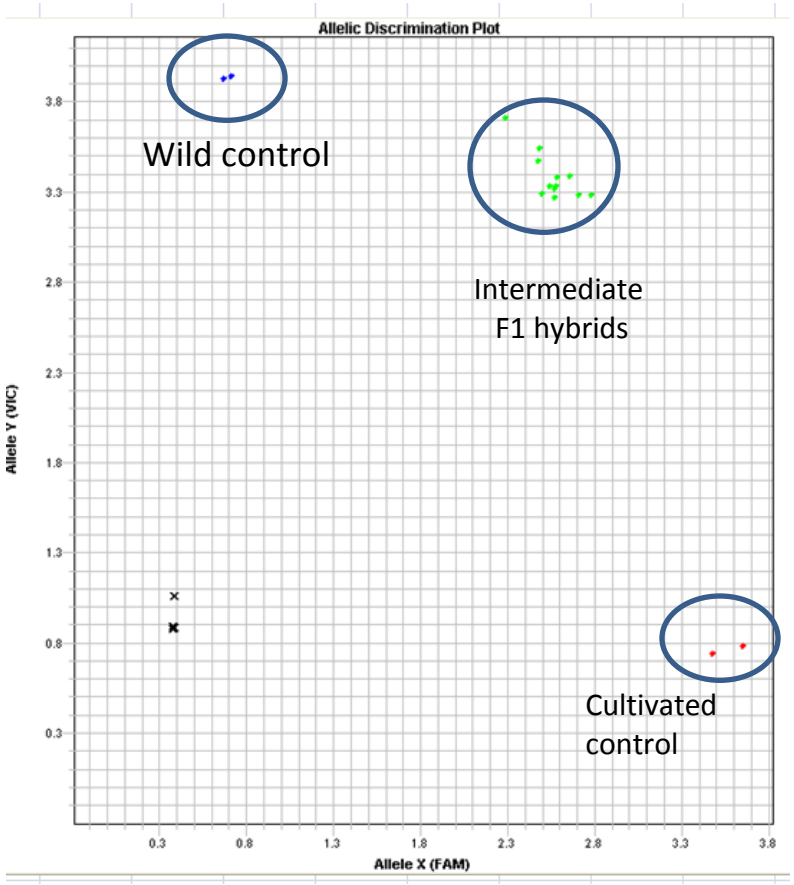


Genotypes	Total	WT allele	Cultivated allele	Both
Cultivated lines	30	0	30	0
Wild individuals	193	165	0	28

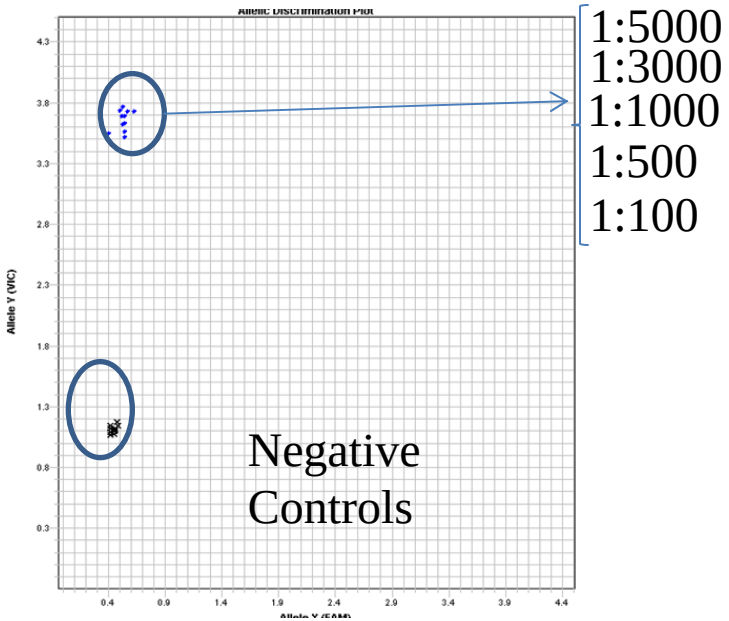




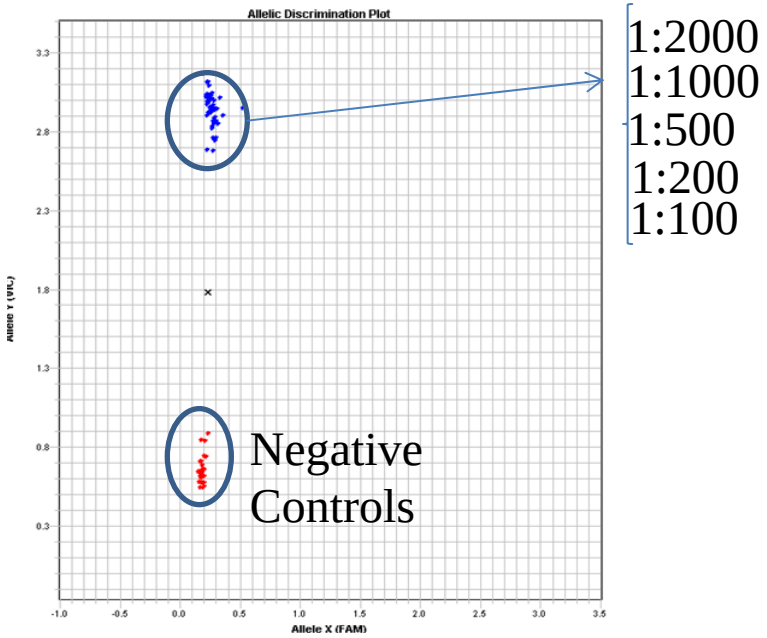
BDI021 is capable of identifying intermediate genotypes



Testing the limit of detection for the assay

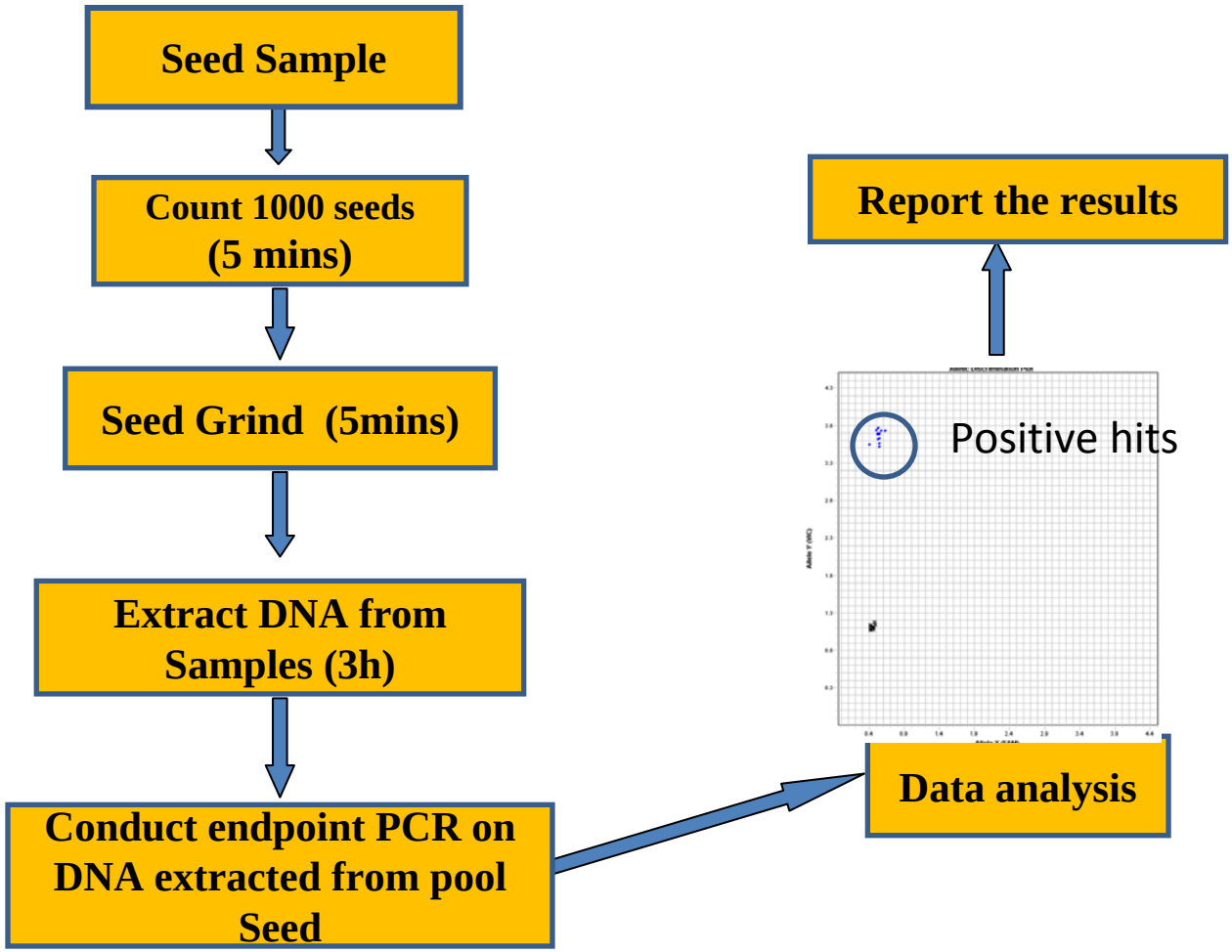


DNA Spike

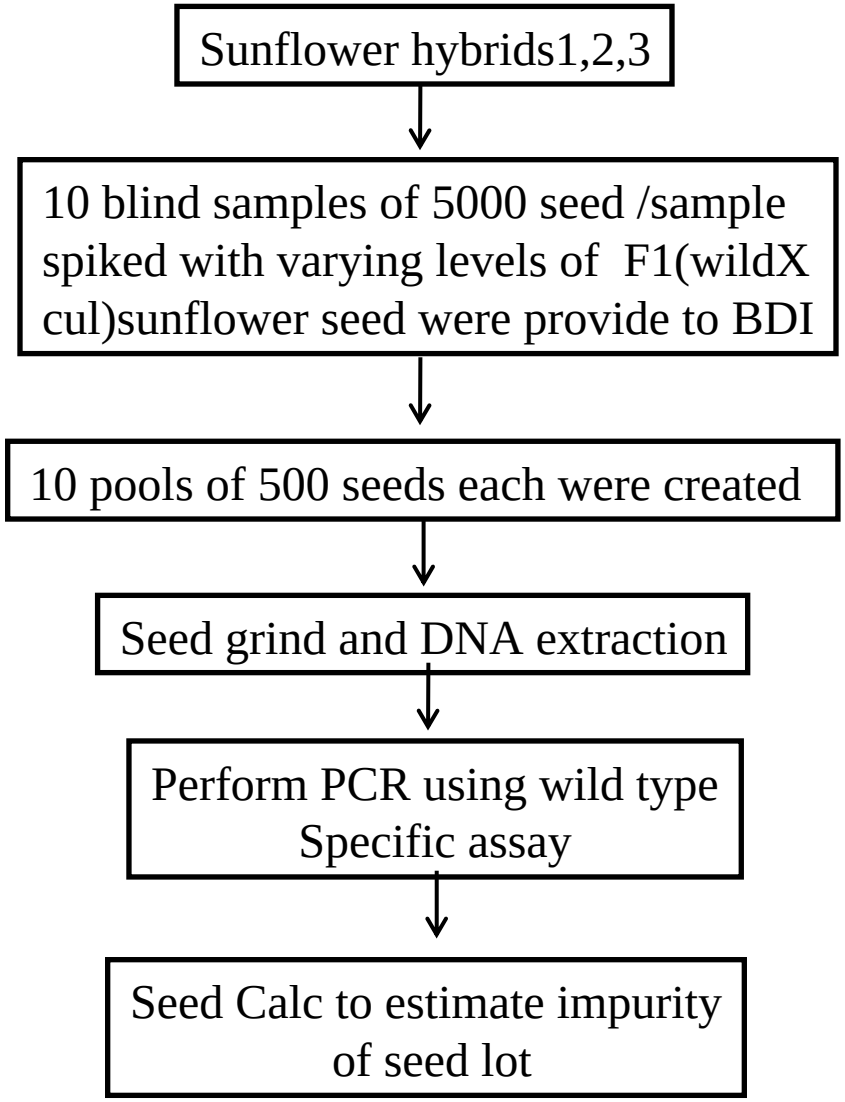


Seed Spike

Key steps in performing *Bonafide BDI*™ – WildSUN



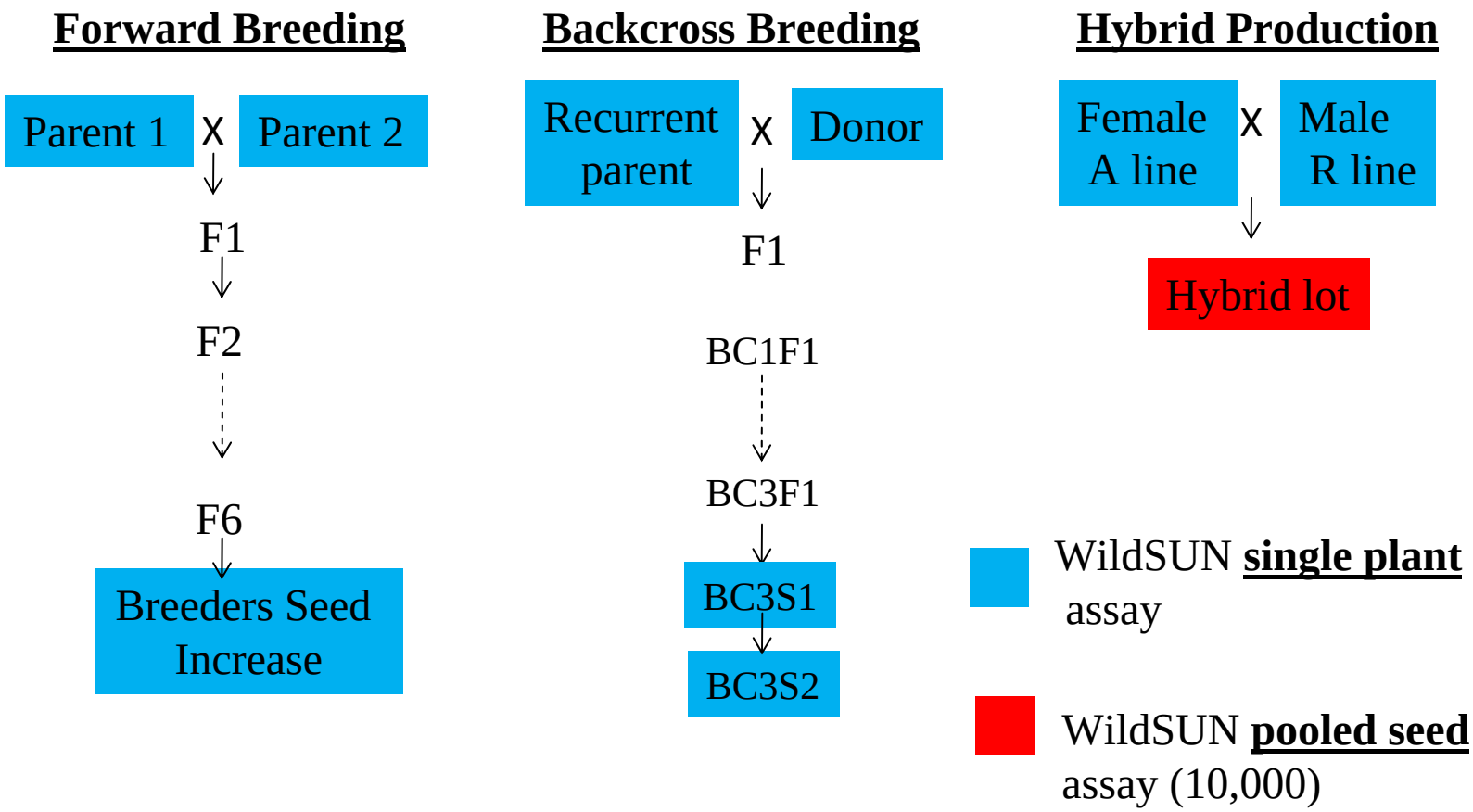
Validation of assay performance in a pooled seed test



WildSUN Validation results

Sample	sample call	Computed % in sample	actual %	difference (%)	Upper bound of True % Impurity
R3 sample 1	1 of 10	0.02	0.02	0.00	0.10
R3 sample 2	2 of 10	0.04	0.04	0.00	0.14
R3 sample 3	3 of 10	0.07	0.06	0.01	0.19
R3 sample 4	3 of 10	0.07	0.10	-0.03	0.19
R3 sample 5	0 of 10	0.00	0.00	0.00	0.06
R3 sample 6	8 of 10	0.32	0.20	0.12	0.66
R3 sample 7	5 of 10	0.14	0.14	0.00	0.30
R3 sample 8	0 of 10	0.00	0.00	0.00	0.06
R3 sample 9	8 of 10	0.32	0.50	-0.18	0.66
R3 sample 10	8 of 10	0.32	0.30	0.02	0.66
R3 control	0 of 10	0.00	0.00	0.00	0.06

Potential stages for WildSUN test application in sunflower breeding programs



Conclusion

- WildSun is a robust alternative methodology to determine the % contamination of wild sunflower in cultivated sunflower.

- WildSun test can be performed on individual plants as well as on seed lots, hence, a useful tool for both plant breeders and seed producers.

- Unlike GOT, WildSun test is less time consuming & does not involve germination of seeds.