UPOV
International Union for the Protection of New Varieties of Plants

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Technical Working Party on Testing Methods and Techniques **TWM/1/19**

First Session
Virtual meeting, September 19 to 23, 2022

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VARIETY TRACER: FRAUDULENT USE OF PARENTAL LINES

Document prepared by an expert from the Netherlands

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The annex to this document contains a copy of a presentation on “Variety Tracer: Fraudulent use of parental lines”, prepared by an expert from the Netherlands, to be made at the first session of the TWM.

[Annex follows]

Variety Tracer

Fraudulent use of parental lines

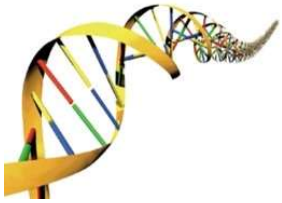
With examples in pepper and broccoli

UPOV-TWM/1 – September 19-23, 2022

DNA as a tool to find your parents

DNA Onbekend - Corrie en Gerard: worstelen met vragen

DNA Onbekend: Cyril en Yvonne - wie is mijn vader?



DNA Onbekend: Jolanda en Marijke - wie is mijn vader en wie is mijn opa?

DNA Onbekend - Thea en Pieter op zoek naar de waarheid

DNA Onbekend - AVROTROS.nl

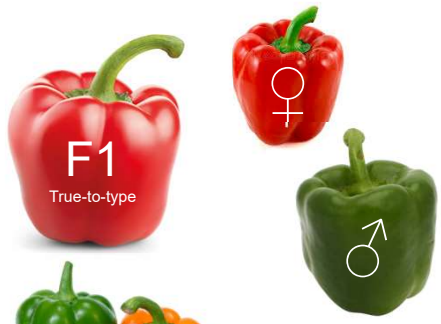
Variety Tracer

How to proof that a protected parent is (illegally) used to produce a F1-hybrid variety?

Strategies:
1. Find inbred plants in the F1 and compare with potential true-to-type parents.
2. Statistical 'parent-offspring' test.
3. Multiple Sequence Alignments – inheritance of haploblocks.

Example 1

Collect material: Principle delivers…



Reference varieties as controls
F1-hybrids from same parents
(full-sibs/half-sibs)
Non-related varieties

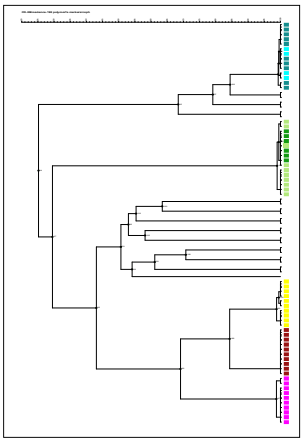
Find inbreds

		M1	M2	M3	M4	M5
Parent A	plant 1					
	plant 2					
Parent B	plant 1					
	plant 2					
Parent C	plant 1					
	plant 2					
Parent D	plant 1					
	plant 2					
Hybrid	plant 1					
	plant 2					
	plant 3					
	plant 4					
	plant 5					
	plant 6					
	plant 7					
	plant 8					
	plant 9					
	plant 10					
	plant 11					
	plant 12					
	plant 13					
	plant 14					
	plant 15					
	plant 16					

1. Identification of inbred plants
2. Growing trial (Morphology)
3. Genotyping test (DNA)

XX YY XY

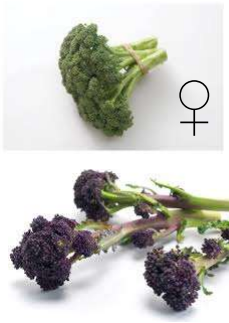
Genetic research - genotyping



- Which DNA marker? Which technology? How many markers? Whole Genome Sequencing?
- Comparing is always relative
- HOW is not crucial
- WITH WHAT *is* crucial

Example 2

Collect material: Principle delivers…



Reference varieties as controls
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Statistical 'Parent-Offspring'-test

- Input DNA information: Brassica-specific genotyping 15K array with 13714 SNPs
- Looked at the possible genetic contribution of each individual potential parent to the genotype of each offspring plant (F1-hybrid).

Parent	Offspring	Score
XX	XX	1
XX	XY	1
XX	YY	0
XY	XX	1
XY	XY	1
XY	YY	1
YY	XX	0
YY	XY	1
YY	YY	1

Can this parent have contributed to this F1 offspring plant?

$$C = b / (b+c)$$

Where C is the contribution of one potential parent to a single offspring F1 hybrid variety, *b* is the number of 1s and *c* is the total number of 0s for all SNPs between the parent and the offspring.

Max C = 0,5

Statistical 'Parent-Offspring'-test

	F1-suspected-sample 1	F1-suspected-sample 2	F1-suspected (harvested material)	F1-true-to-type-principle	F1-true-to-type (DUS material)	'X'	'Y'	'A'	'B'	'C'	'D'	'E'	'Z'
	1a	1b	1c	4	5	8	10	14	15	16	17	18	13
2	0,499	0,499	0,499	0,499	0,499	0,499	0,433	0,419	0,428	0,434	0,432	0,433	0,438
3	0,475	0,474	0,474	0,499	0,499	0,455	0,469	0,451	0,470	0,473	0,466	0,466	0,439
9	0,454	0,454	0,454	0,458	0,458	0,499	0,468	0,447	0,461	0,460	0,465	0,466	0,449
11	0,457	0,457	0,456	0,459	0,458	0,454	0,499	0,467	0,488	0,471	0,483	0,483	0,450
12	0,461	0,460	0,460	0,468	0,468	0,469	0,499	0,456	0,465	0,463	0,472	0,499	0,455

4/5 = 2 + 3

8 = 2 + 9

10 = 11 + 12

CONTROLS

1abc = 2?

Research question

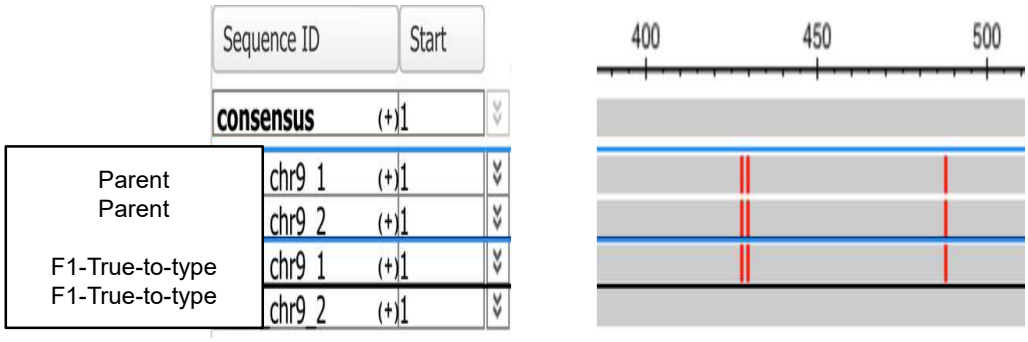
Smoking-Gun Evidence

Multiple Sequence Alignments – inheritance of haploblocks (physically linked SNPs) from parent to offspring

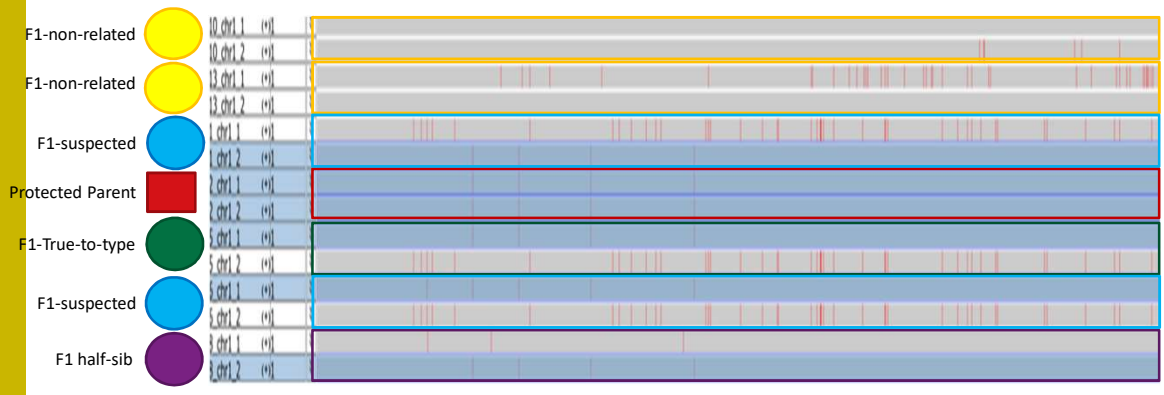
What do we need?

- WGS: DNA sequences of long strands of DNA (e.g. nanopore)
- Reliable and high quality reference genome for mapping.
- Identification of regions with physically linked SNPs
- High coverage and high reliable sequencing quality (Illumina)
- Highly skilled bioinformaticians with a lot of spare time
- Enough space for data storage

MSA



MSA - Smoking-Gun Evidence



Summary

- There are several strategies to proof that a protected parent has (illegally) been used to produce a F1-hybrid.
- The relative simple and cost-effective strategies need inbred plants and/or produces statistical evidence.
- The innovative and expensive strategy produces ‘Smoking-Gun’ evidence.

Acknowledgements

Principles

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