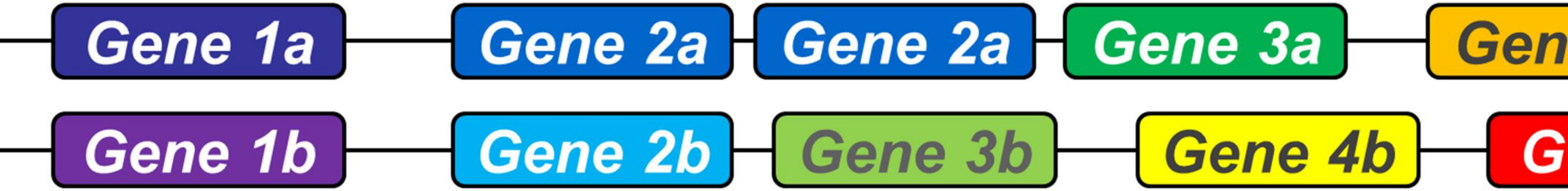
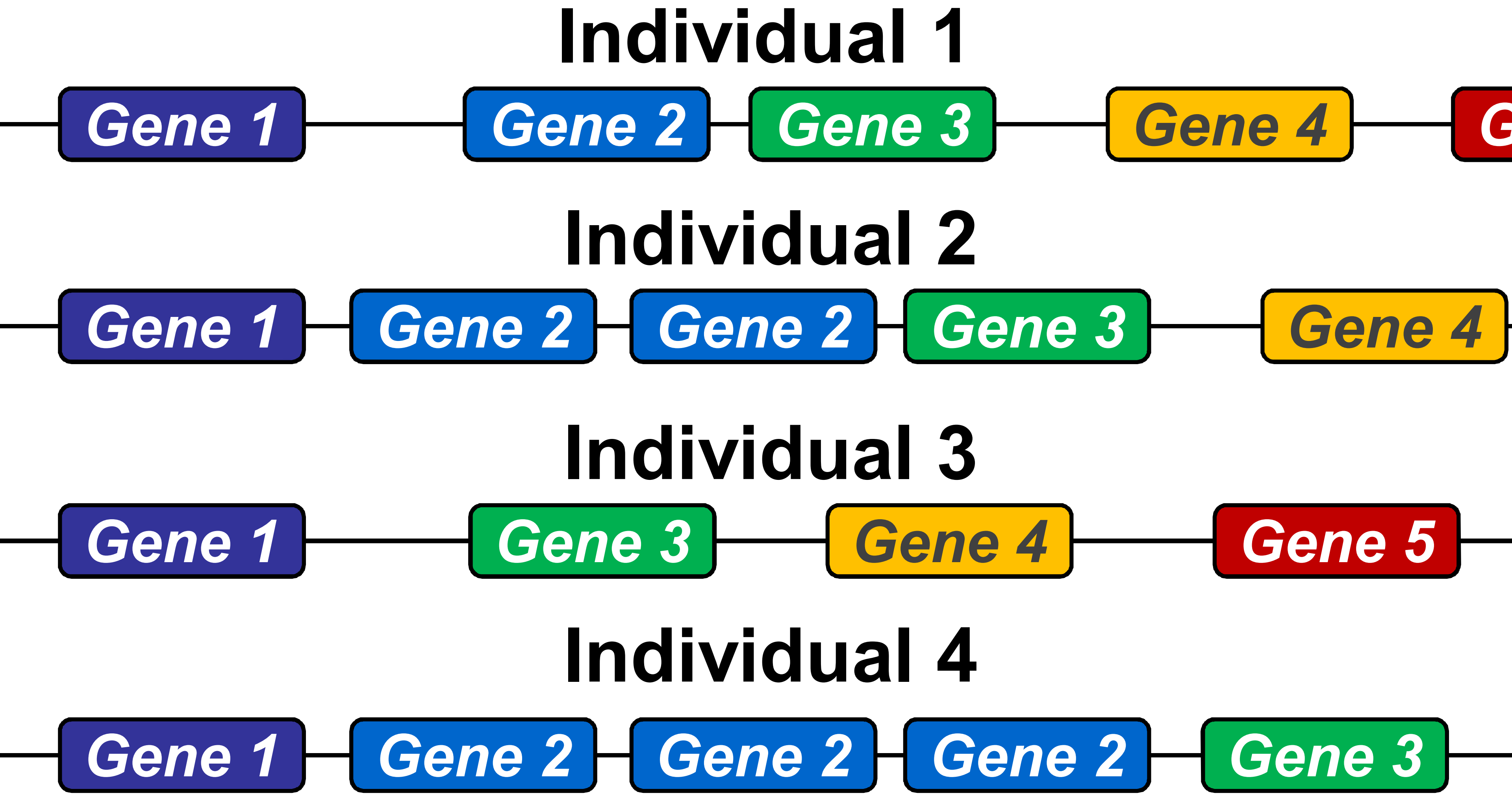


Methods to determine copy number variation in *Brassica* species

Dr. Sarah Schiessl-Weidenweber

Justus Liebig University Giessen

What is copy number variation (CNV)?



Individual 1

Gene 1 Gene 2 Gene 3 Gene 4 G

Individual 2

Gene 1 Gene 2 Gene 2 Gene 3 Gene 4

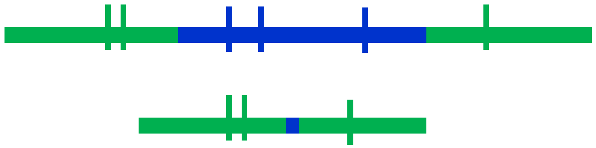
Individual 3

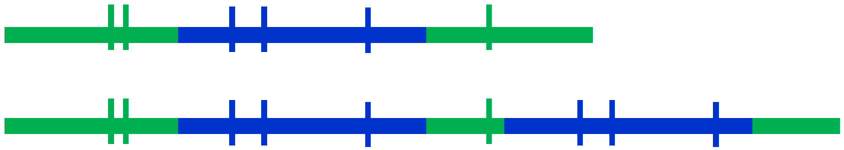
Gene 1 Gene 3 Gene 4 Gene 5

Individual 4

Gene 1 Gene 2 Gene 2 Gene 2 Gene 3

CNVs come from genomic rearrangements

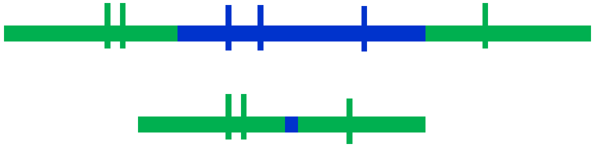




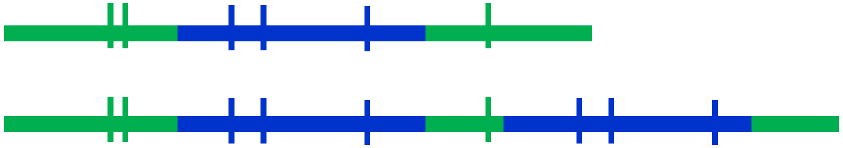
Deletion

Duplication

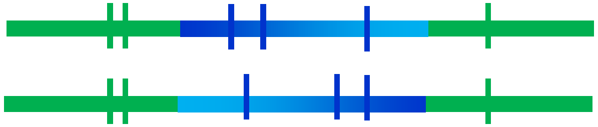
CNVs come from genomic rearrangements



Deletion

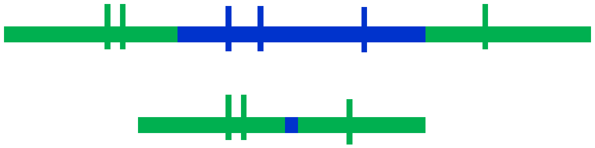


Duplication

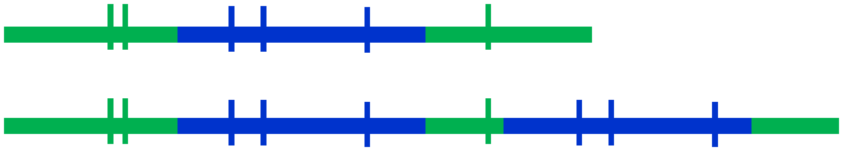


Inversion

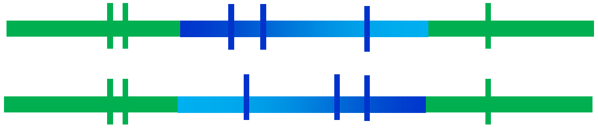
CNVs come from genomic rearrangements



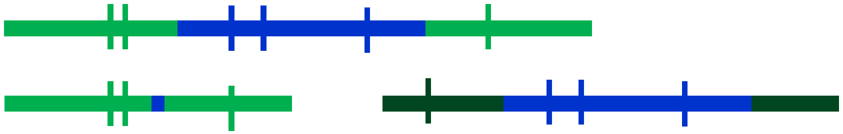
Deletion



Duplication

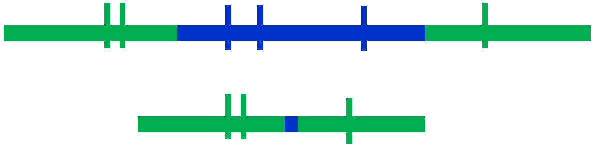


Inversion

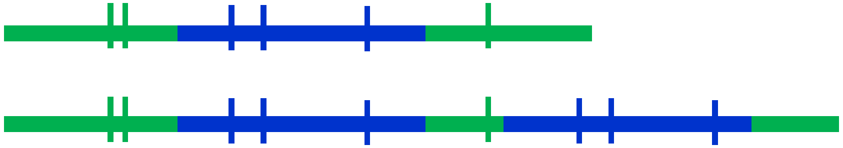


Translocation

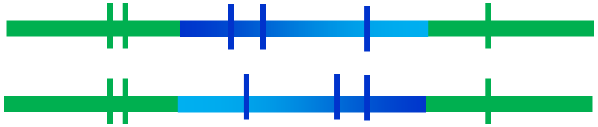
CNVs come from genomic rearrangements



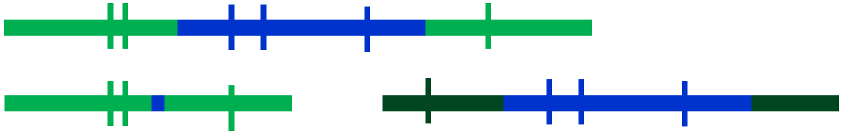
Deletion



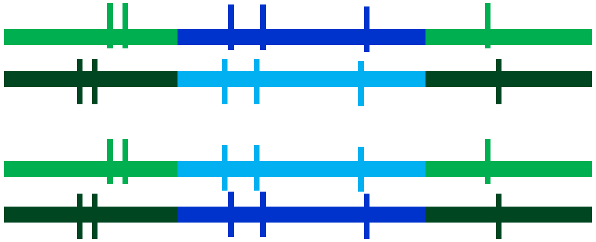
Duplication



Inversion

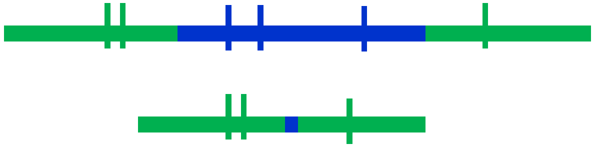


Translocation

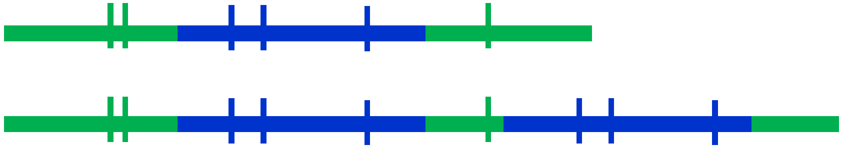


Exchange

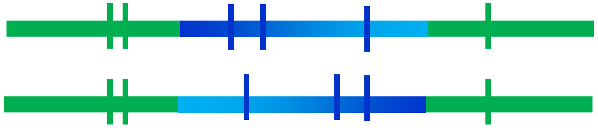
CNVs come from genomic rearrangements



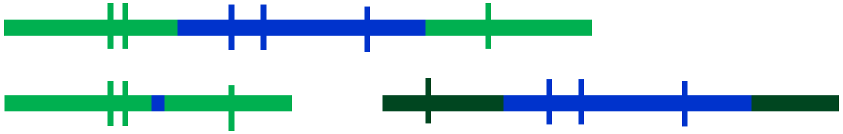
Deletion



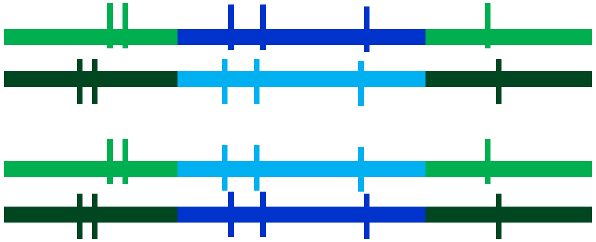
Duplication



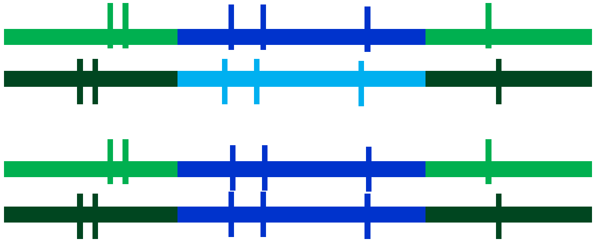
Inversion



Translocation

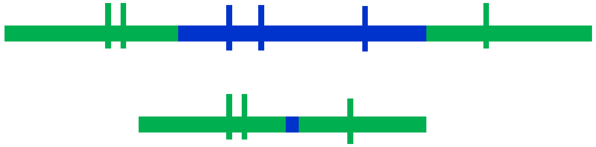


Exchange

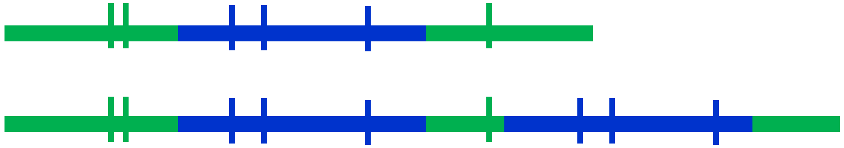


Conversion

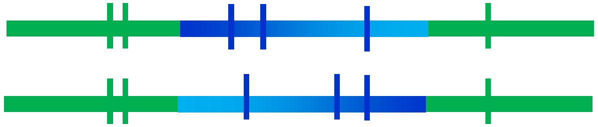
…but not all rearrangements change CN!



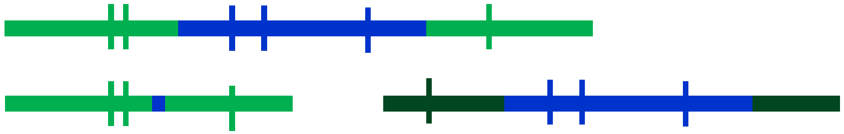
Δcopy number: -1



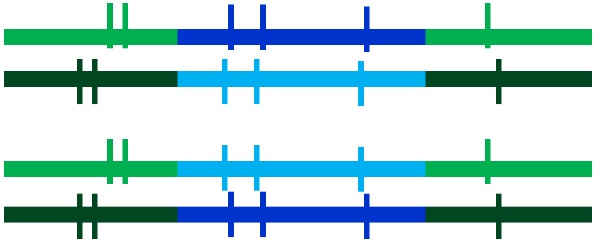
Δcopy number: +1



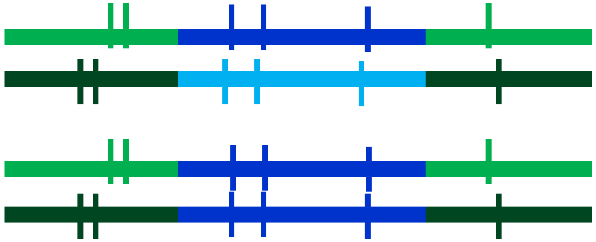
Δcopy number: 0



Δcopy number: 0



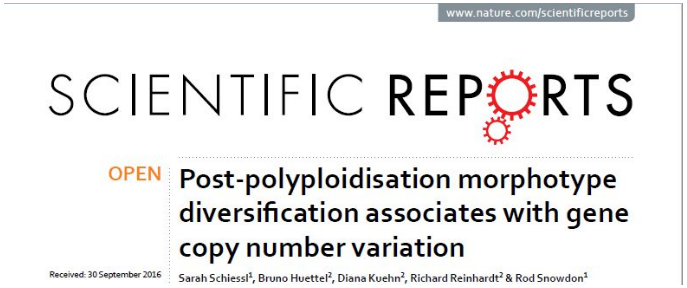
Δcopy number: 0



Δcopy number: -1/+1

CNVs can have a strong phenotypic impact

Flowering time



www.nature.com/scientificreports

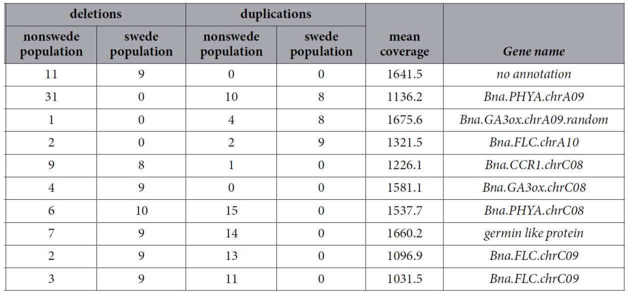
SCIENTIFIC REPORTS

OPEN

Post-polyploidisation morphotype diversification associates with gene copy number variation

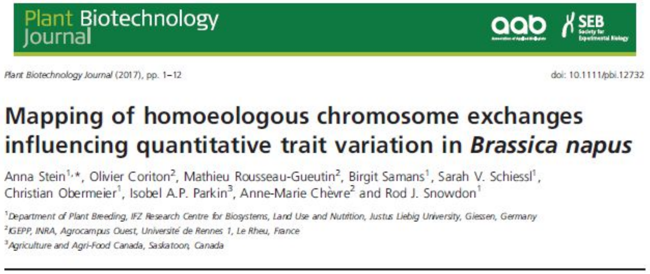
Received: 30 September 2016

Sarah Schiessl[1], Bruno Huettel[2], Diana Kuehn[2], Richard Reinhardt[2] & Rod Snowdon[1]



deletions		duplications		mean coverage	*Gene name*
nonswede population	swede population	nonswede population	swede population		
11	9	0	0	1641.5	*no annotation*
31	0	10	8	1136.2	*Bna.PHYA.chrA09*
1	0	4	8	1675.6	*Bna.GA3ox.chrA09.random*
2	0	2	9	1321.5	*Bna.FLC.chrA10*
9	8	1	0	1226.1	*Bna.CCR1.chrC08*
4	9	0	0	1581.1	*Bna.GA3ox.chrC08*
6	10	15	0	1537.7	*Bna.PHYA.chrC08*
7	9	14	0	1660.2	*germin like protein*
2	9	13	0	1096.9	*Bna.FLC.chrC09*
3	9	11	0	1031.5	*Bna.FLC.chrC09*

Seed quality



Plant Biotechnology Journal

Plant Biotechnology Journal (2017), pp. 1–12

doi: 10.1111/pbi.12732

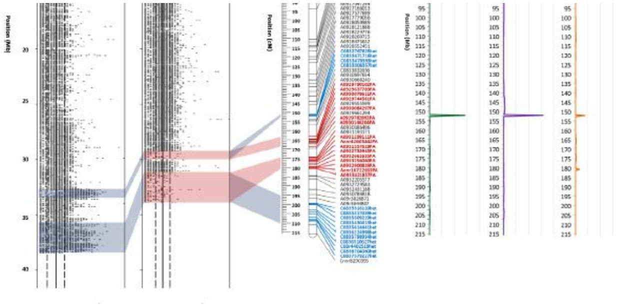
Mapping of homoeologous chromosome exchanges influencing quantitative trait variation in *Brassica napus*

Anna Stein[1,*], Olivier Coriton[2], Mathieu Rousseau-Gueutin[2], Birgit Samans[1], Sarah V. Schiessl[1], Christian Obermeier[1], Isobel A.P. Parkin[3], Anne-Marie Chèvre[2] and Rod J. Snowdon[1]

[1] *Department of Plant Breeding, IFZ Research Centre for Biosystems, Land Use and Nutrition, Justus Liebig University, Giessen, Germany*

[2] *IGEPP, INRA, Agrocampus Ouest, Université de Rennes 1, Le Rheu, France*

[3] *Agriculture and Agri-Food Canada, Saskatoon, Canada*



Chlorophyll content

Molecular Plant
Research Article

CellPress
PARTNER JOURNAL

Deletion of a Stay-Green Gene Associates with Adaptive Selection in *Brassica napus*

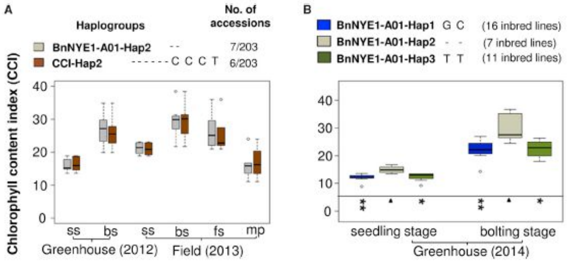
Lunwen Qian[1], Kai Voss-Fels[1], Yixin Cui[2], Habib U. Jan[1], Birgit Samans[1], Christian Obermeier[1], Wei Qian[2] and Rod J. Snowdon[1,*]

[1] Department of Plant Breeding, IFZ Research Centre for Biosystems, Land Use and Nutrition, Justus Liebig University, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany

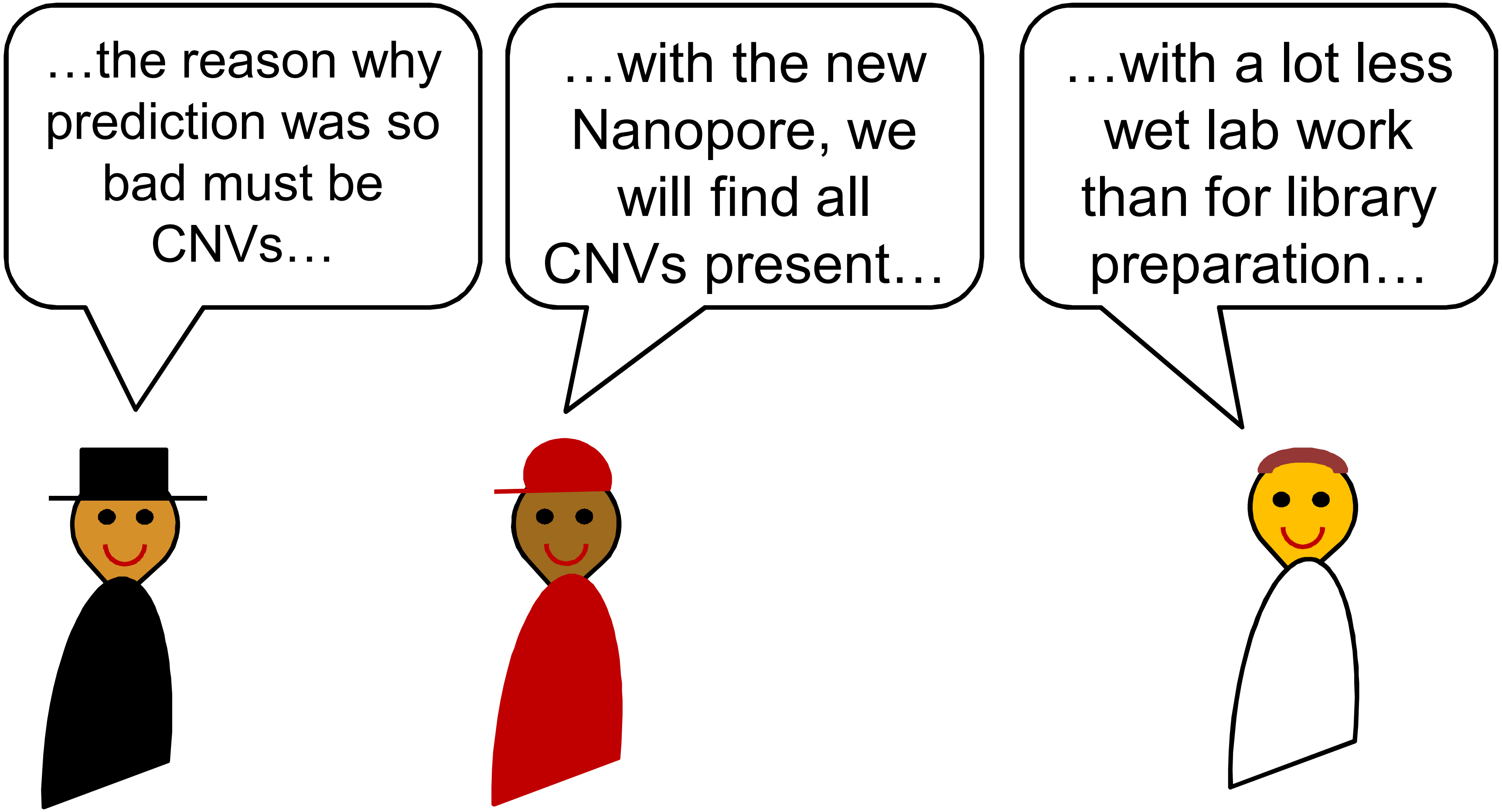
[2] College of Agronomy and Biotechnology, Southwest University, 400716 Chongqing, China

*Correspondence: Rod J. Snowdon (rod.snowdon@agrar.uni-giessen.de)

http://dx.doi.org/10.1016/j.molp.2016.10.017



The age of CNVs has started…



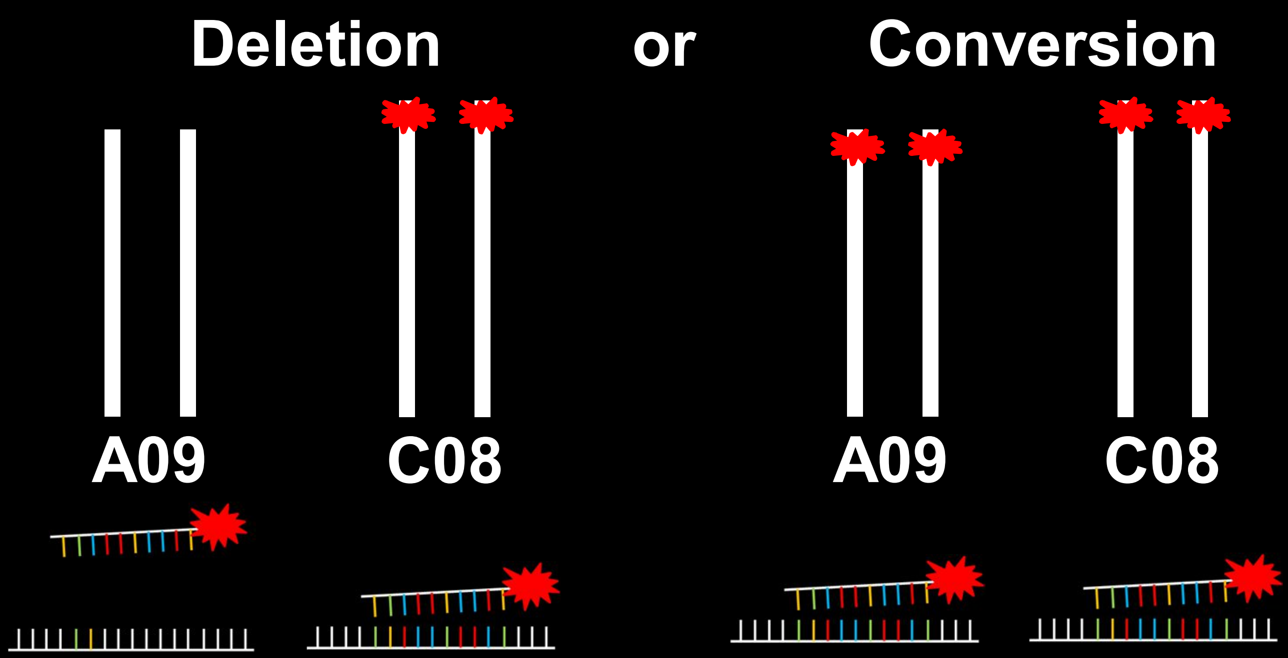
CNVs can be detected via various methods

- **Hybridization**
  - via FISH
  - via SNP arrays
- **PCR** (qPCR, fragment presence)
- **Sequencing**
  - Short read sequencing
  - Long read sequencing

General decision rules

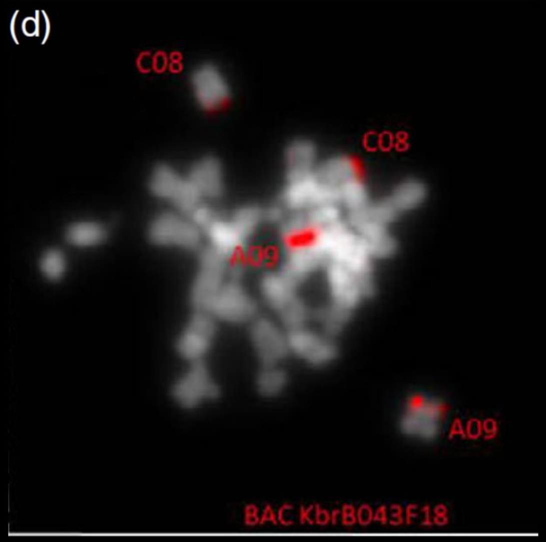
1. Do not rely on a single technique! Cooperate if necessary.
2. Use replicates and controls!
3. Think of hypothesis, budget, time, experience, bioinformatics…

FISH for CNV detection



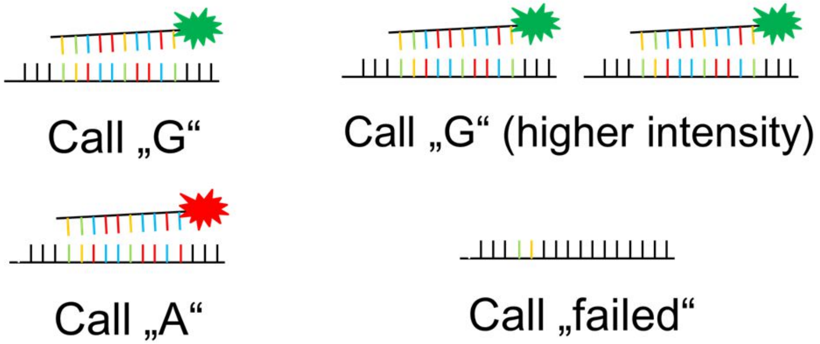
Deletion or **Conversion**

A09 **C08** **A09** **C08**



Stein et al., 2017, Plant Biotechnol J

SNP arrays for CNV detection

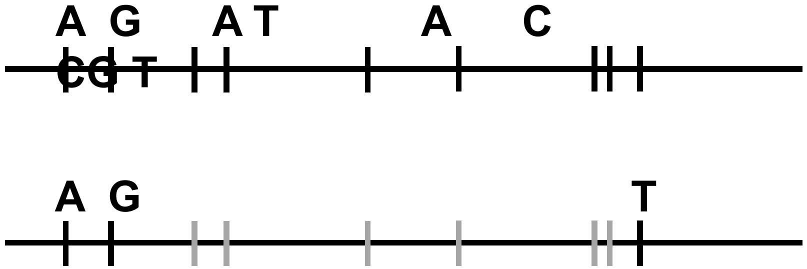


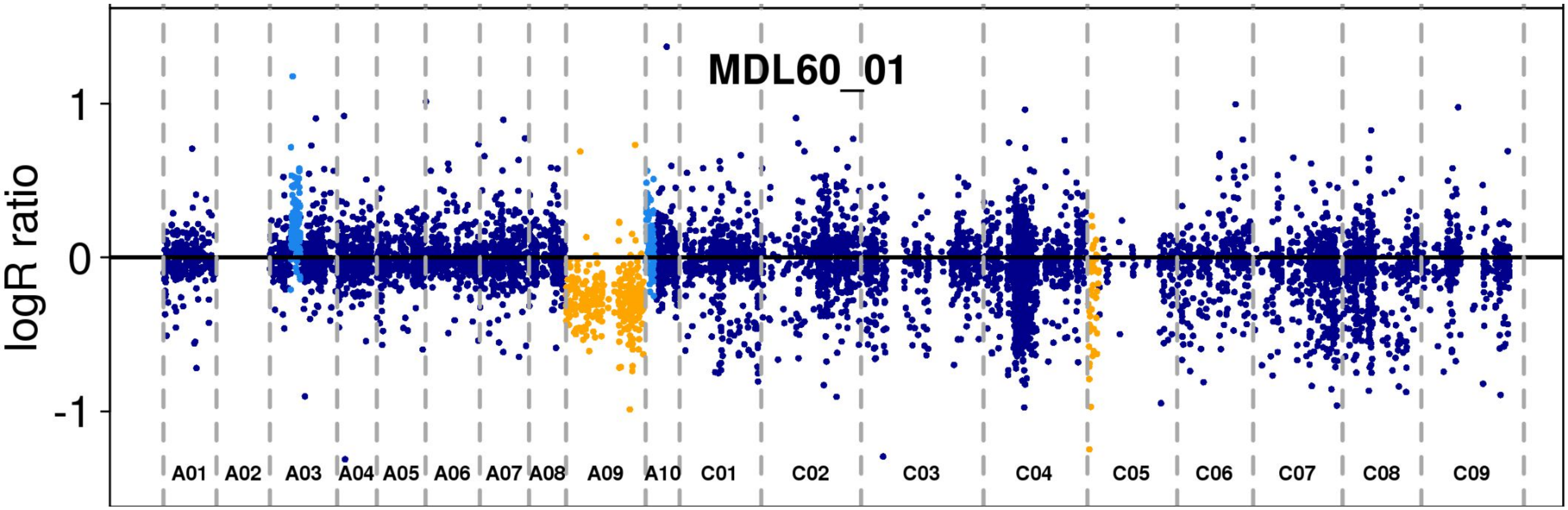
Call „G“

Call „G“ (higher intensity)

Call „A“

Call „failed“

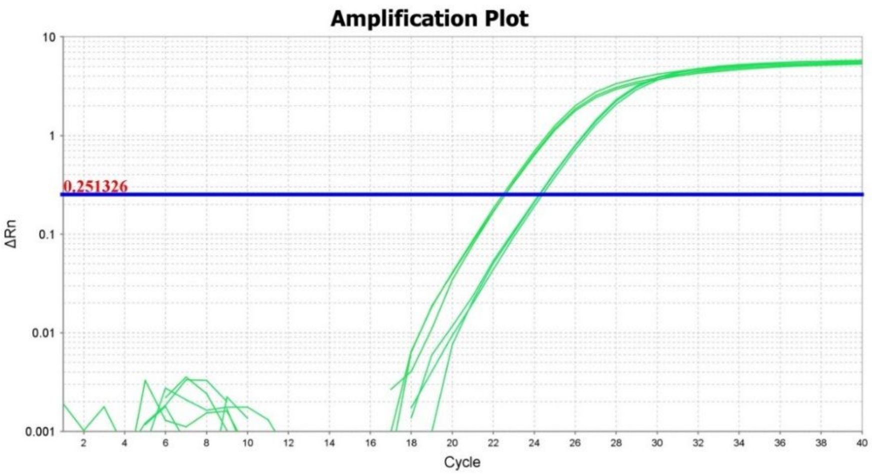


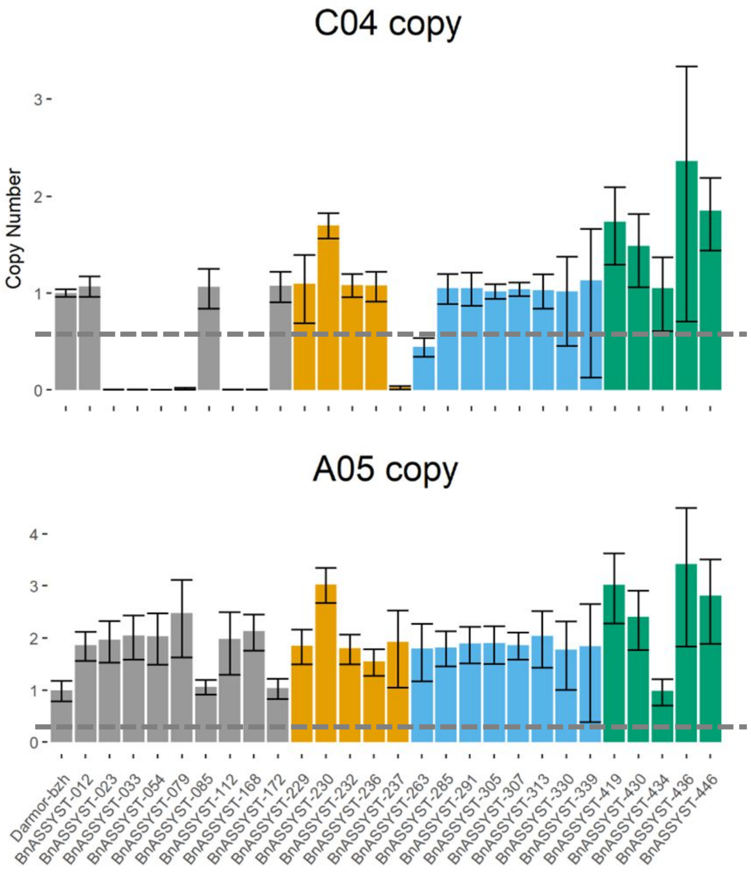


Mwathi et al., 2019, under review

qPCR for CNV detection

Time until exponential amplification ~ amount of input DNA ~ copy number





Copy number variation at the *HvCBF4–HvCBF2* genomic segment is a major component of frost resistance in barley

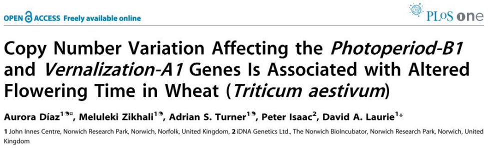
Enrico Francia[1,2] · Caterina Morcia[3] · Marianna Pasquariello[1,4] ·
Valentina Mazzamurro[1] · Justyna Anna Milc[1,2] · Fulvia Rizza[3] · Valeria Terzi[3] ·
Nicola Pecchioni[1,2,5]

PNAS

Aluminum tolerance in maize is associated with higher *MATE1* gene copy number

Lyza G. Maron[a,1], Claudia T. Guimarães[b], Matias Kirst[c,d], Patrice S. Albert[e], James A. Birchler[e], Peter J. Bradbury[a,f], Edward S. Buckler[a,f], Alison E. Coluccio[a], Tatiana V. Danilova[e,2], David Kudrna[g], Jurandir V. Magalhaes[b], Miguel A. Piñeros[a], Michael C. Schatz[h], Rod A. Wing[g], and Leon V. Kochian[a,1]

[a]Robert W. Holley Center for Agriculture and Health, US Department of Agriculture–Agricultural Research Service, and [f]Institute for Genomic Diversity, Cornell University, Ithaca, NY 14853; [b]Embrapa Maize and Sorghum, 35701-970, Sete Lagoas, Minas Gerais, Brazil; [c]School of Forest Resources and Conservation, University of Florida, Gainesville, FL 32611; [d]University of Florida Genetics Institute, University of Florida, Gainesville, FL 32610; [e]Division of Biological Sciences, University of Missouri, Columbia, MO 65211; [g]Arizona Genomics Institute, School of Plant Sciences, University of Arizona, Tucson, AZ 85705; and [h]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724



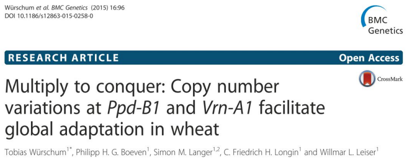
OPEN ACCESS Freely available online

PLoS one

Copy Number Variation Affecting the *Photoperiod-B1* and *Vernalization-A1* Genes Is Associated with Altered Flowering Time in Wheat (*Triticum aestivum*)

Aurora Díaz[1]¤, Meluleki Zikhali[1], Adrian S. Turner[1], Peter Isaac[2], David A. Laurie[1]*

1 John Innes Centre, Norwich Research Park, Norwich, Norfolk, United Kingdom, 2 iDNA Genetics Ltd., The Norwich BioIncubator, Norwich Research Park, Norwich, United Kingdom



Würschum *et al. BMC Genetics* (2015) 16:96
DOI 10.1186/s12863-015-0258-0

BMC Genetics

RESEARCH ARTICLE Open Access

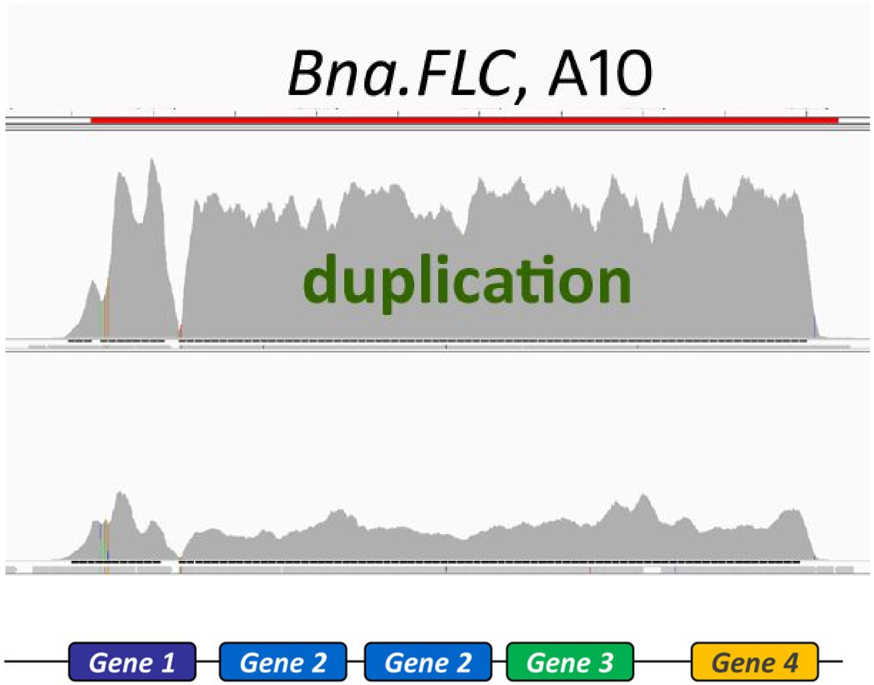
CrossMark

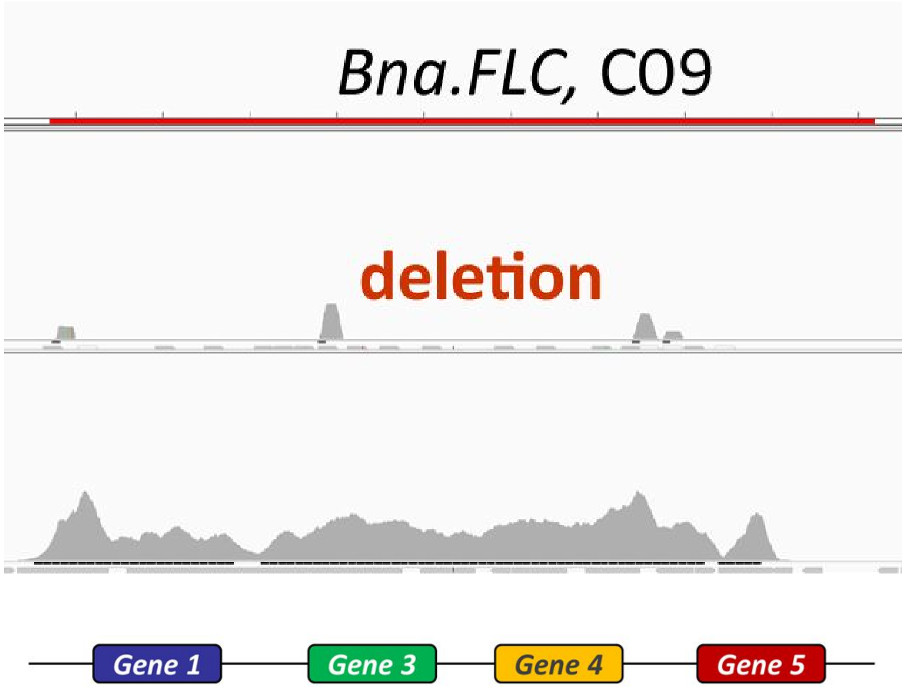
Multiply to conquer: Copy number variations at *Ppd-B1* and *Vrn-A1* facilitate global adaptation in wheat

Tobias Würschum[1*], Philipp H. G. Boeven[1], Simon M. Langer[1,2], C. Friedrich H. Longin[1] and Willmar L. Leiser[1]

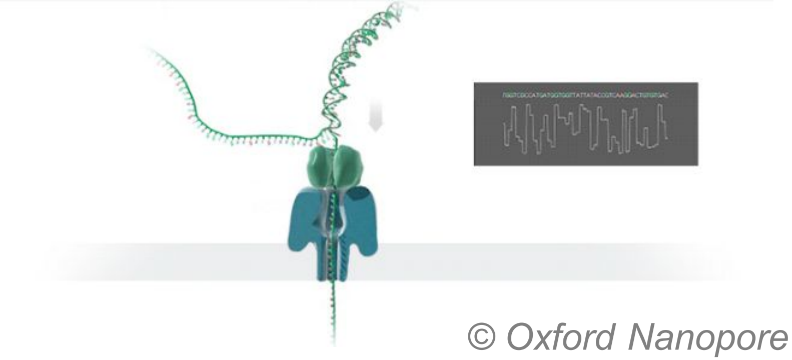
A. Mariette, 2017

Short read sequencing for CNV detection

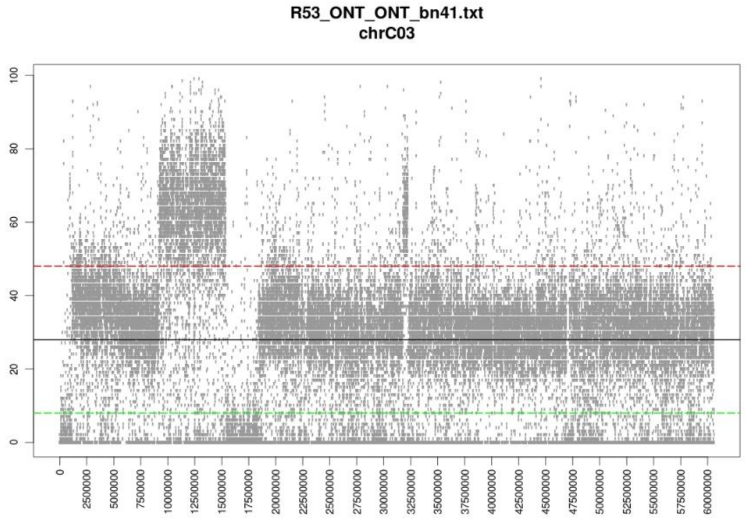




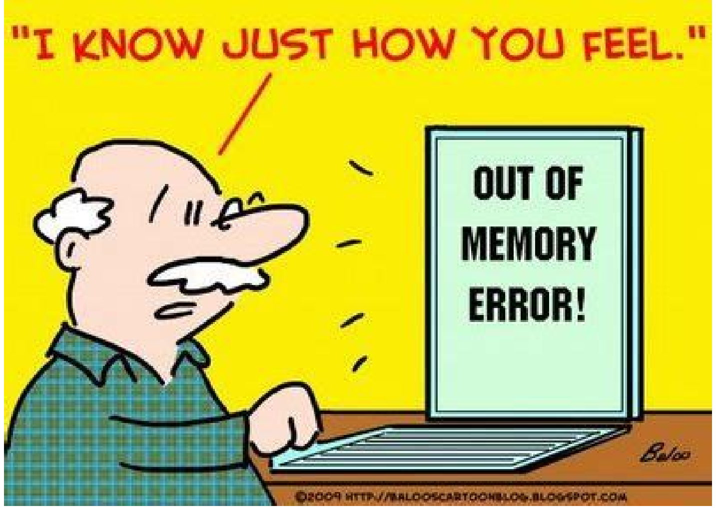
Long read sequencing for CNV detection



© Oxford Nanopore



Courtesy of H. Chawla

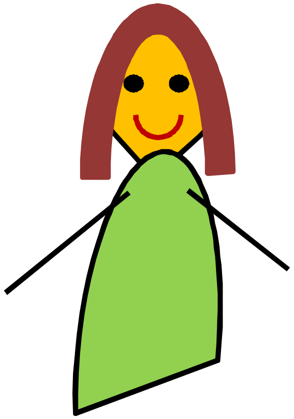


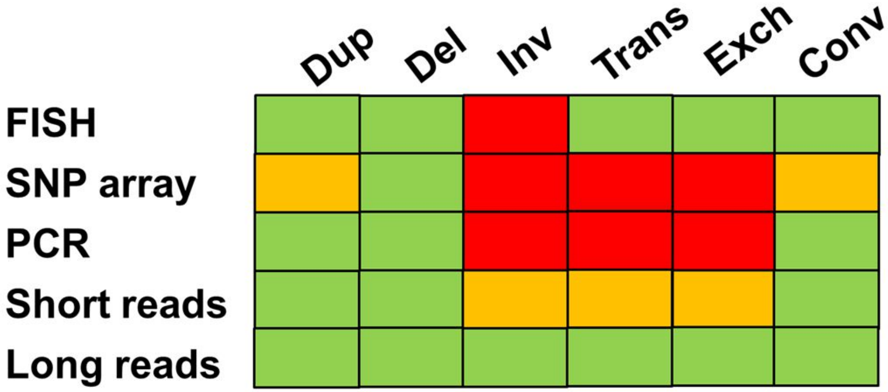
Example 1: As a basic researcher

Example 1: As a basic researcher

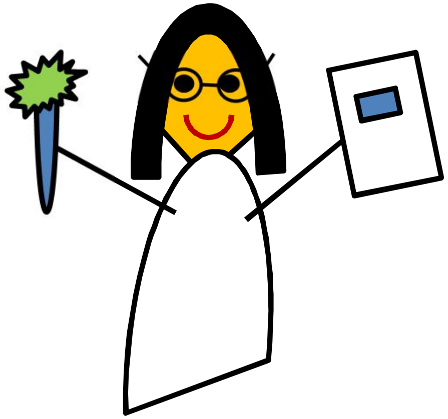
What do you want to find out?

I want to know which, where and how exactly homeologous exchanges happen.

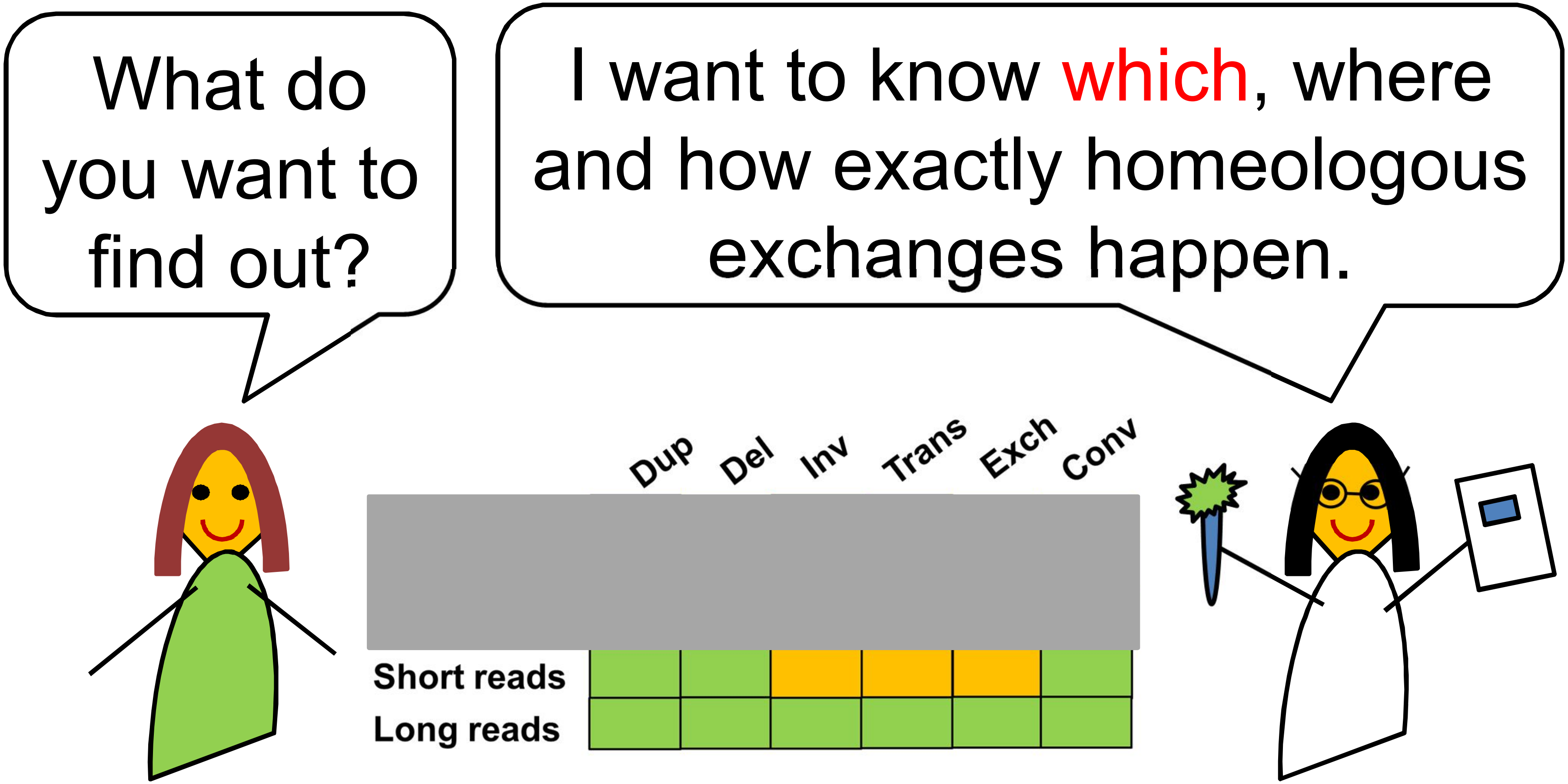




	Dup	Del	Inv	Trans	Exch	Conv
FISH	🟩	🟩	🟥	🟩	🟩	🟩
SNP array	🟨	🟩	🟥	🟥	🟥	🟨
PCR	🟩	🟩	🟥	🟥	🟥	🟩
Short reads	🟩	🟩	🟨	🟨	🟨	🟩
Long reads	🟩	🟩	🟩	🟩	🟩	🟩



Example 1: As a basic researcher

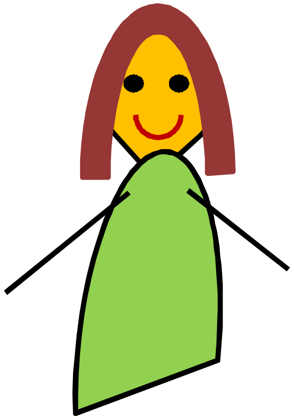


	Dup	Del	Inv	Trans	Exch	Conv
Short reads	🟩	🟩	🟧	🟧	🟧	🟩
Long reads	🟩	🟩	🟩	🟩	🟩	🟩

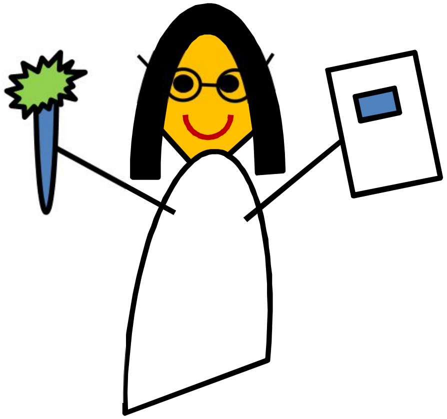
Example 1: As a basic researcher

What do you want to find out?

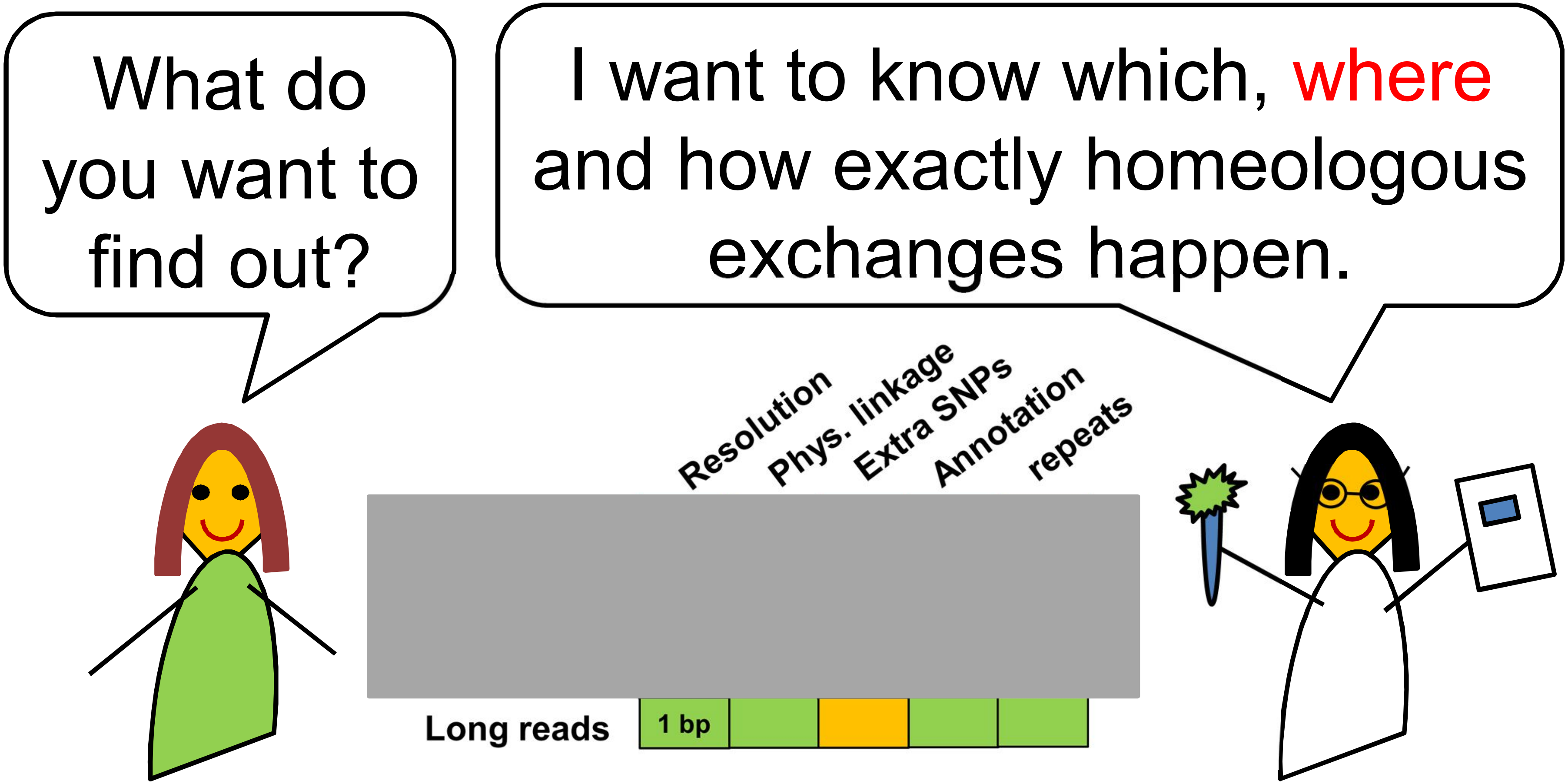
I want to know which, where and how exactly homeologous exchanges happen.



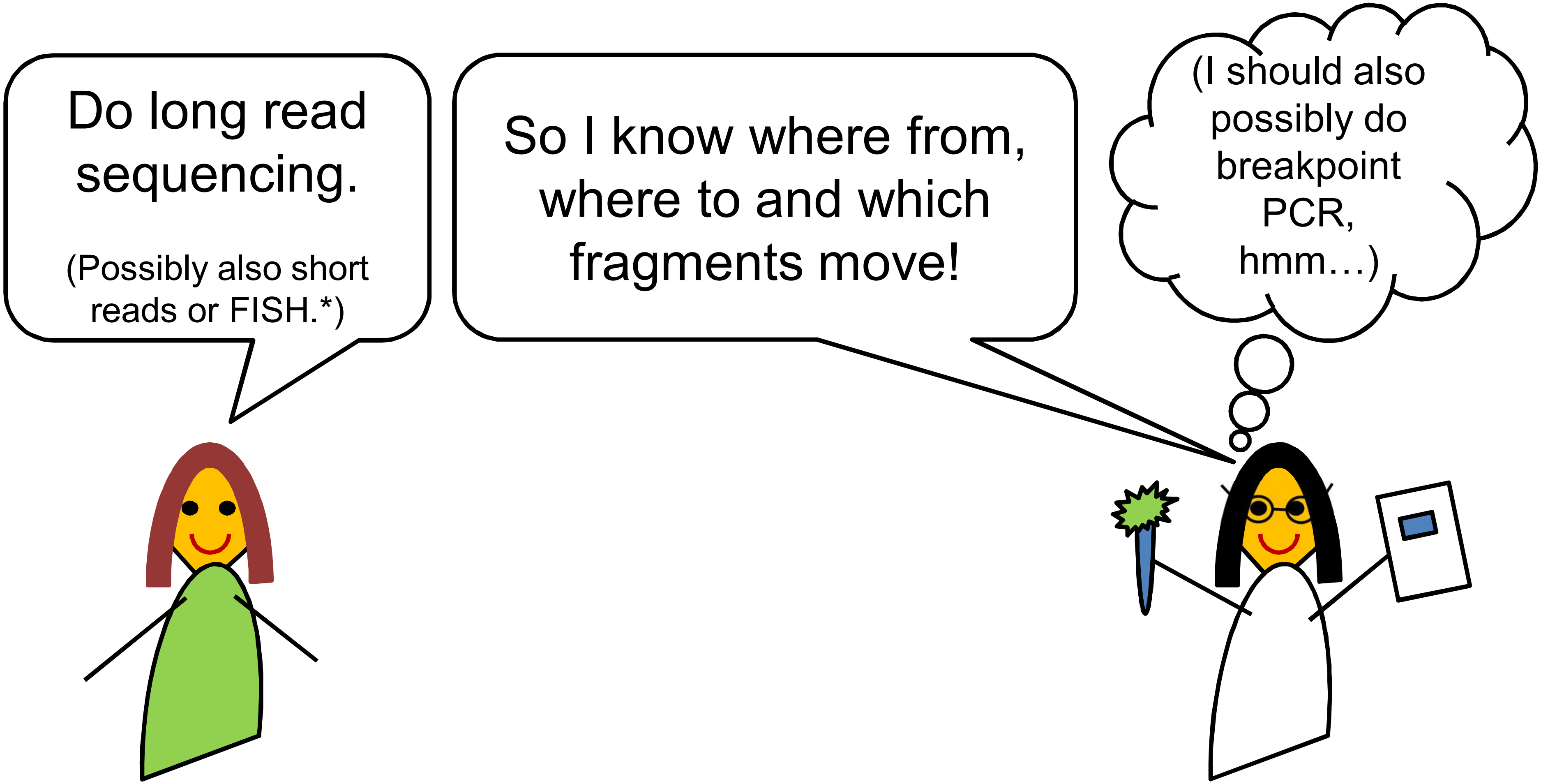
	Resolution	Phys. linkage	Extra SNPs	Annotation	repeats
FISH	🟥 5 Mb	🟩	🟥	🟥	🟧
SNP array	🟥 1 Mb	🟥	🟩	🟧	🟥
PCR	🟧 0.1 kb	🟧	🟥	🟧	🟧
Short reads	🟩 1 bp	🟧	🟩	🟩	🟥
Long reads	🟩 1 bp	🟩	🟧	🟩	🟩



Example 1: As a basic researcher

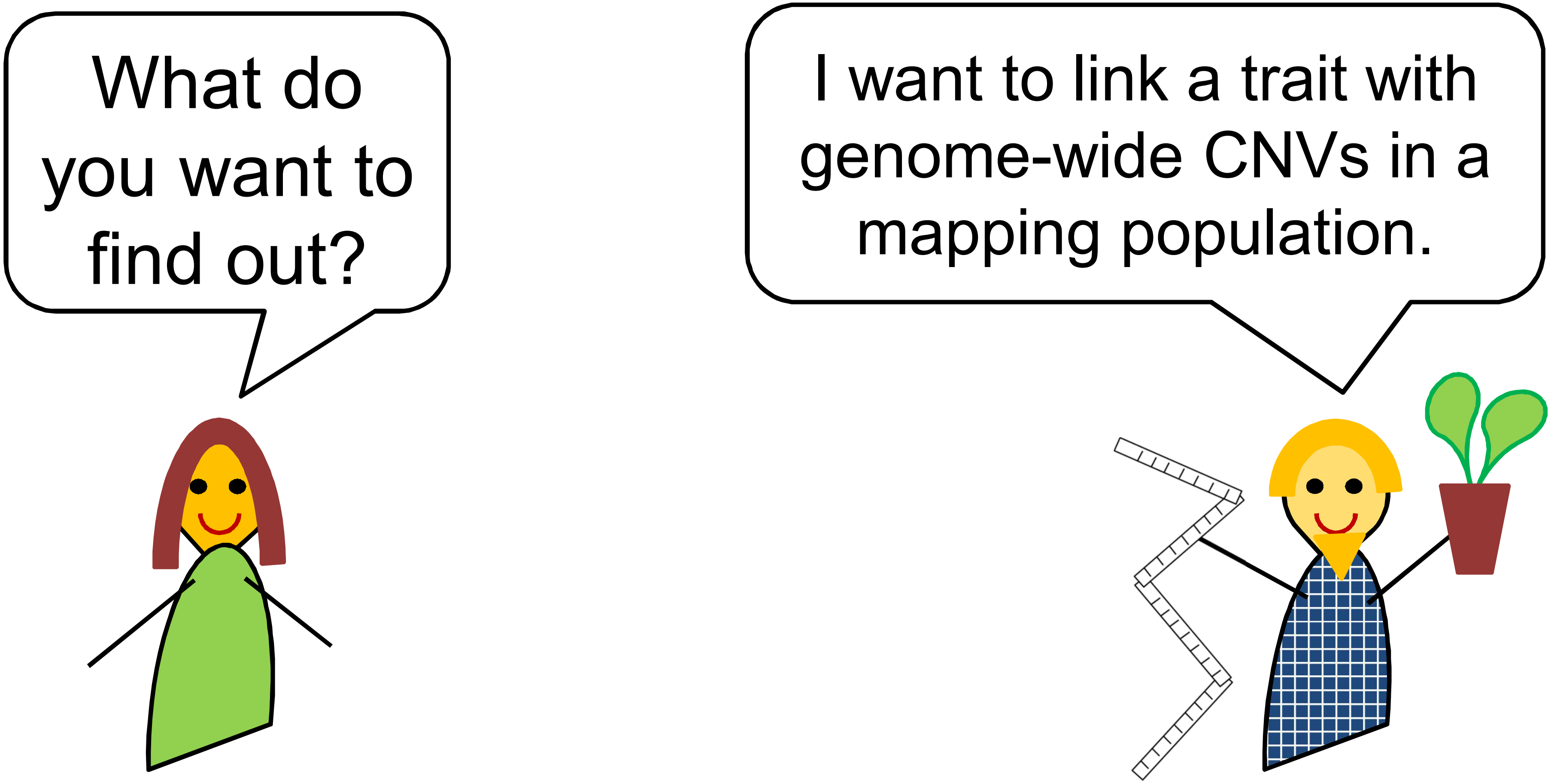


Example 1: As a basic researcher



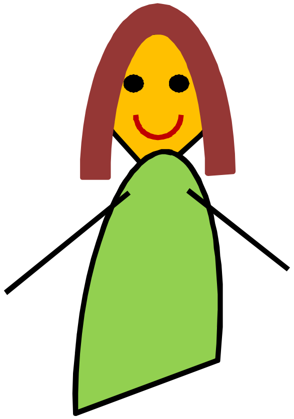
* because of general rule 1

Example 2: As an applied scientist

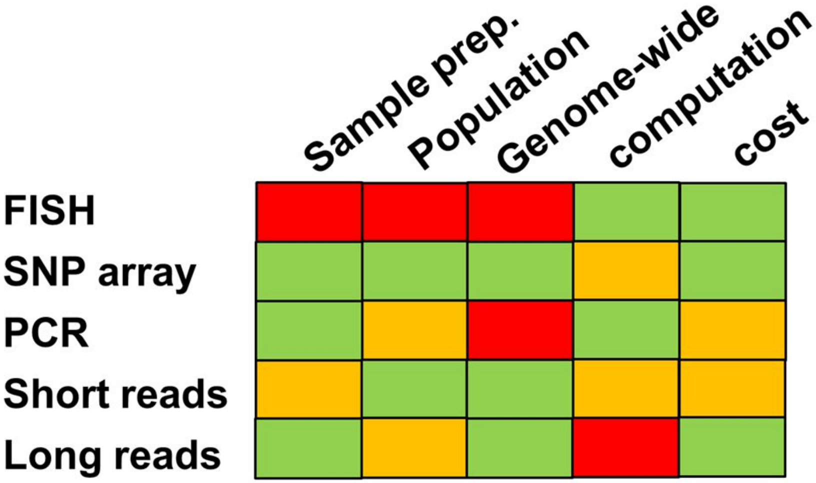


Example 2: As an applied scientist

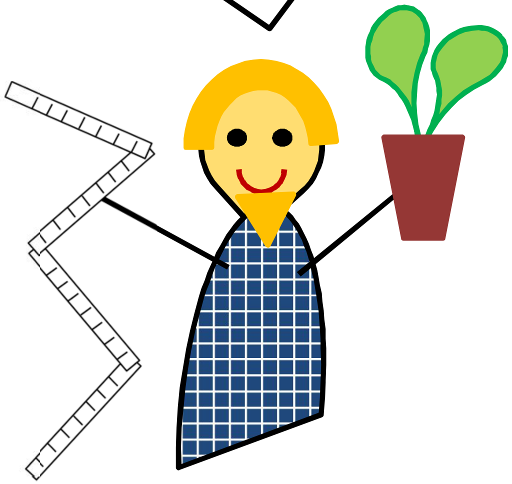
What do you want to find out?



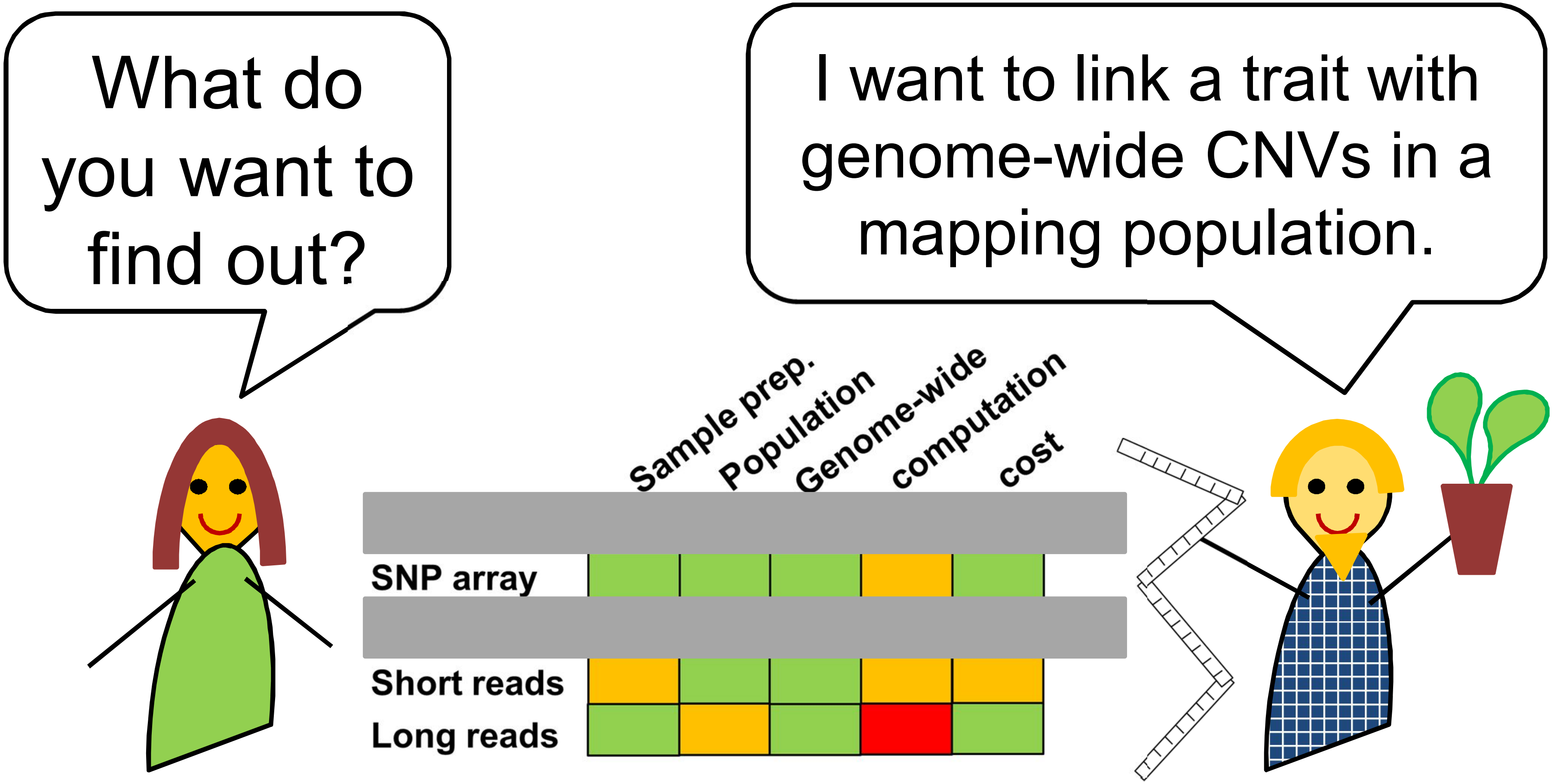
I want to link a trait with genome-wide CNVs in a mapping population.



	Sample prep.	Population	Genome-wide	computation	cost
FISH	🟥	🟥	🟥	🟩	🟩
SNP array	🟩	🟩	🟩	🟨	🟩
PCR	🟩	🟨	🟥	🟩	🟨
Short reads	🟨	🟩	🟩	🟨	🟨
Long reads	🟩	🟨	🟩	🟥	🟩



Example 2: As an applied scientist

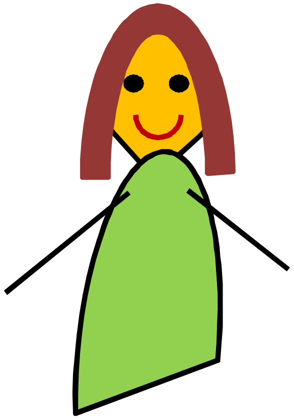


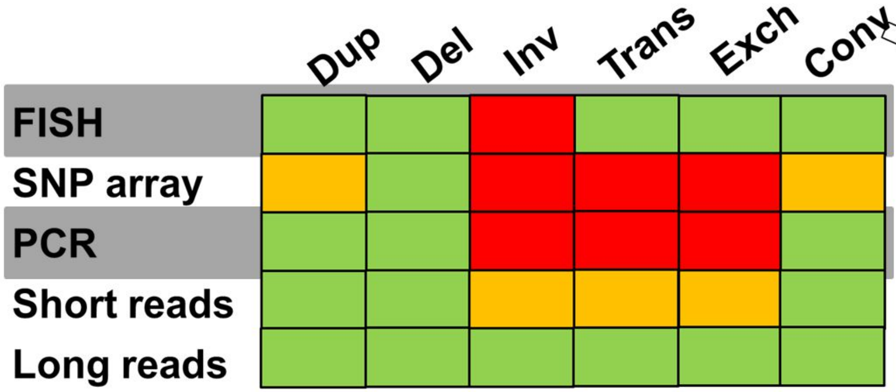
	Sample prep.	Population	Genome-wide	computation	cost
SNP array	🟩	🟩	🟩	🟨	🟩
Short reads	🟨	🟩	🟩	🟨	🟨
Long reads	🟩	🟨	🟩	🟥	🟩

Example 2: As an applied scientist

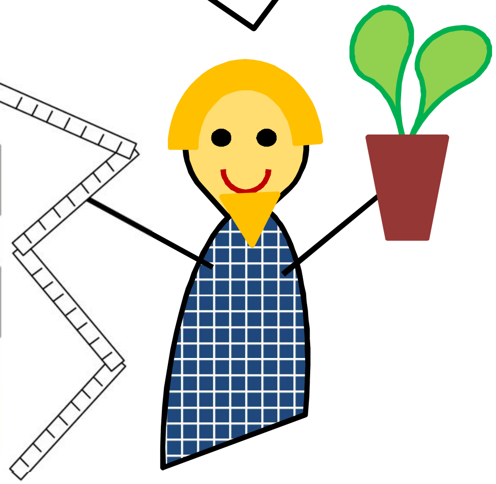
What do you want to find out?

I want to link a trait with genome-wide CNVs in a mapping population.

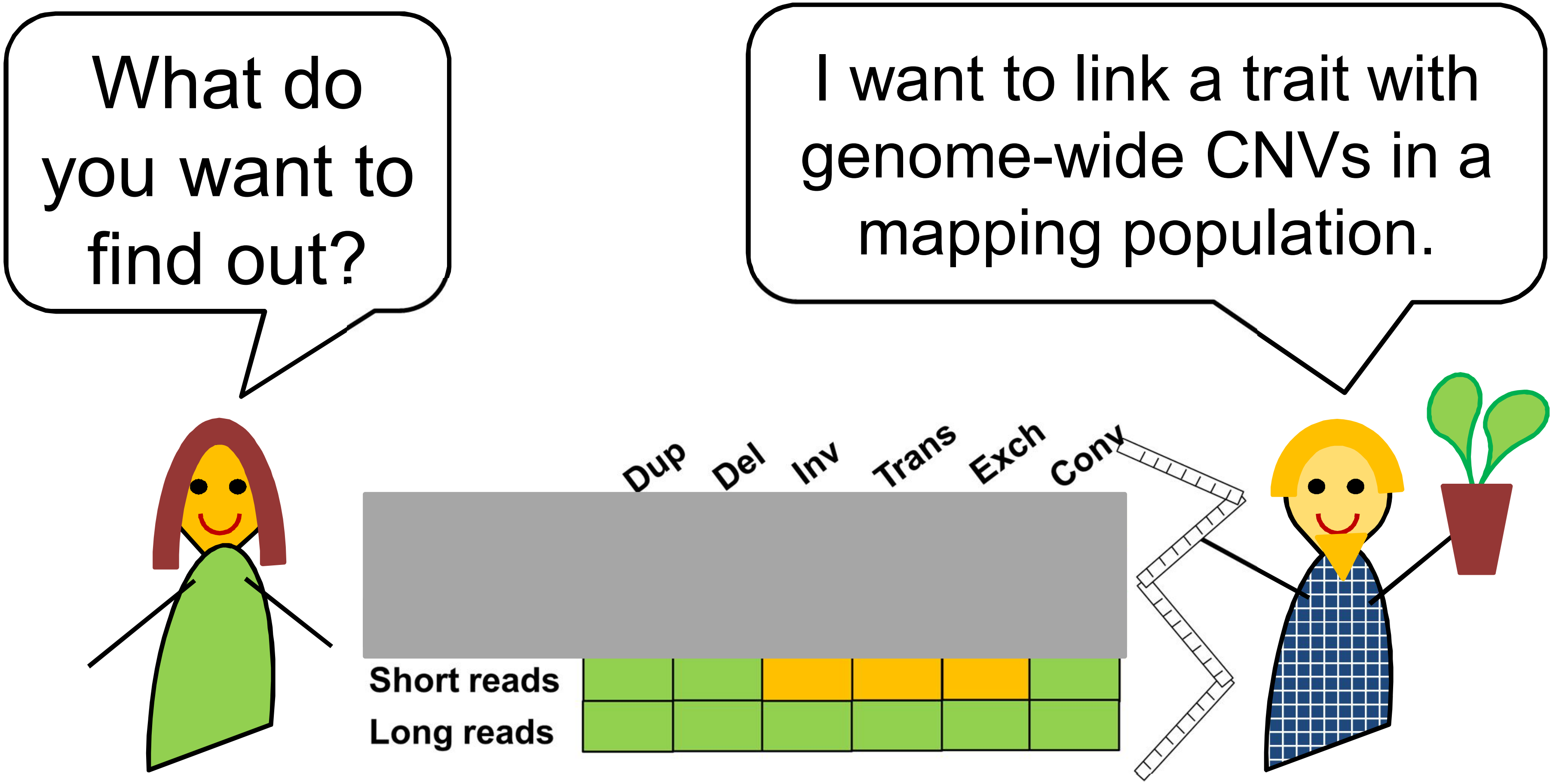




	Dup	Del	Inv	Trans	Exch	Conv
FISH	🟩	🟩	🟥	🟩	🟩	🟩
SNP array	🟨	🟩	🟥	🟥	🟥	🟨
PCR	🟩	🟩	🟥	🟥	🟥	🟩
Short reads	🟩	🟩	🟨	🟨	🟨	🟩
Long reads	🟩	🟩	🟩	🟩	🟩	🟩



Example 1: As an applied scientist

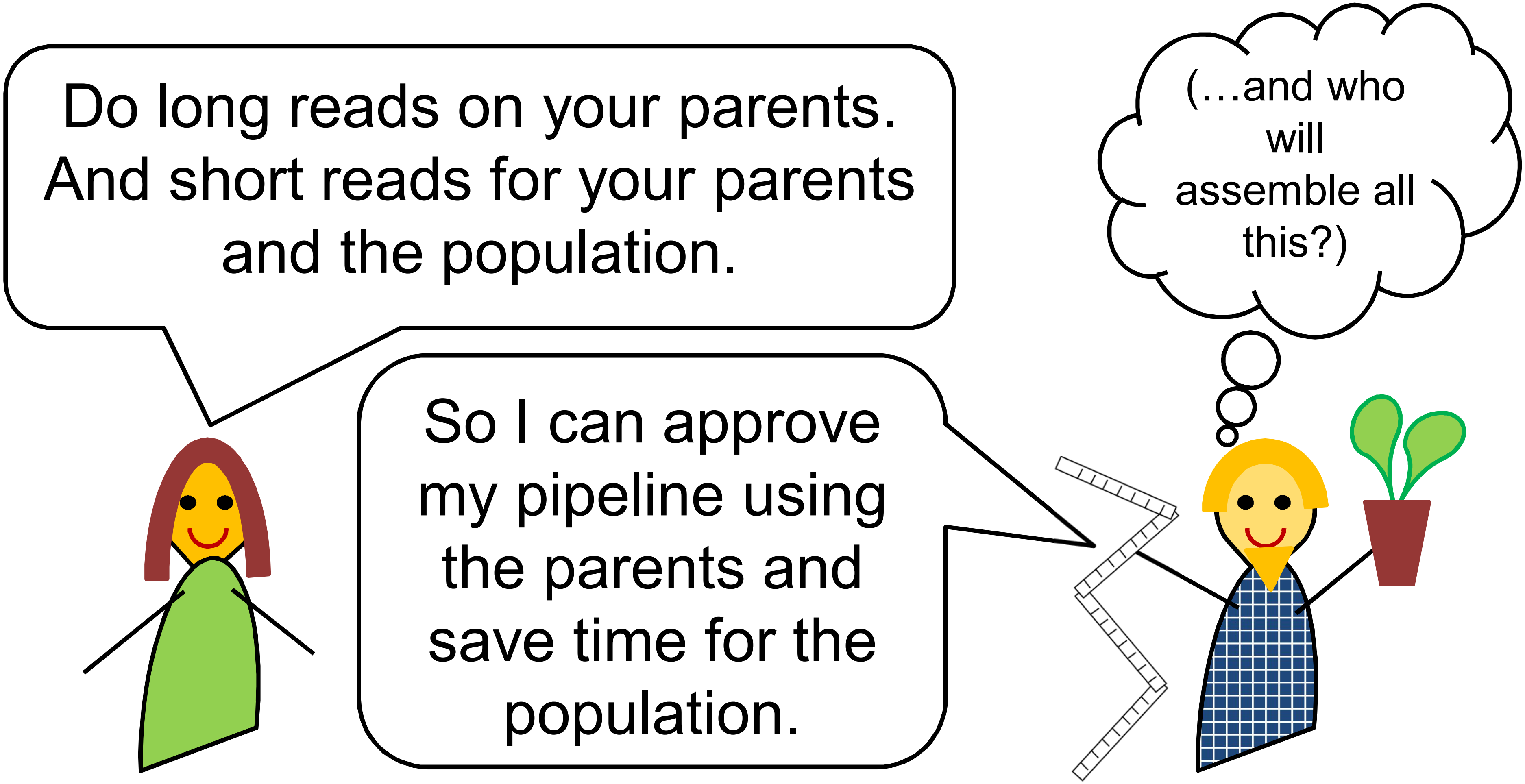


What do you want to find out?

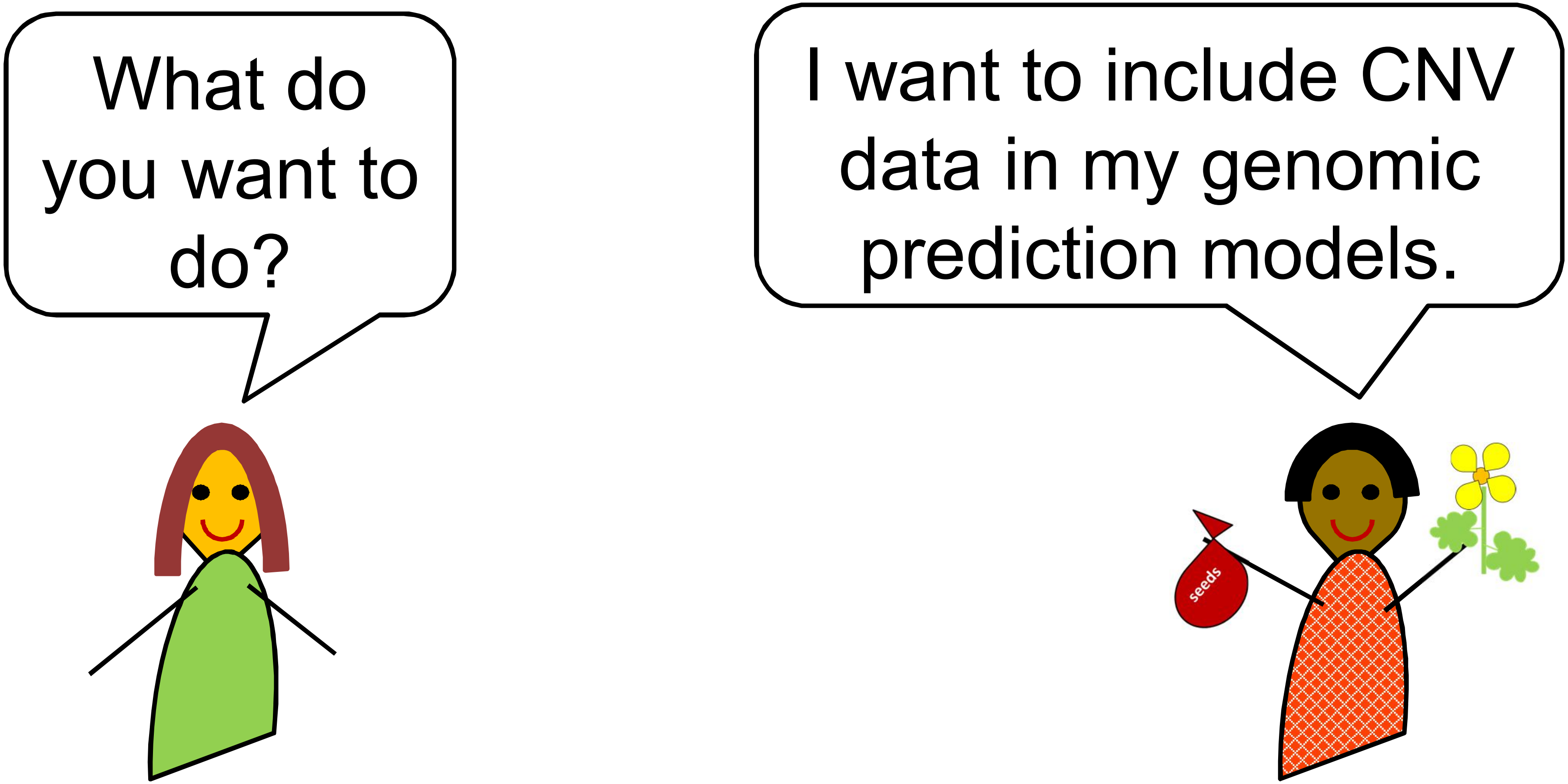
I want to link a trait with genome-wide CNVs in a mapping population.

	Dup	Del	Inv	Trans	Exch	Conv
Short reads	🟩	🟩	🟧	🟧	🟧	🟩
Long reads	🟩	🟩	🟩	🟩	🟩	🟩

Example 2: As an applied scientist



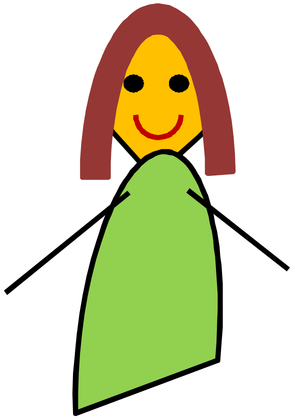
Example 3: As a breeder

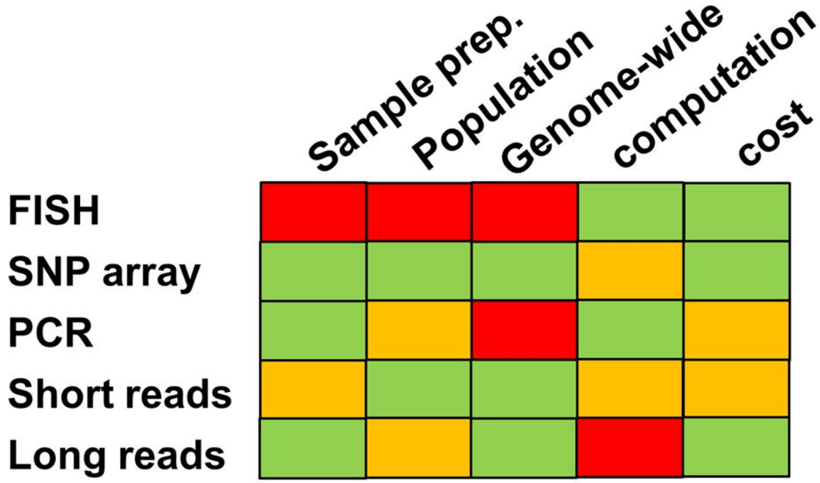


Example 3: As a breeder

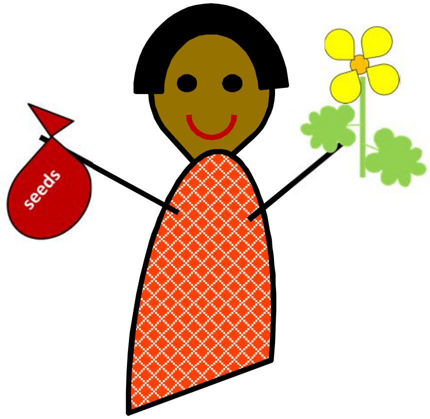
What do you want to do?

I want to include CNV data in my genomic prediction models.

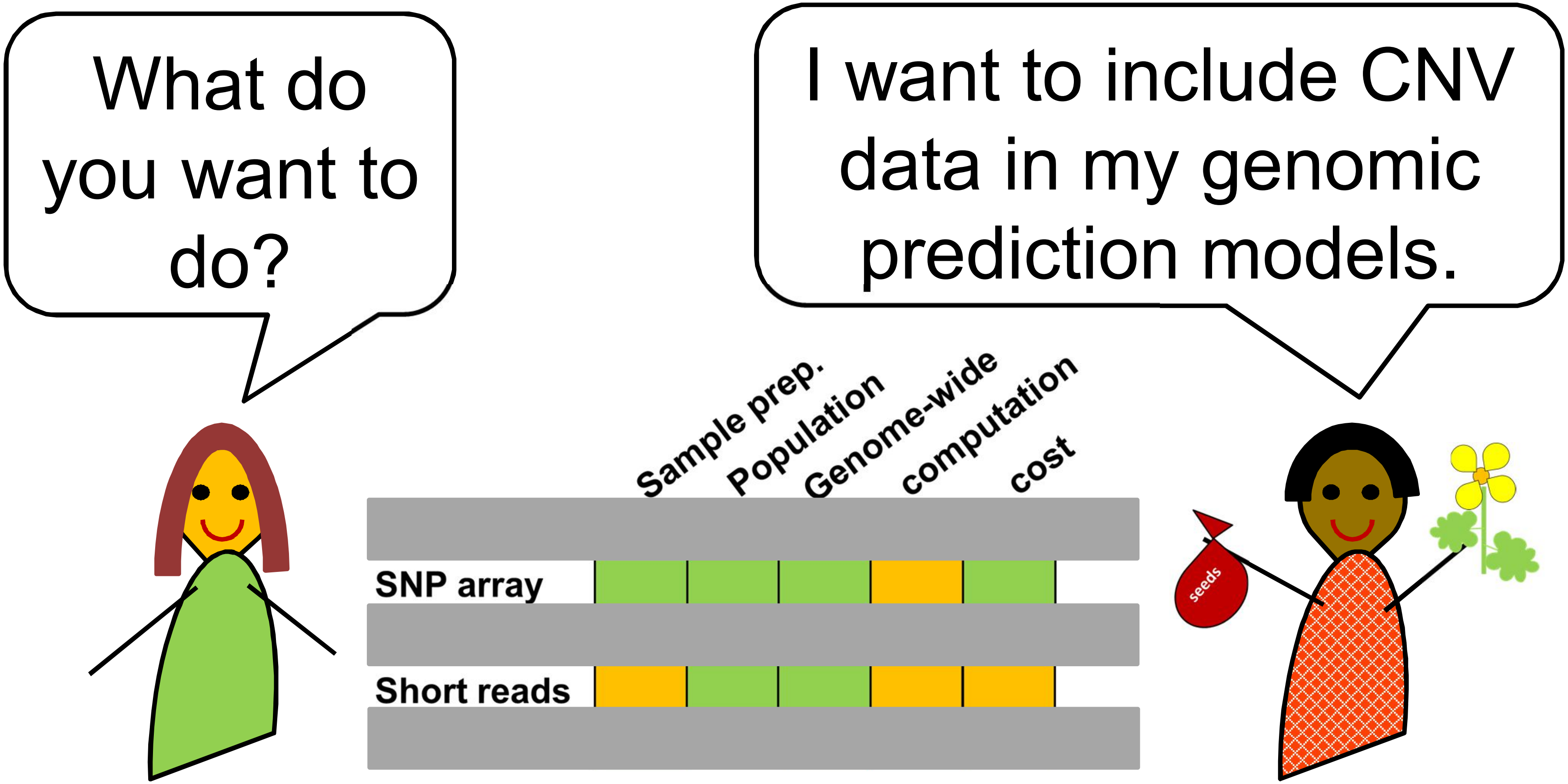




	Sample prep.	Population	Genome-wide	computation	cost
FISH	🟥	🟥	🟥	🟩	🟩
SNP array	🟩	🟩	🟩	🟨	🟩
PCR	🟩	🟨	🟥	🟩	🟨
Short reads	🟨	🟩	🟩	🟨	🟨
Long reads	🟩	🟨	🟩	🟥	🟩



Example 3: As a breeder

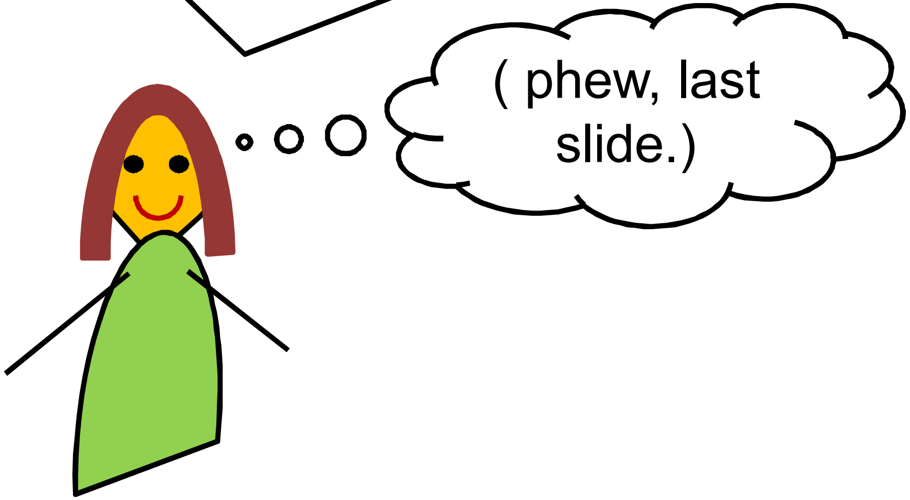


	Sample prep.	Population	Genome-wide	computation	cost
SNP array	🟩	🟩	🟩	🟧	🟩
Short reads	🟧	🟩	🟩	🟧	🟧

Example 3: As a breeder

What do YOU want to find out?

If you plan a project involving CNV determination and you are unsure what to do, I‘m happy to discuss with you.



Thanks to…

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Alban Mariette
Harmeet Chawla
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Elvis Katche
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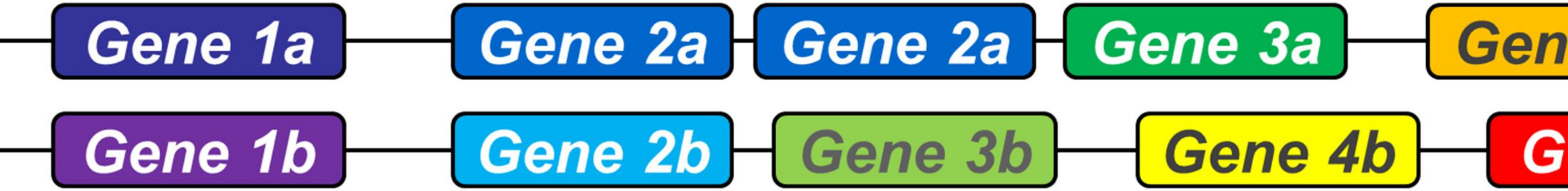

Methods to determine copy number variation in *Brassica* species

Dr. Sarah Schiessl-Weidenweber

Justus Liebig University Giessen