KASP markers for QC in potato and sweet potato

Lessons learned and perspectives

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A need for QC

Vegetative propagation

Mislabeling may happen across the breeding cycle

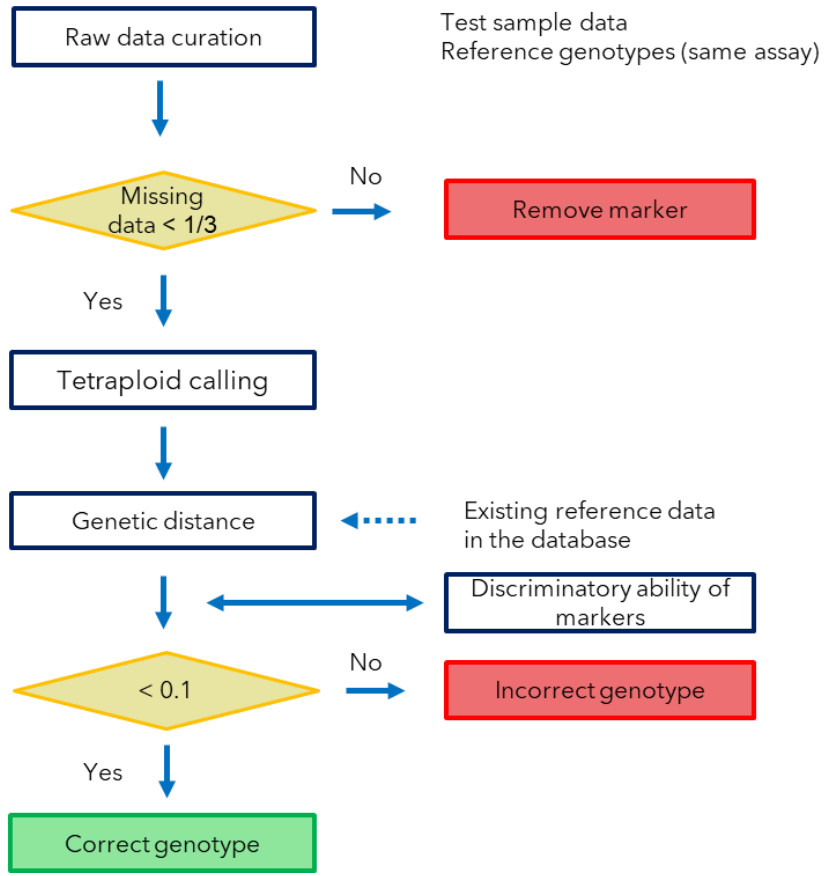
MAS, low selection accuracy, low rate of genetic gain

KASP markers as an option

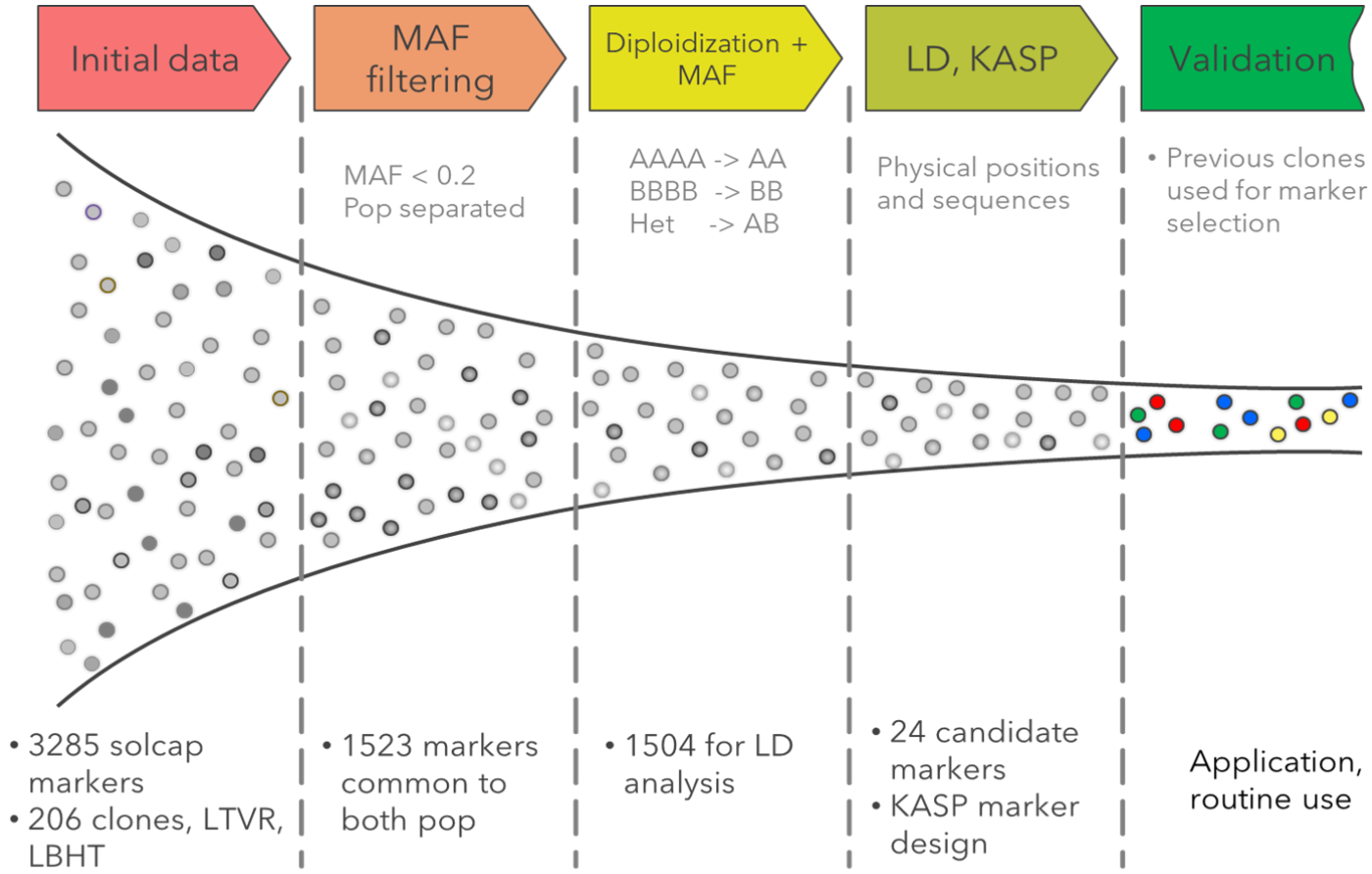
High discriminatory ability, easy to design, low cost, TAT

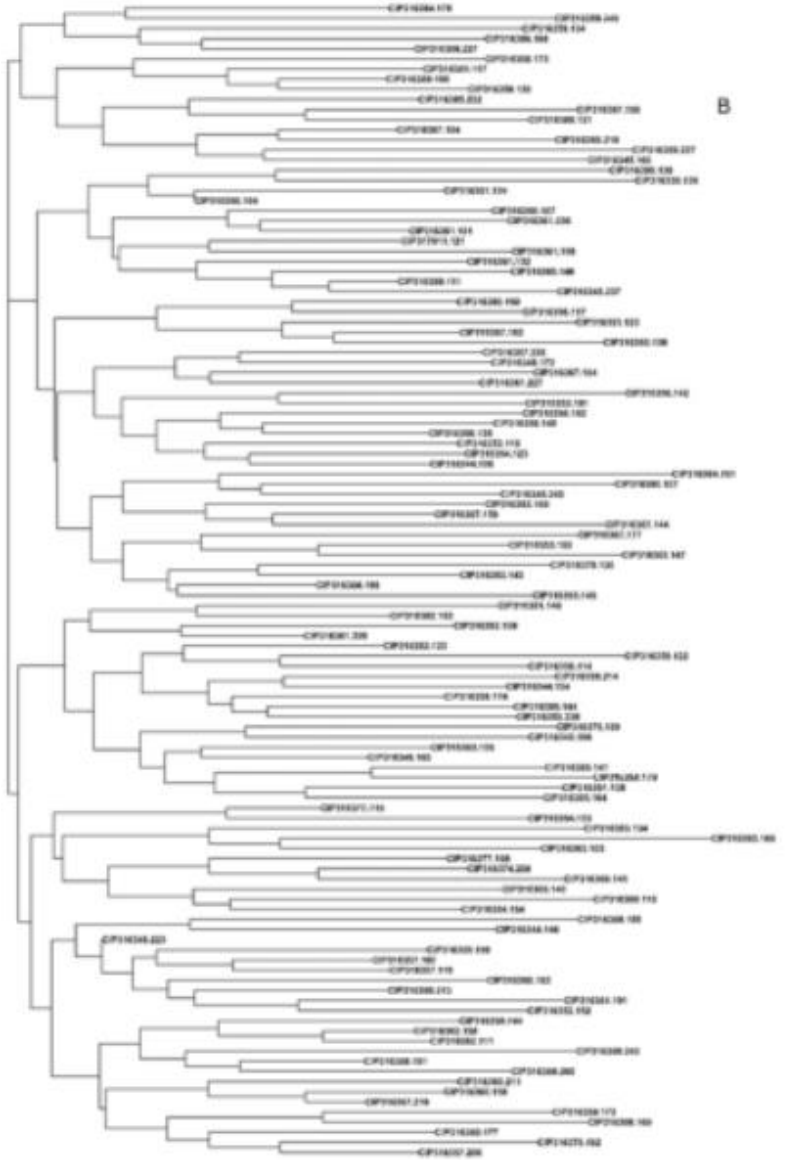
Used in breeding and prebreeding material

Simple analytic workflow to allow fast decision



Potato: panel design





21 markers validated and used along with traits markers
Tetraploid dosage

Potato: routine use, 2021-2023, 5329 samples

batch	#samples	missing geno	missing marker	control
TM0321	376	0.50	4.80	
TM1021	1495	0.00	14.30	
TM1121	372	0.00	0.00	
TM0922	573	0.00	9.50	
TM1122	1385	6.70	14.30	14
TM0223	1128	1.90	9.50	12

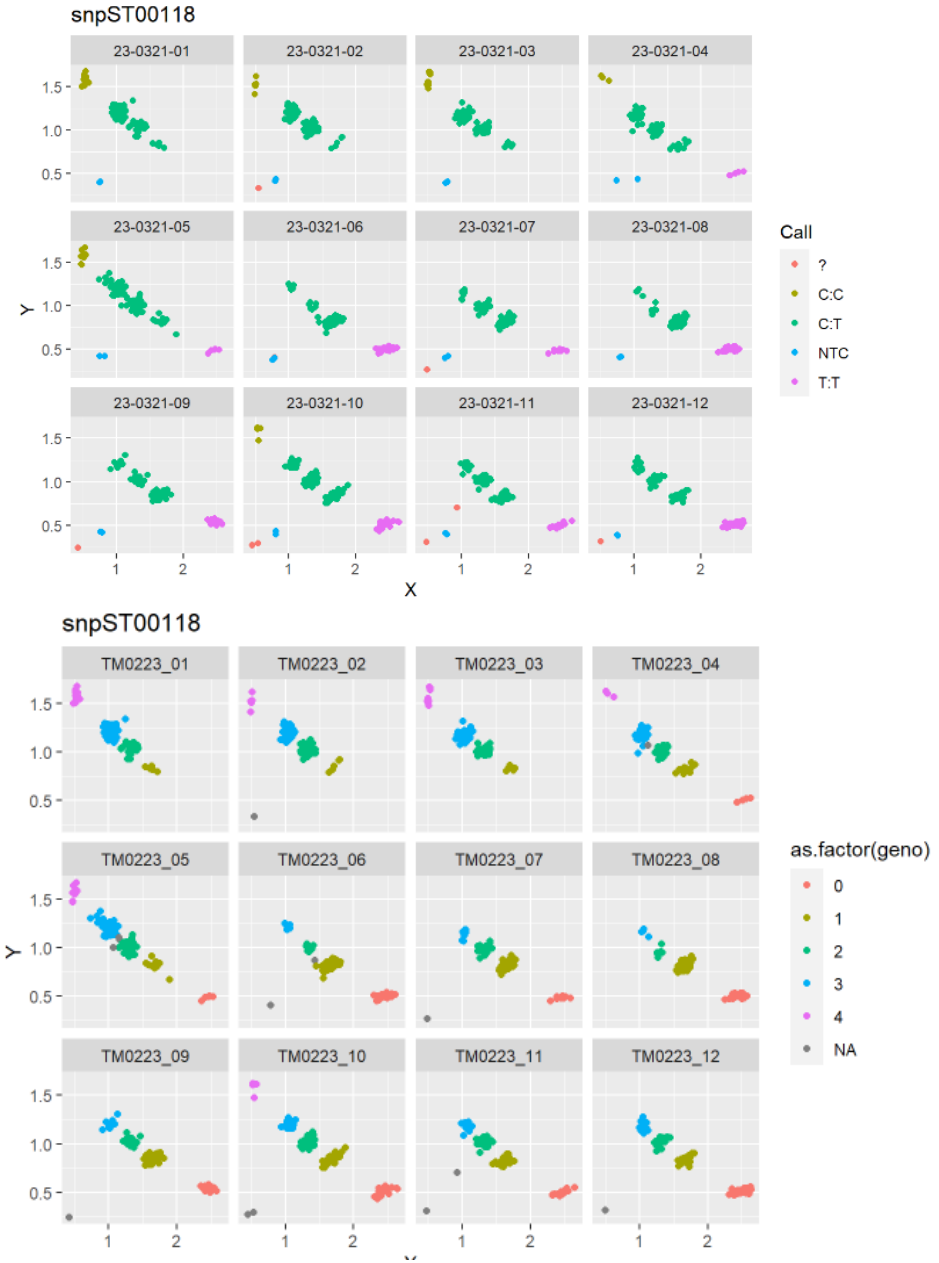
Crossing block
Two breeding pipelines
Prebreeding (2x) material

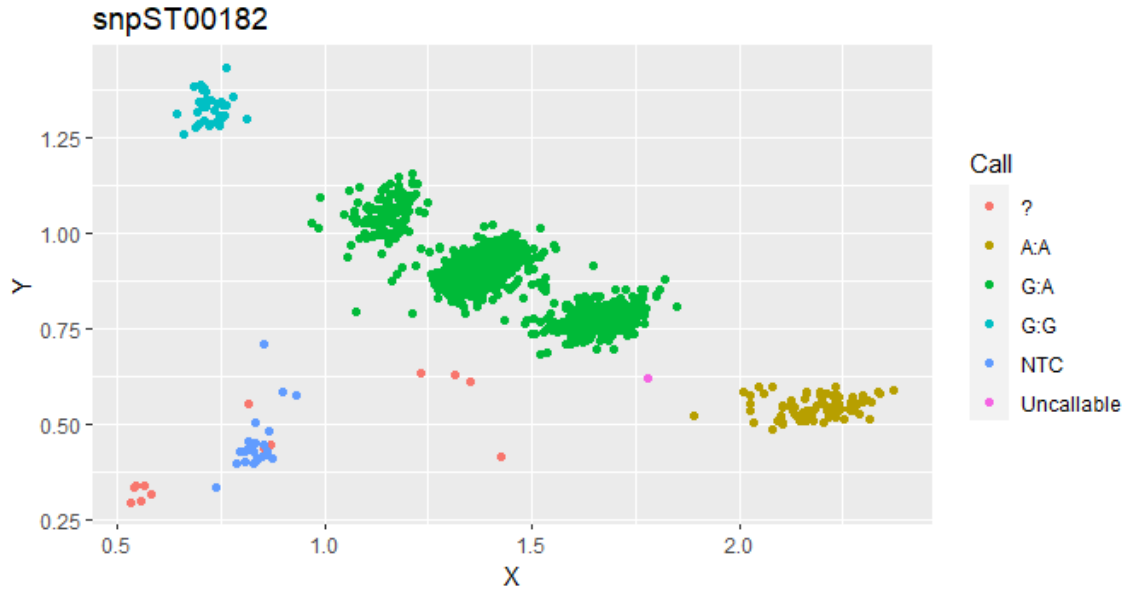
40 parents per pipeline
23 KASP markers

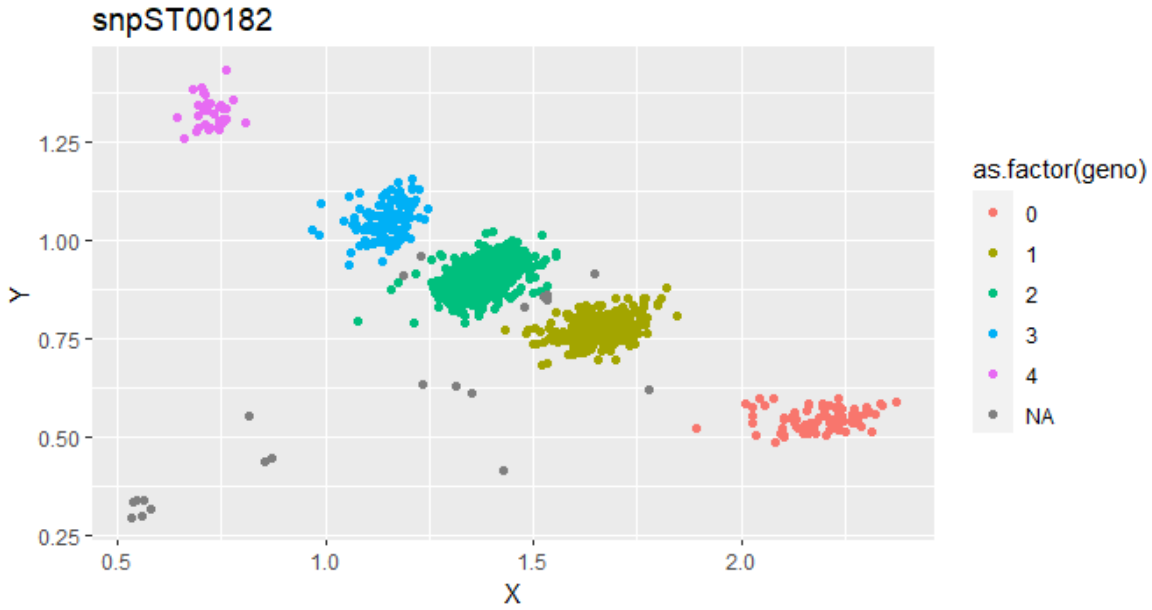
pipeline	plants in CB†	samples†	# markers‡	mislabeled	rate
LTVR	920	900	19	50	5.56
LBHT	920	870	18	131	15.06
Total	1840	1770	37	181	

† Difference due to plant survival in crossing blocks and genotypes with low quality data
‡ markers with low quality data (missing data) were removed

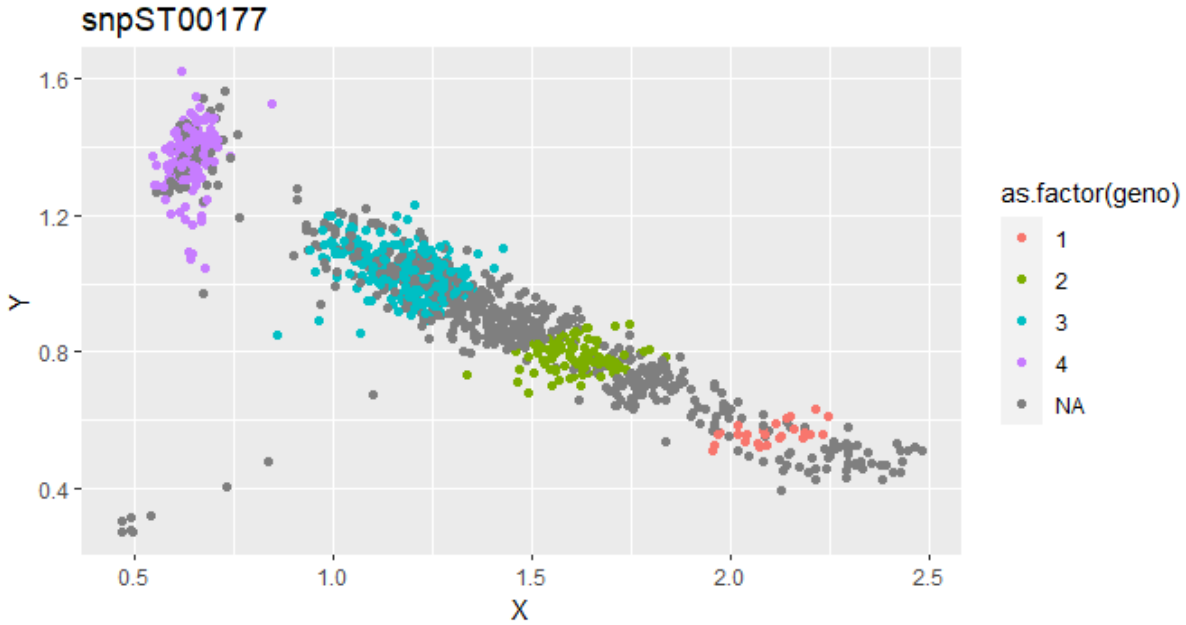
Potato: routine use, good markers

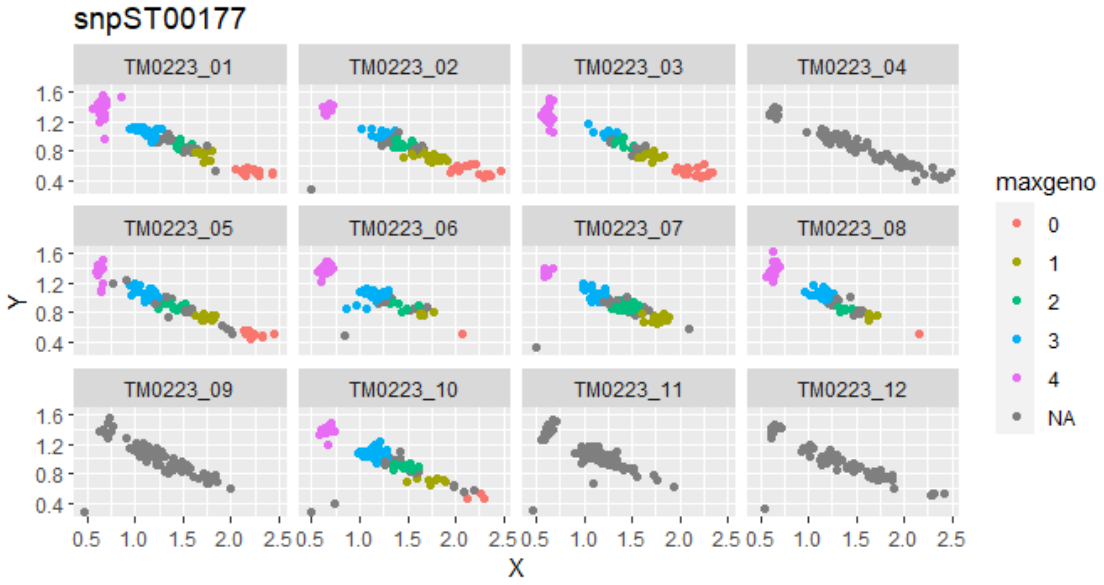


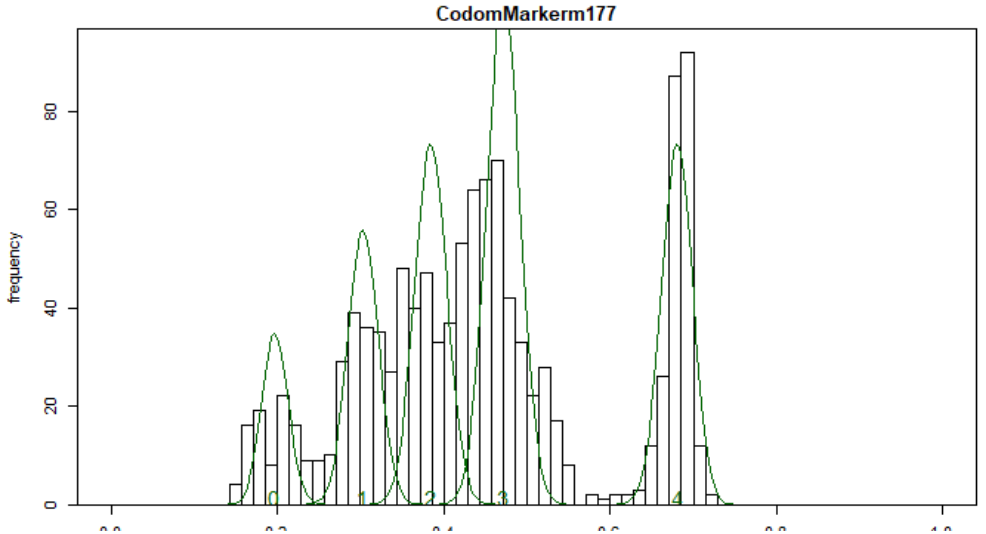




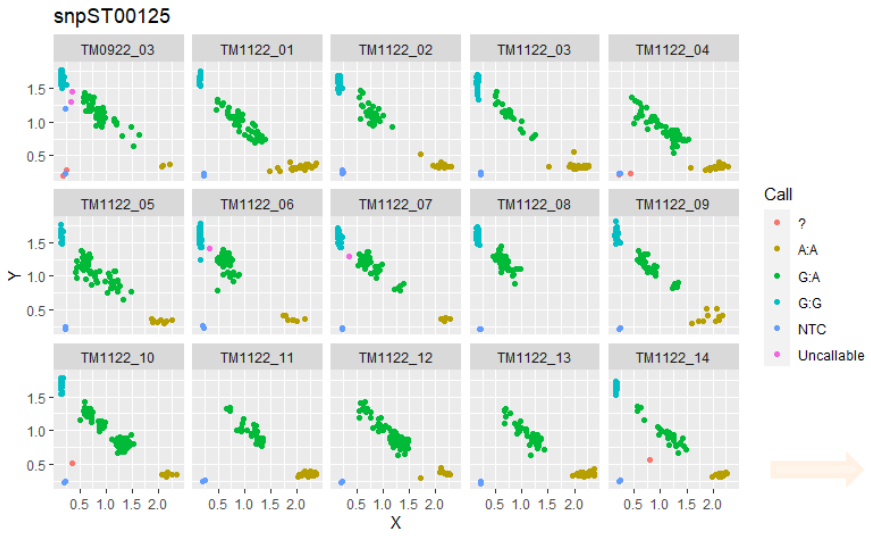
Potato: routine use, complicated markers

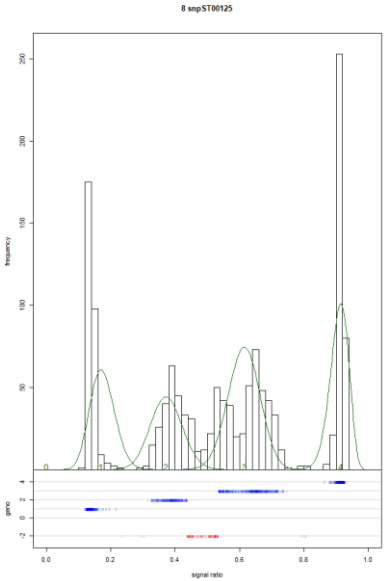


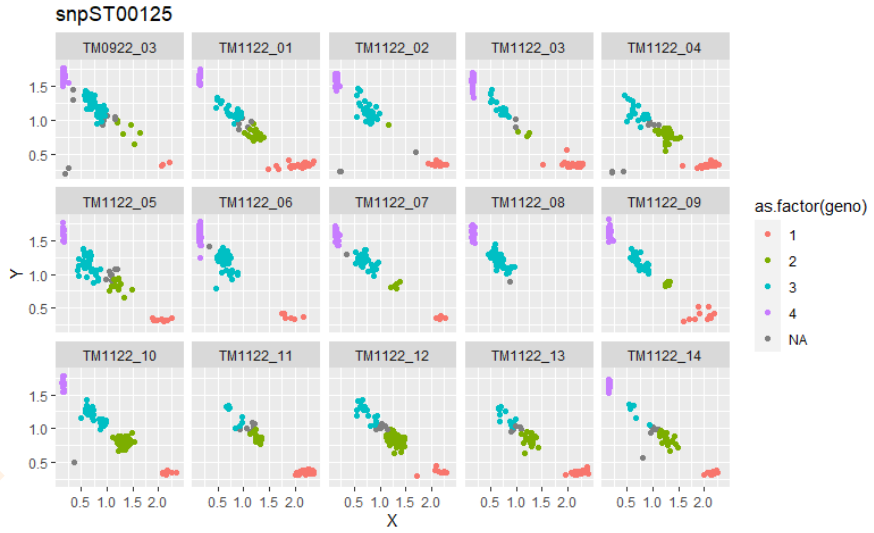


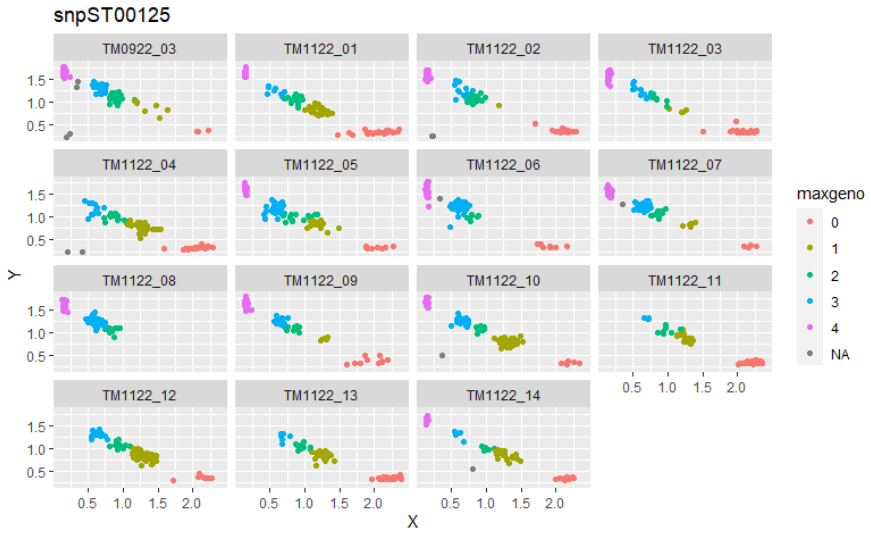


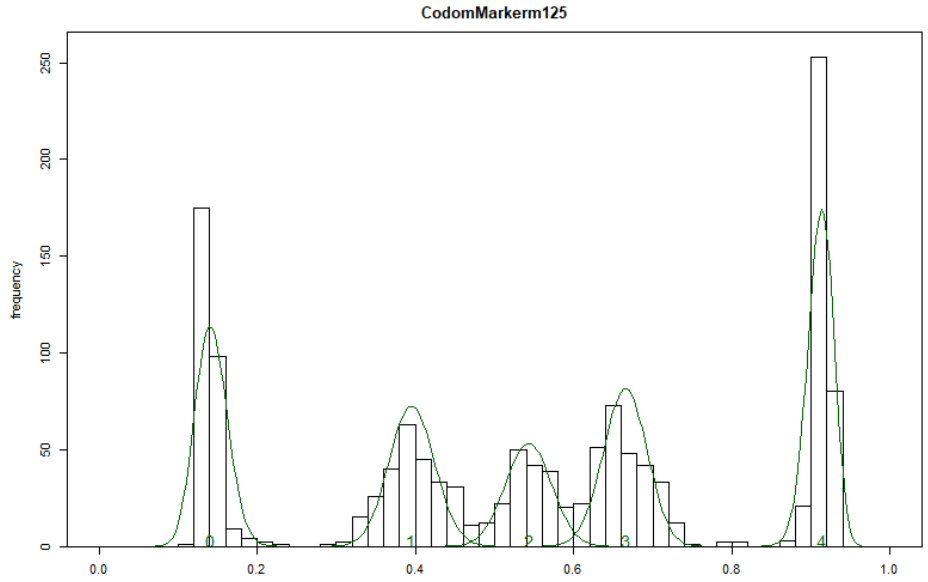
Potato: routine use, correction possible, but tedious and somewhat arbitrary



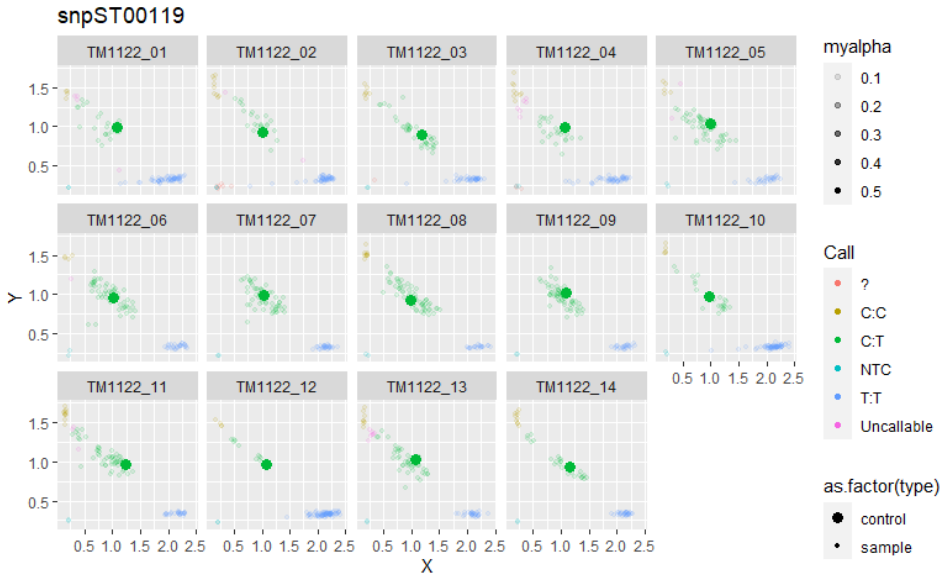


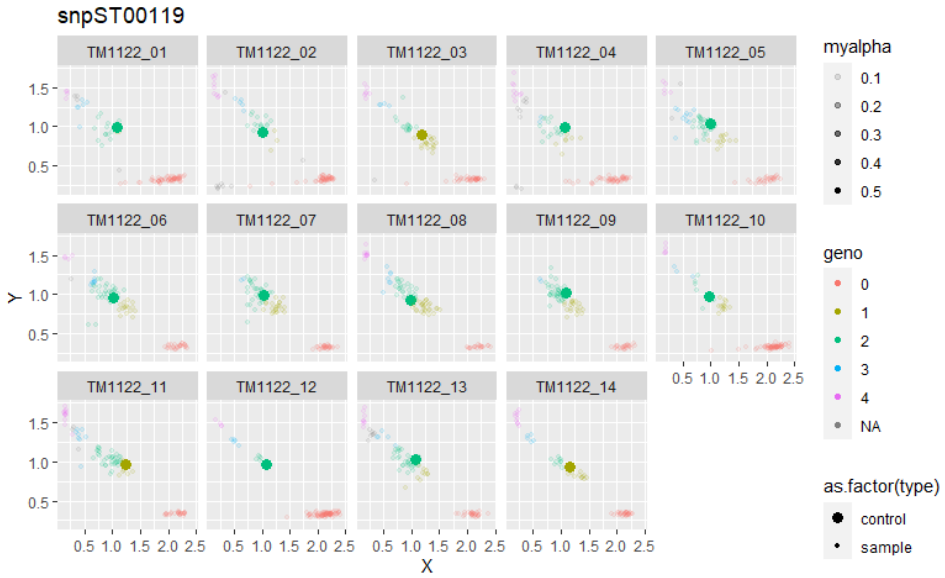


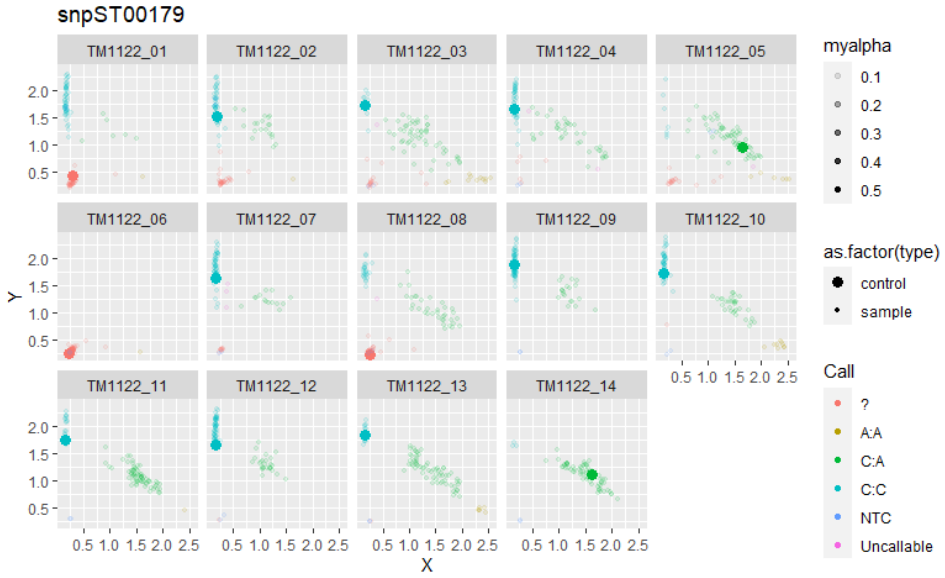


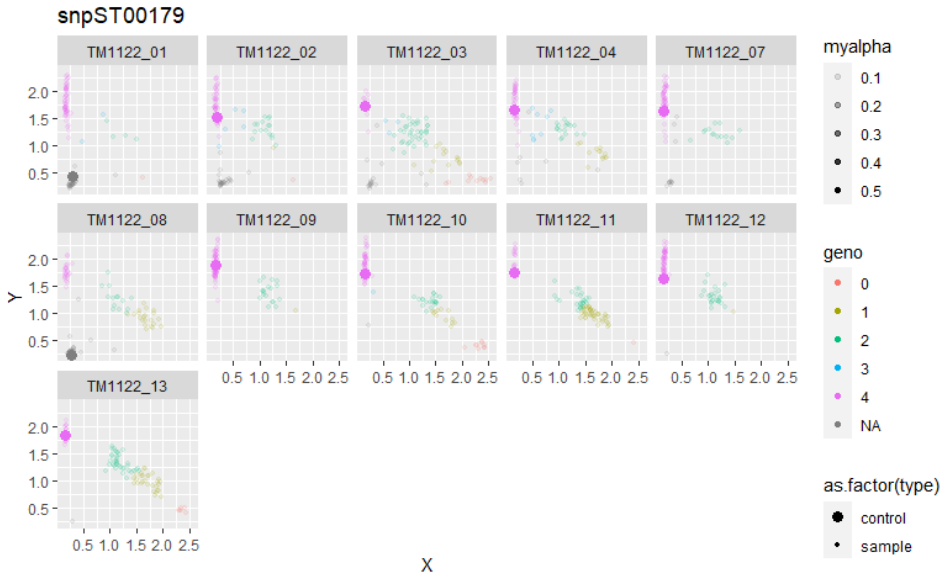


Potato: routine use, using the same control in several plates



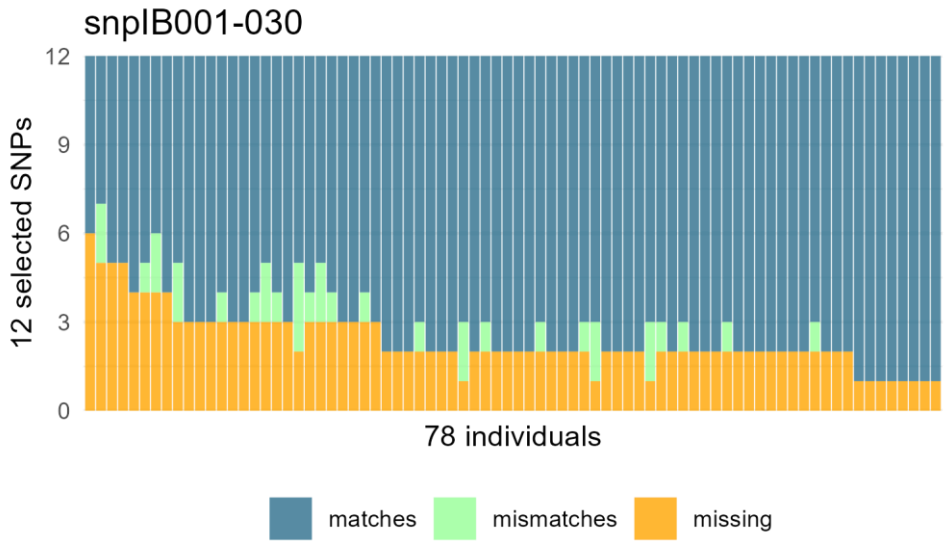




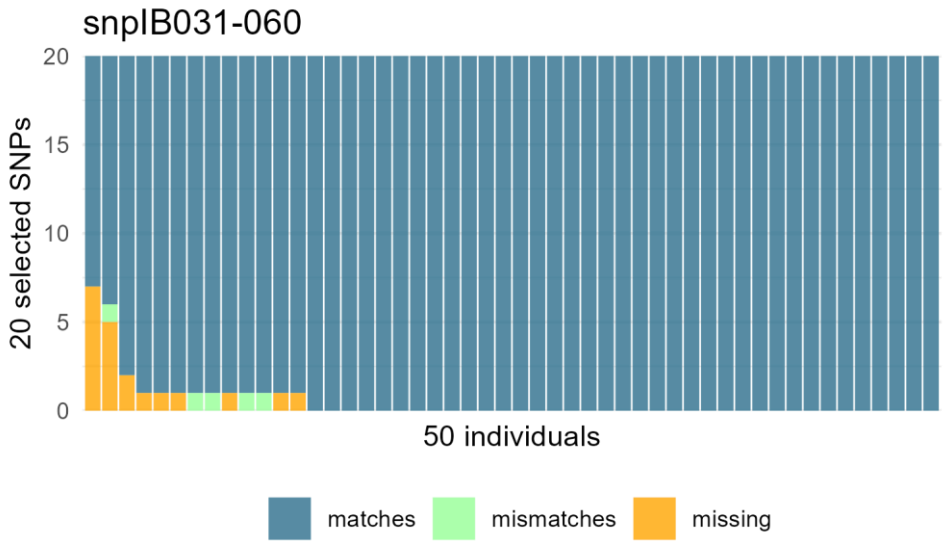


Sweetpotato: verification

- 60 KASP tested: 94 samples
  - ✓ 1st test: snpIB001-030 = 12 ok
    1. Plate 1: silica gel dry + normal buffer
    2. Plate 2: freeze dry + normal buffer
    3. Plate 3: silica gel dry + PVP buffer
    4. Plate 4: freeze dry + PVP buffer

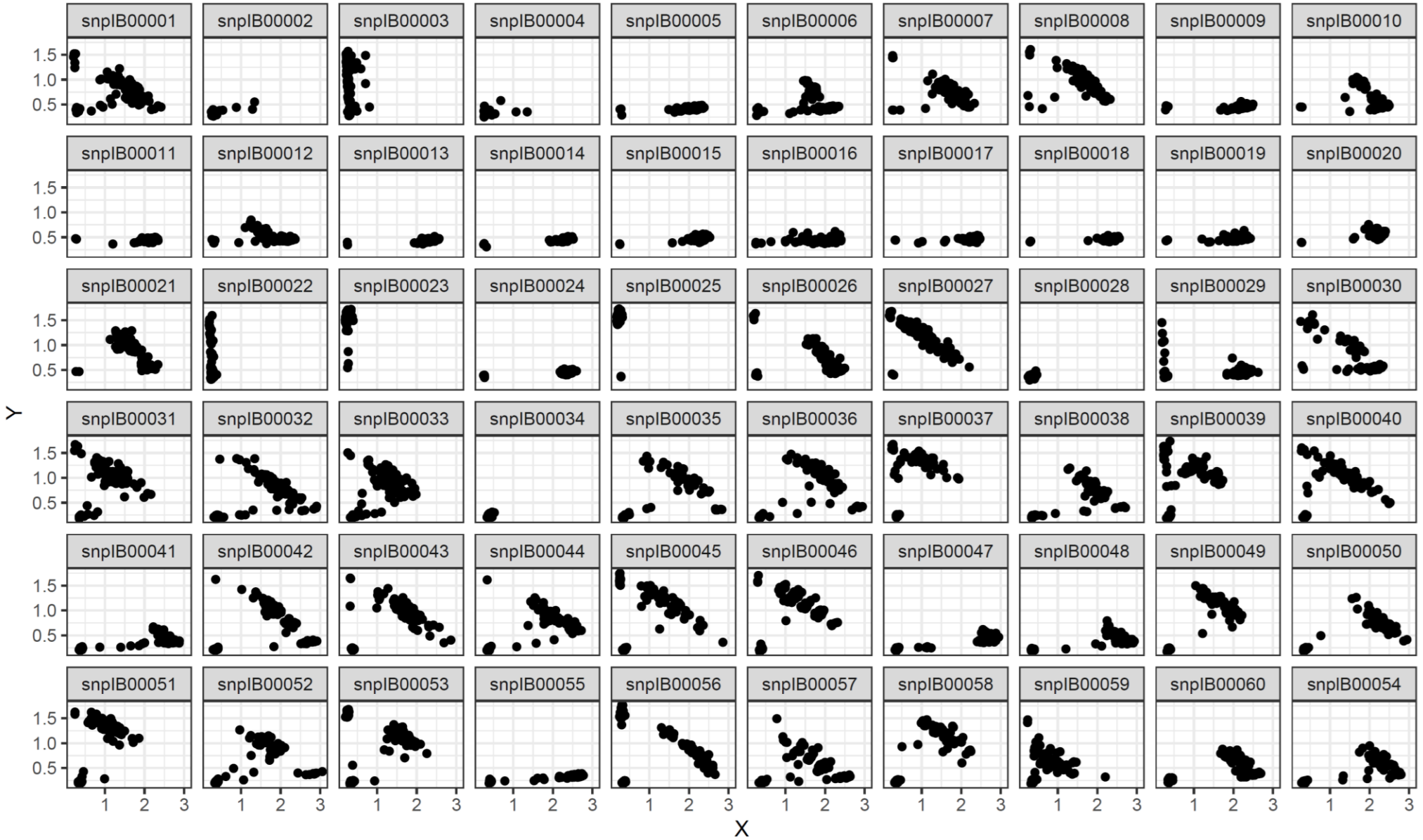


✓ 2nd test: snpIB031-060 = 20 ok
1. Plate 1: DNA dilution 1
2. Plate 2: DNA dilution 2

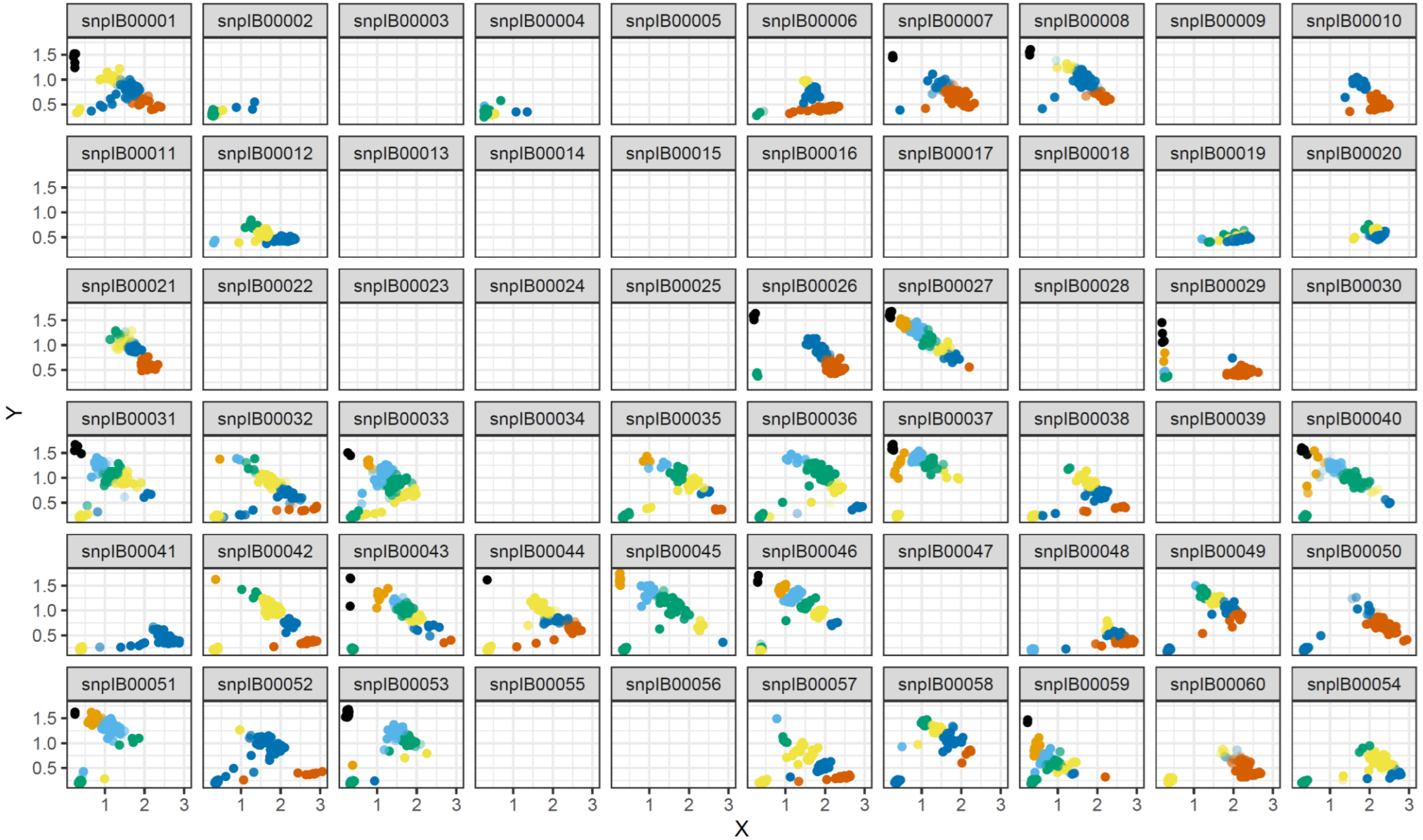


* ok = amplification ok, polymorphism ok

Sweetpotato: verification

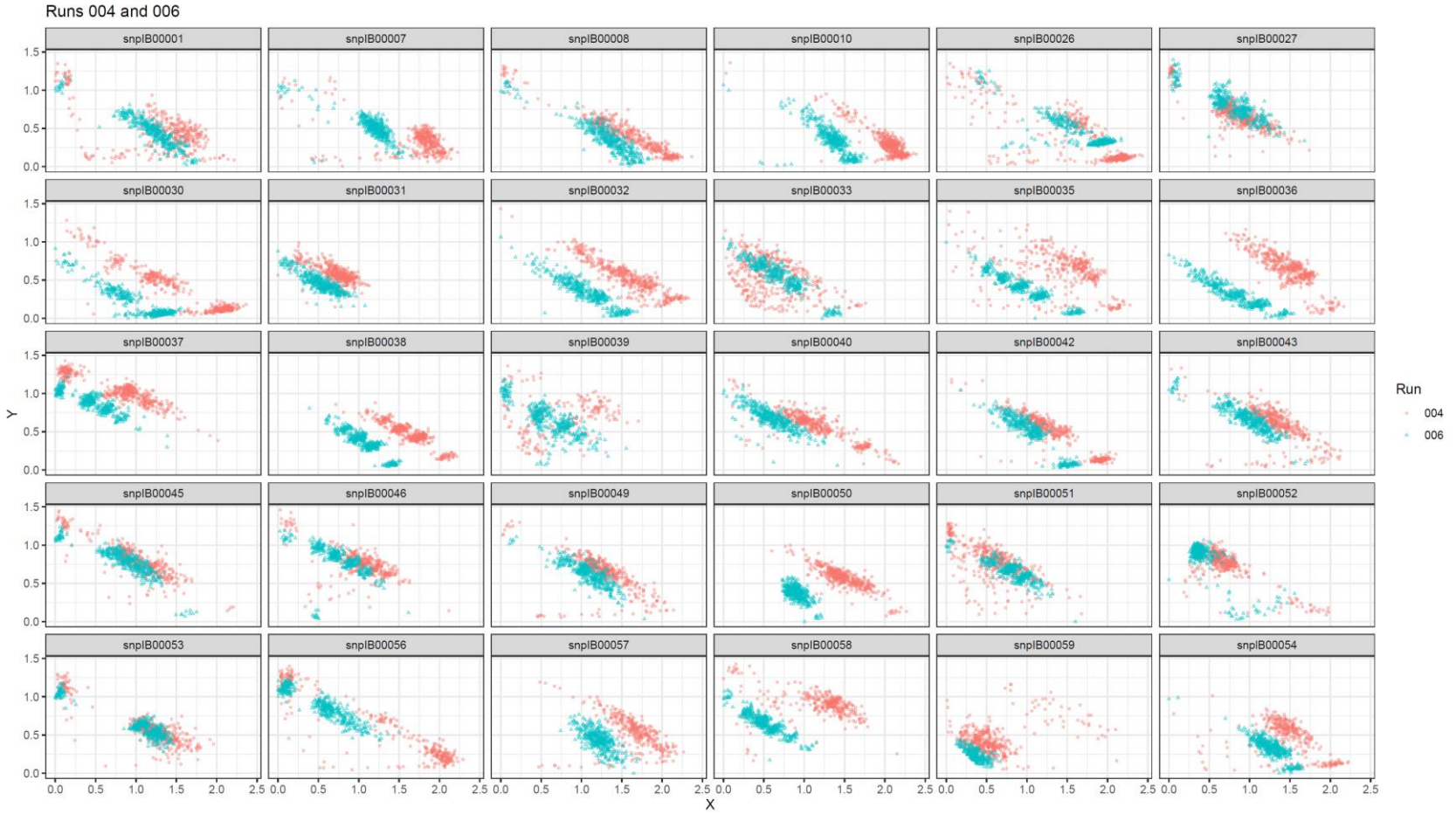


Sweetpotato: verification



Sweetpotato: application

- 30 KASP selected
  - ✓ 1st run: 18 plates from screenhouse
    1. CIP TP = 13 plates (circles)
    2. NaCRRI TP = 5 plates (triangles)
  - ✓ 2nd run: 4 plates from field
    1. CIP TP: 3 plates
    2. NaCRRI TP: 1 plate



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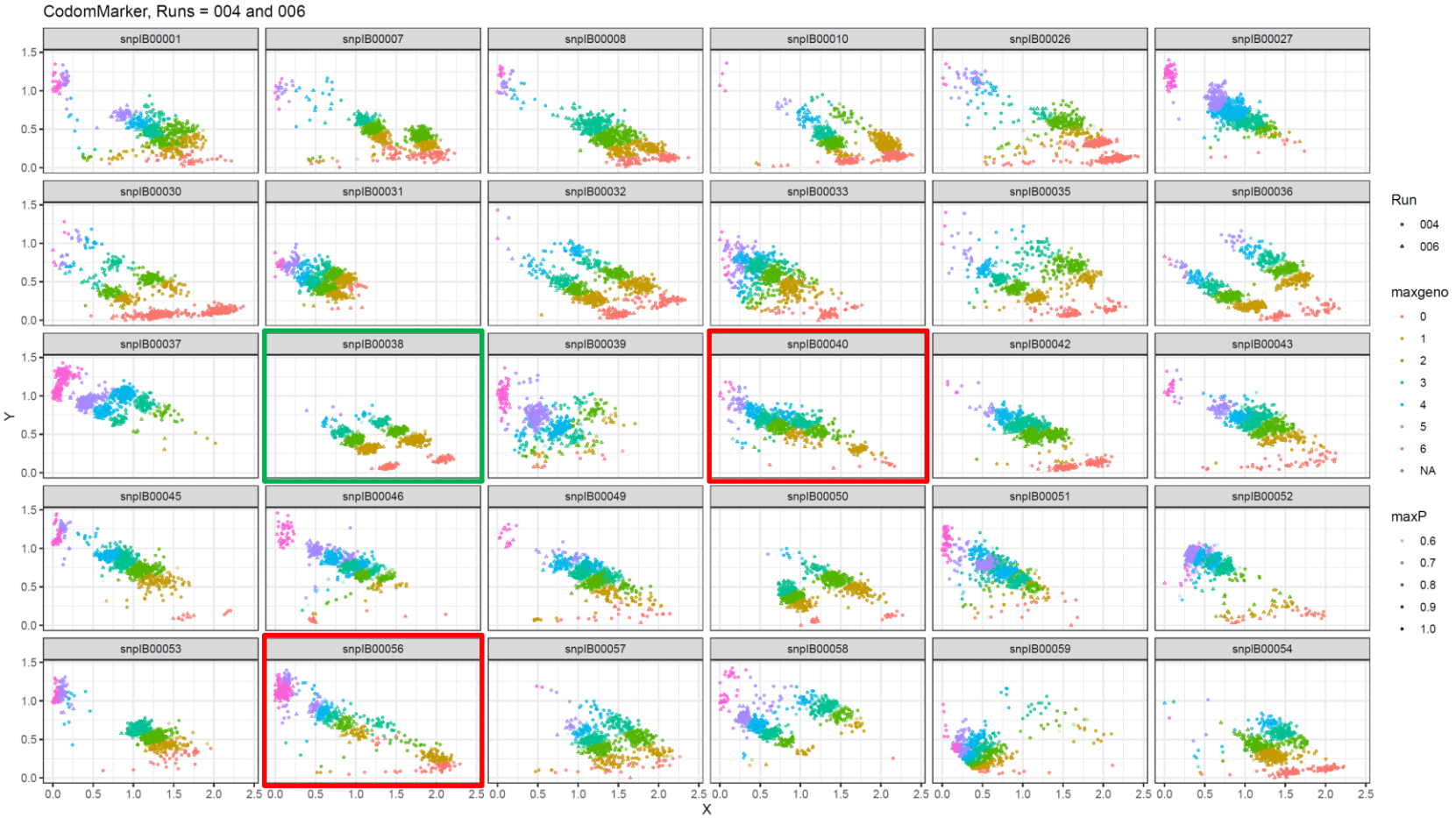
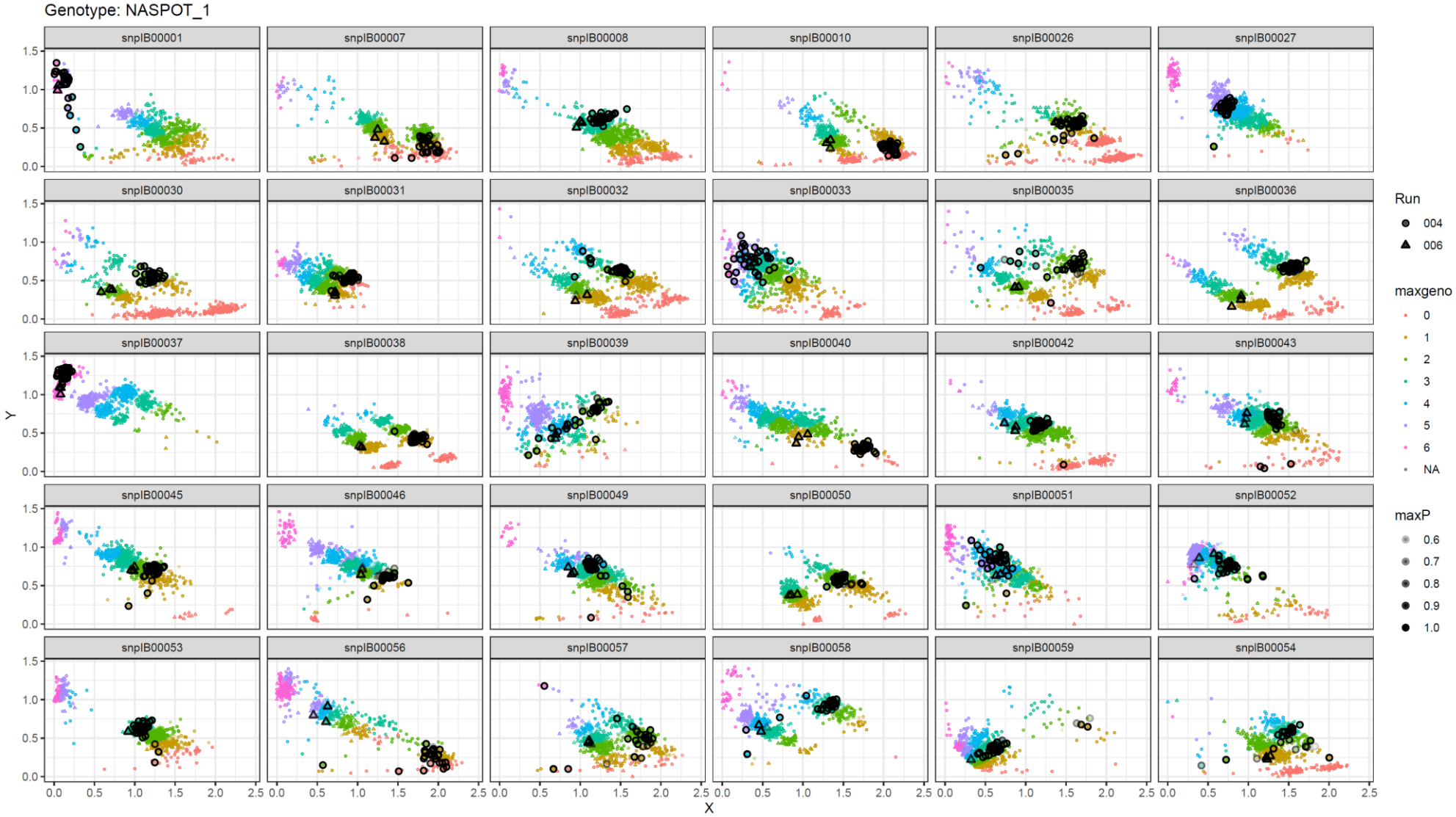


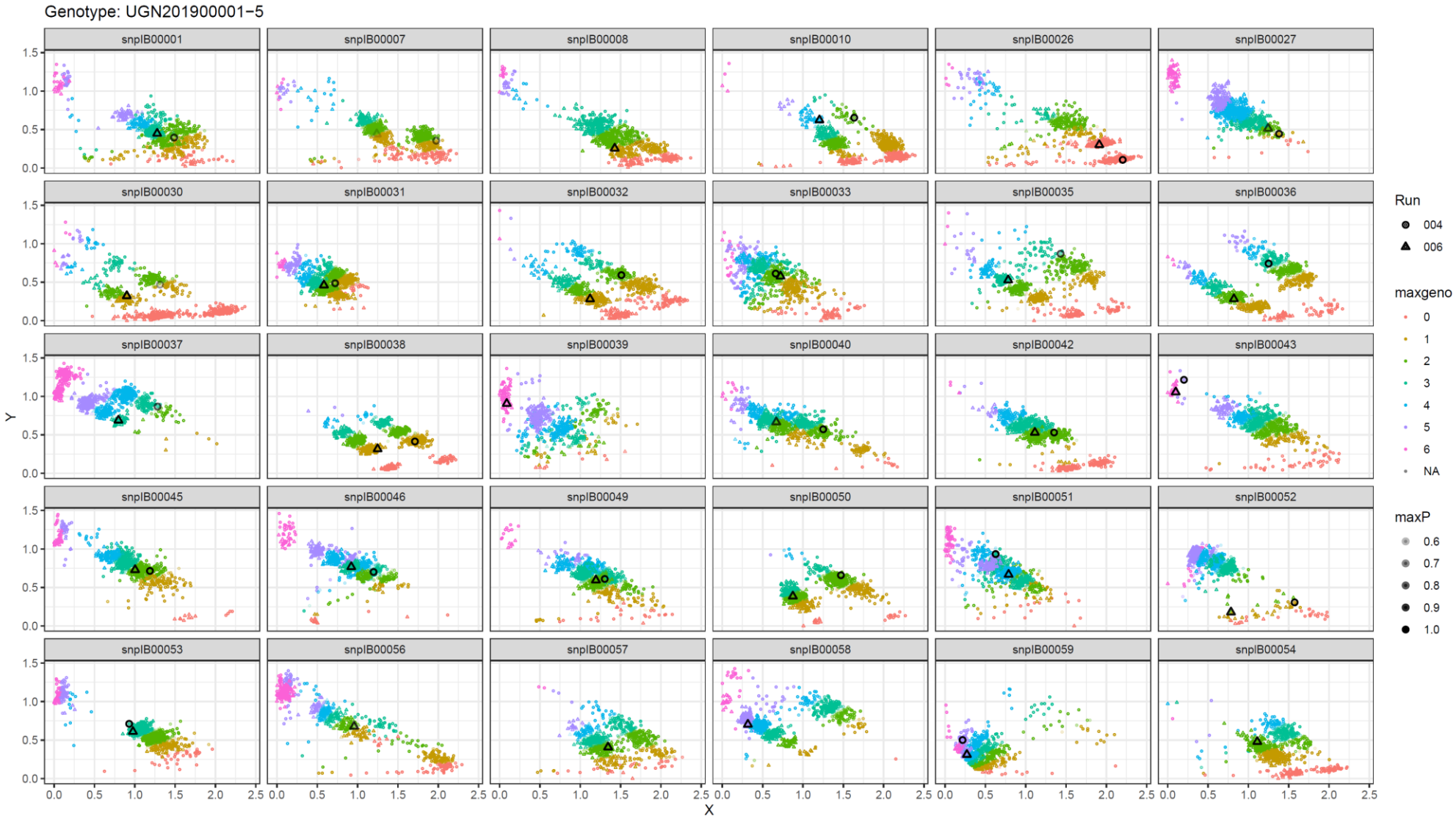
Sweetpotato: application

- 30 KASP selected: e.g. positive control



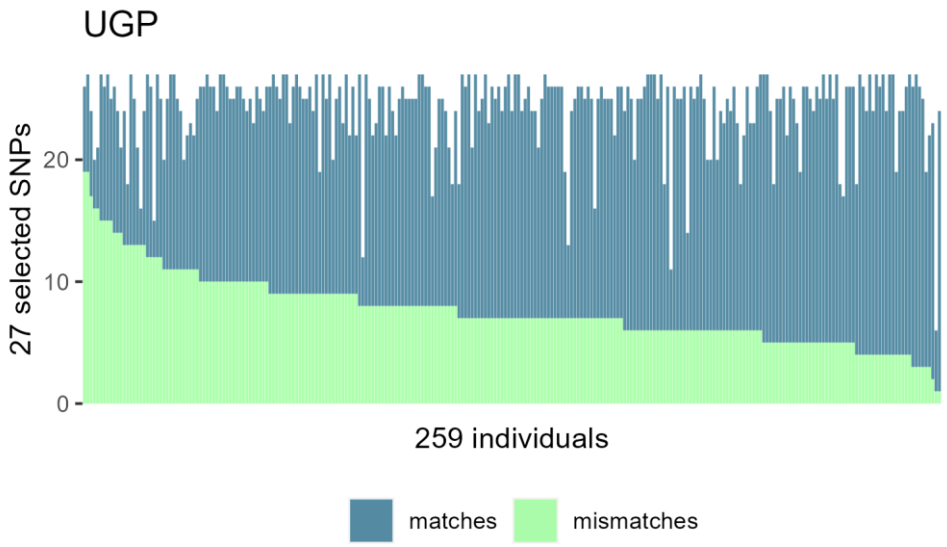
Sweetpotato: application

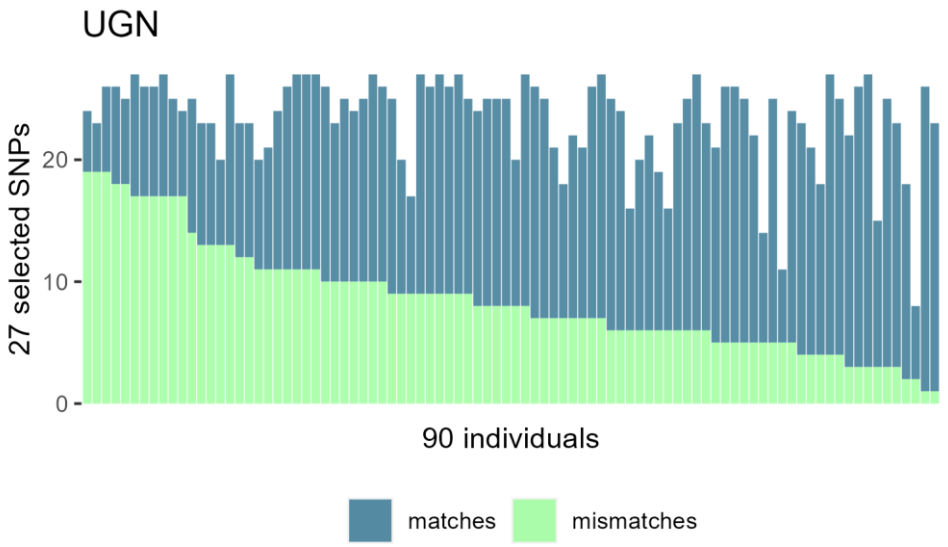
- 30 KASP selected: e.g. random sample



Sweetpotato: application

- 30 KASP selected
  - ✓ 1st run: 18 plates from screenhouse
    1. CIP TP (UGP) = 13 plates
    2. NaCRRI TP (UGN) = 5 plates
  - ✓ 2nd run: 4 plates from field
    1. CIP TP (UGP): 3 plates
    2. NaCRRI TP (UGN): 1 plate





Conclusions

- KASP markers demonstrate significant discriminatory ability, making them suitable for identity analyses for low ploidy crops.
- The control genotype had different calls for the same marker across various plates, emphasizing the need for marker stability.
- Continued efforts are required to identify and select markers that maintain consistent performance over time.
- A strategic effort would be designing a DArTag panels for QC analysis in polyploids
- Given the experience gained with KASP markers, 100-150 (and trait) markers would be enough for QC analysis, and parental verification.