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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA

WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES AND DNA PROFILING IN PARTICULAR

Eleventh Session
Madrid, September 16 to 18, 2008

ADDENDUM

PREPARATION OF GUIDELINE FOR METHOD VALIDATION OF DNA IDENTIFICATION FOR THE ENFORCEMENT OF PLANT BREEDER'S RIGHTS IN JAPAN

Document prepared by experts from Japan

Preparation of Guideline for Method Validation of DNA Identification for the Enforcement of PBRs

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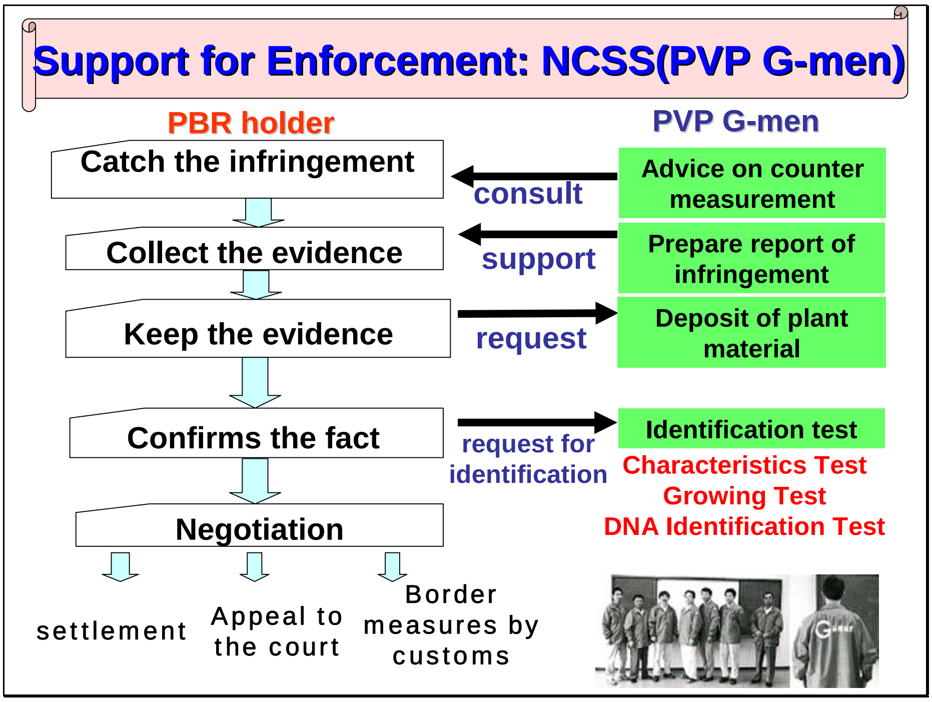
Madrid, September 16-18, 2008

11th Working Group on Biochemical and Molecular Techniques and DNA Profiling in Particular (BMT)

Support for Enforcement: NCSS(PVP G-men)



PBR holder

- Catch the infringement
- Collect the evidence
- Keep the evidence
- Confirms the fact
- Negotiation
  - settlement
  - Appeal to the court
  - Border measures by customs

PVP G-men

- Advice on counter measurement (consult)
- Prepare report of infringement (support)
- Deposit of plant material (request)
- Identification test (request for identification)
  - Characteristics Test
  - Growing Test
  - DNA Identification Test

Variety Identification Services Provided by NCSS

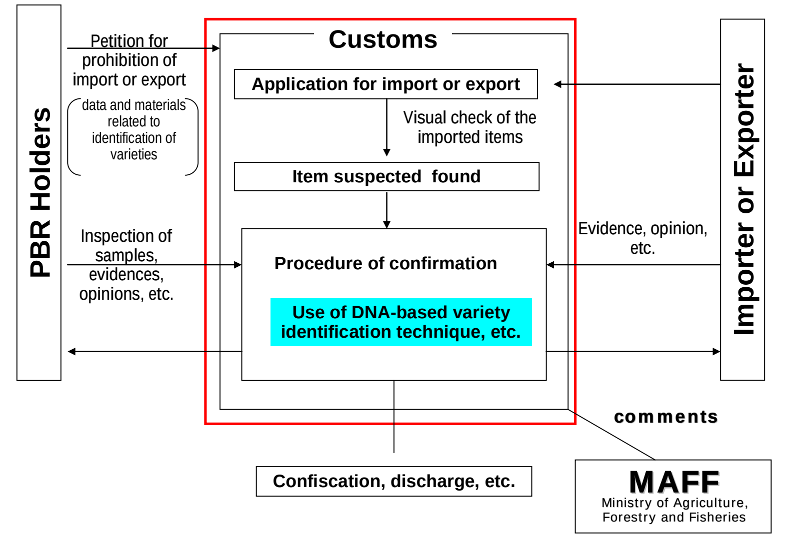
- Characteristics Test

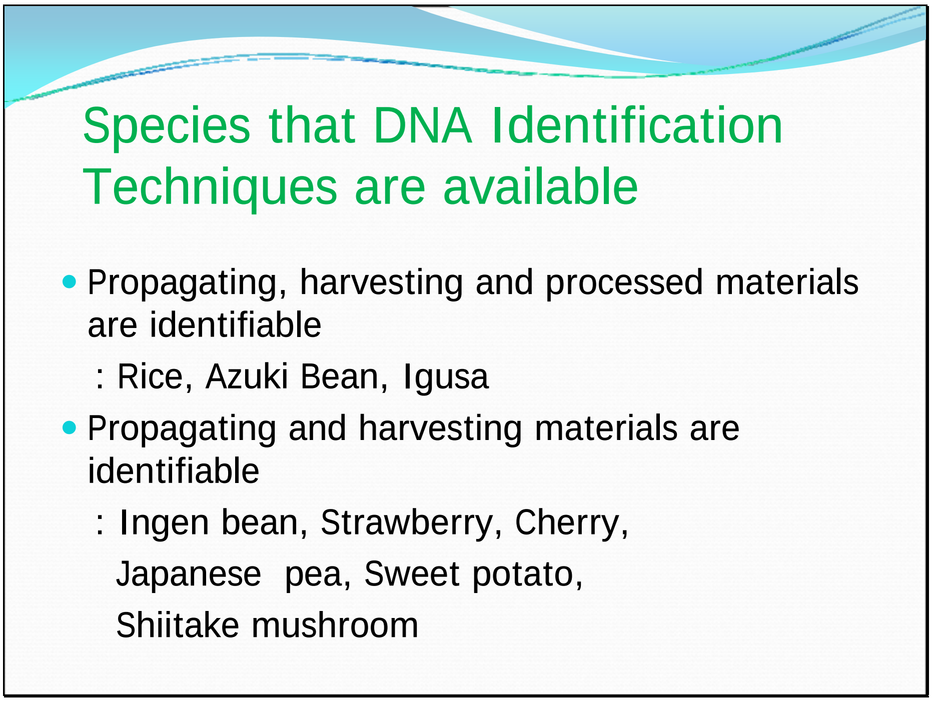
  Result is gained very quickly, but may not correct
- Growing Test

  Result is more precise, but takes longer time
- DNA Identification Test

  Quick and precise

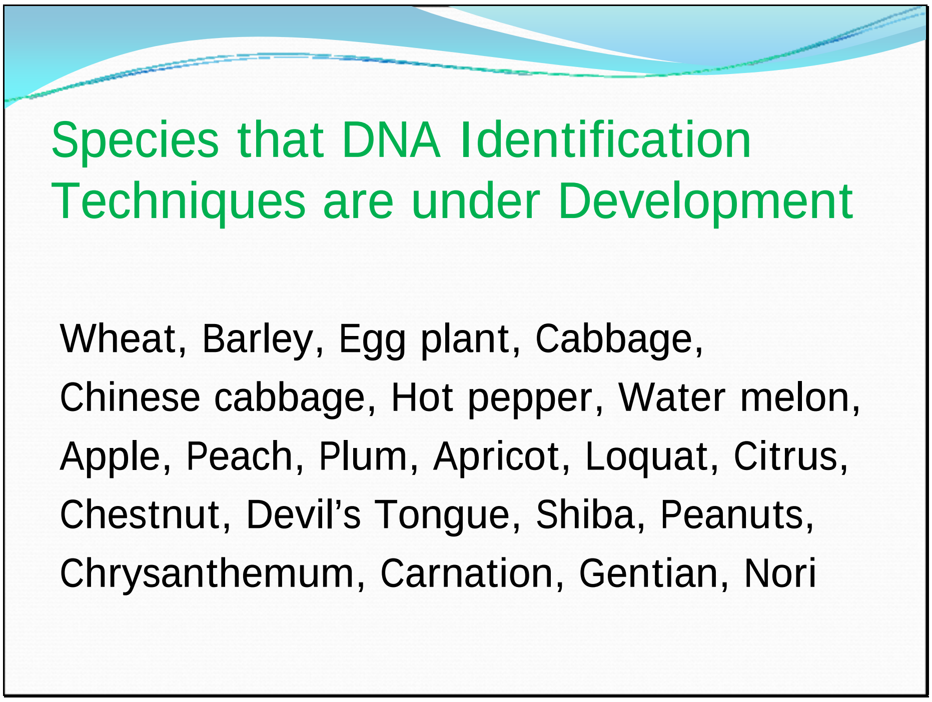
"Border Measures" Control of Items infringing PBR





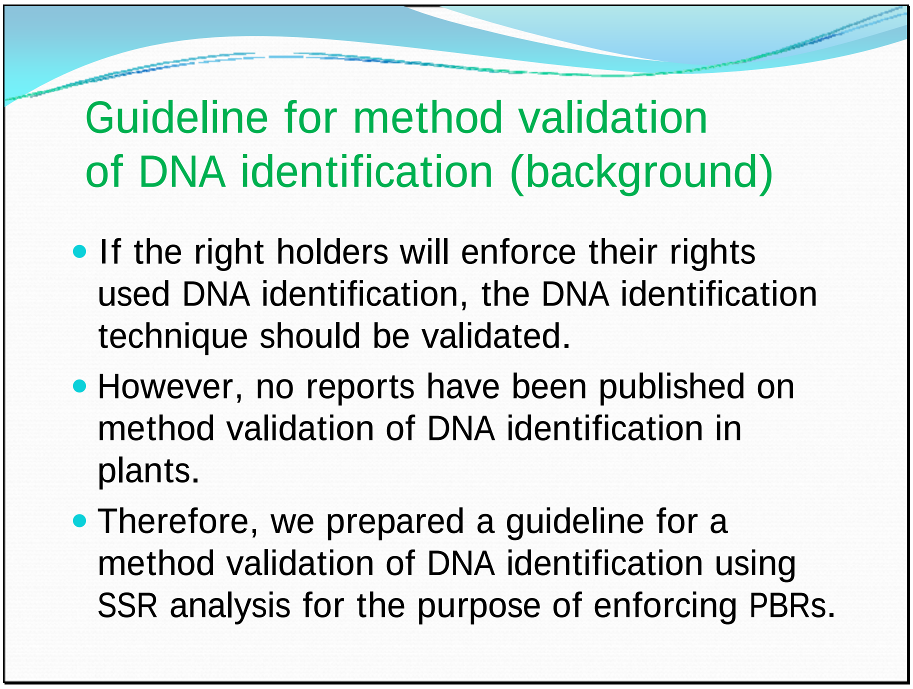
Species that DNA Identification Techniques are available

- Propagating, harvesting and processed materials are identifiable
  : Rice, Azuki Bean, Igusa
- Propagating and harvesting materials are identifiable
  : Ingen bean, Strawberry, Cherry,
  Japanese pea, Sweet potato,
  Shiitake mushroom



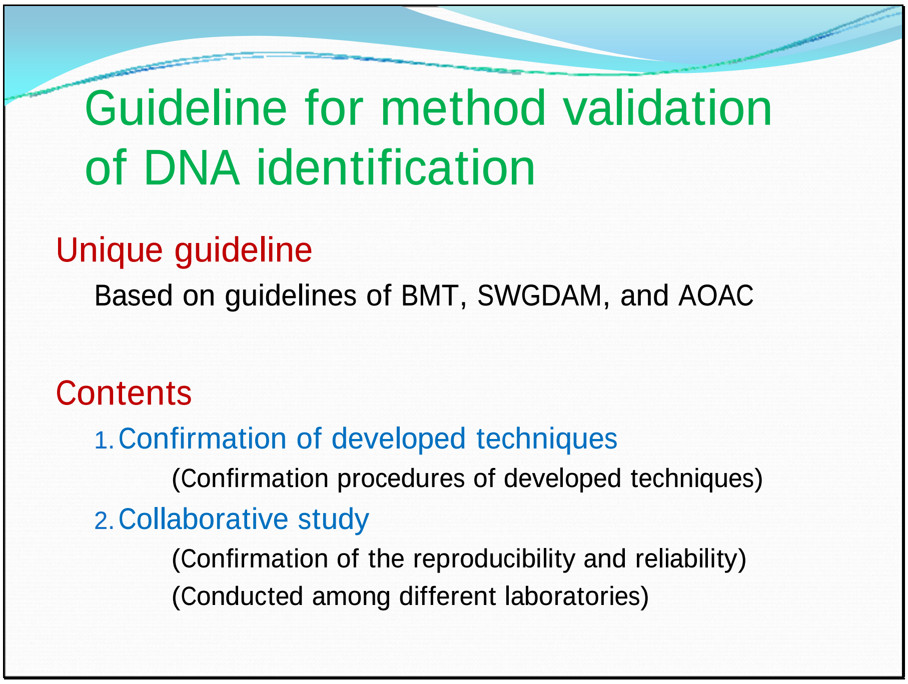
Species that DNA Identification Techniques are under Development

Wheat, Barley, Egg plant, Cabbage,
Chinese cabbage, Hot pepper, Water melon,
Apple, Peach, Plum, Apricot, Loquat, Citrus,
Chestnut, Devil’s Tongue, Shiba, Peanuts,
Chrysanthemum, Carnation, Gentian, Nori



Guideline for method validation of DNA identification (background)

- If the right holders will enforce their rights used DNA identification, the DNA identification technique should be validated.
- However, no reports have been published on method validation of DNA identification in plants.
- Therefore, we prepared a guideline for a method validation of DNA identification using SSR analysis for the purpose of enforcing PBRs.



Guideline for method validation of DNA identification

Unique guideline

Based on guidelines of BMT, SWGDAM, and AOAC

Contents

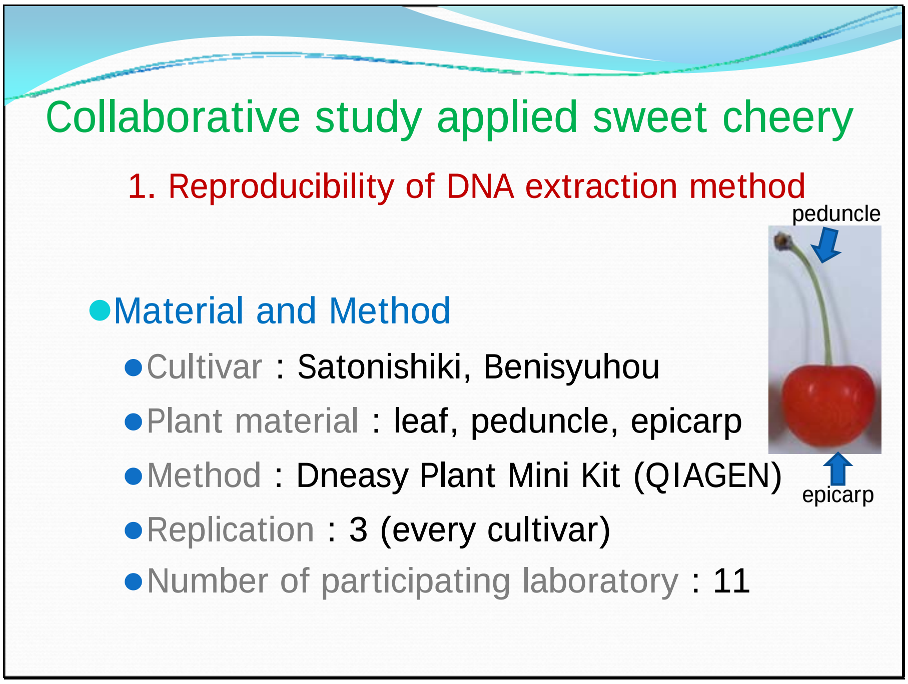
1. Confirmation of developed techniques
    (Confirmation procedures of developed techniques)
2. Collaborative study
    (Confirmation of the reproducibility and reliability)
    (Conducted among different laboratories)

Validation of DNA identification method for sweet cherries

- DNA identification of sweet cherry (*Prunus avium* L.) has published in MAFF’s home page (http://www.hinsyu.maff.go.jp/).
- We conducted method validation of DNA identification of sweet cherry according to the guideline.

Confirmation of developed techniques applied sweet cheery

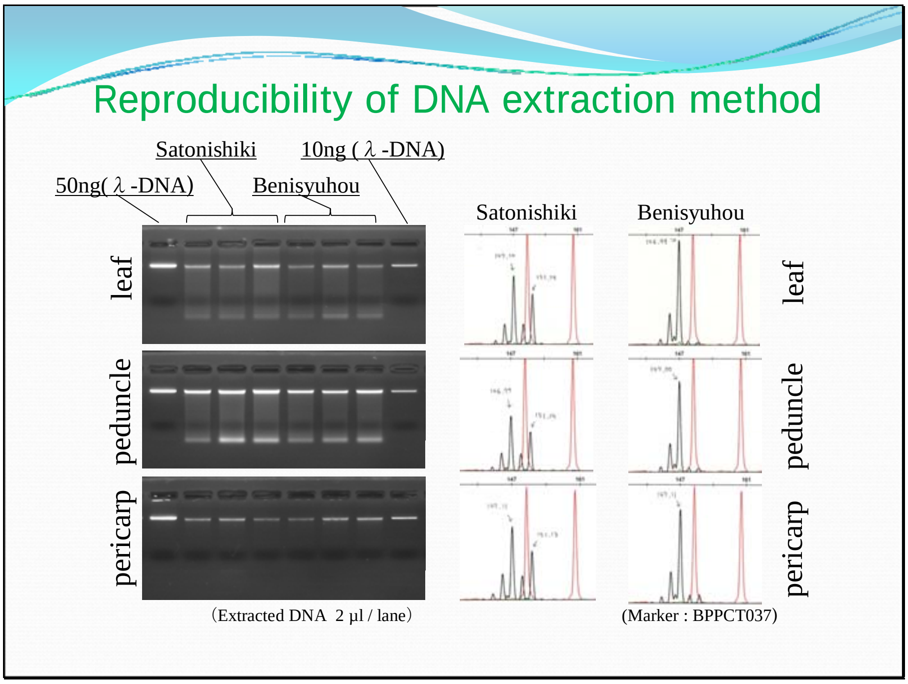
- Information of subject plant
  - Plant materials used for analysis
  - Selection of varieties for reference alleles
- SSR marker
  - Selection of SSR markers
  - Confirmation of null allele
- DNA extraction
  - Tissues used for extraction
  - Condition of samples for DNA extraction
- PCR condition
- Peer-reviewed publication



Collaborative study applied sweet cheery

1. Reproducibility of DNA extraction method

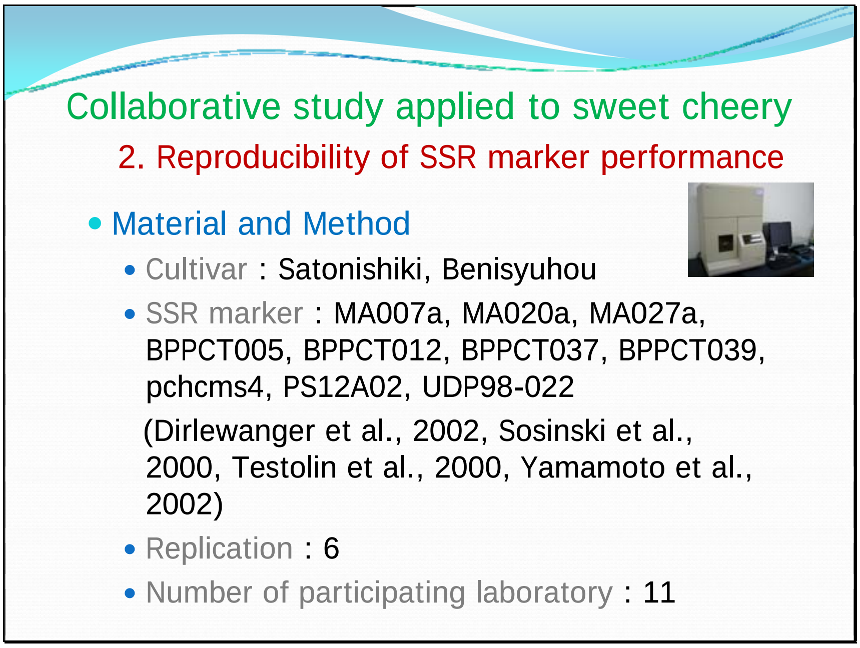
- Material and Method
  - Cultivar : Satonishiki, Benisyuhou
  - Plant material : leaf, peduncle, epicarp
  - Method : Dneasy Plant Mini Kit (QIAGEN)
  - Replication : 3 (every cultivar)
  - Number of participating laboratory : 11



Reproducibility of DNA extraction method

（Extracted DNA 2 µl / lane）

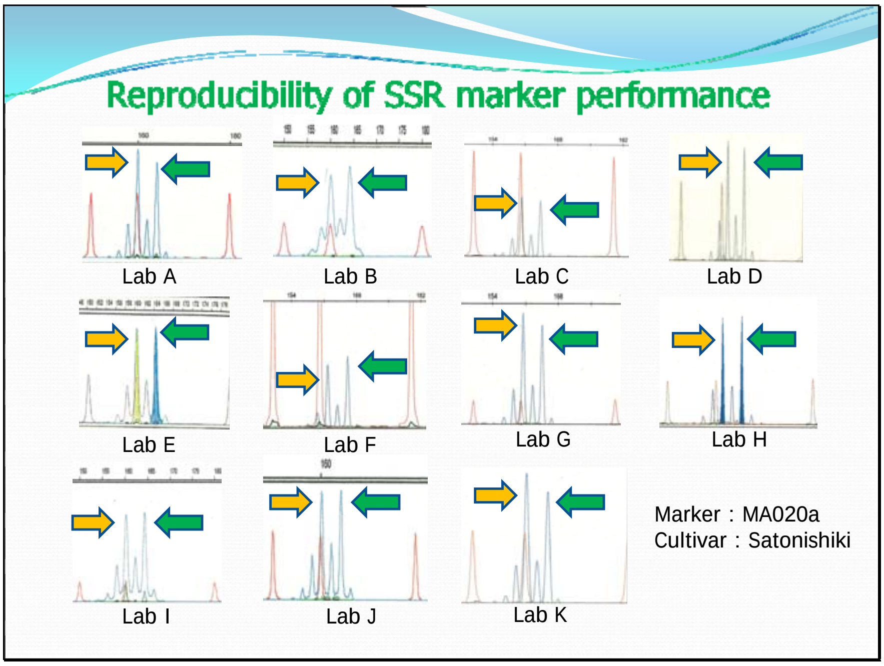
(Marker : BPPCT037)



Collaborative study applied to sweet cheery

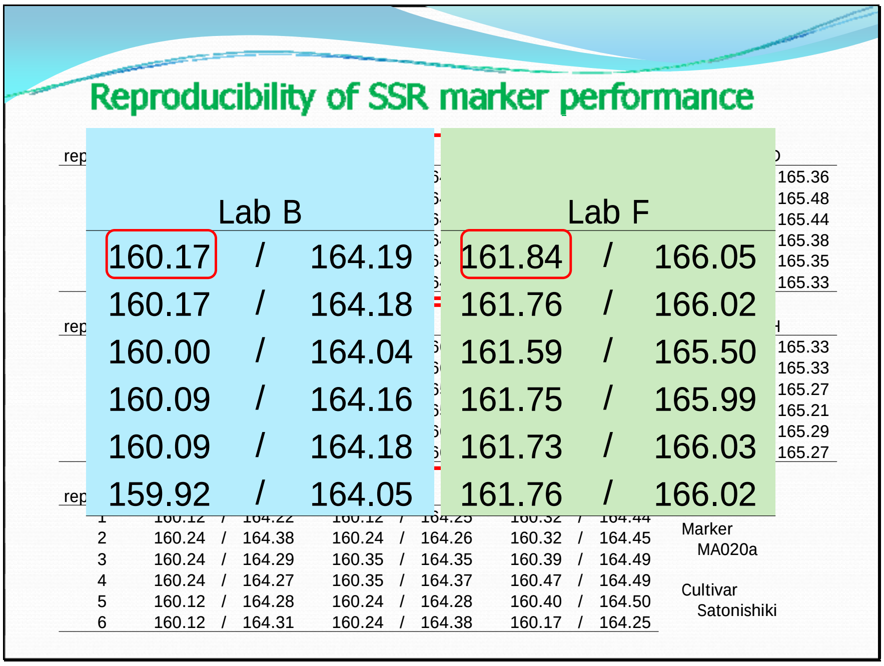
2. Reproducibility of SSR marker performance

- Material and Method
  - Cultivar : Satonishiki, Benisyuhou
  - SSR marker : MA007a, MA020a, MA027a, BPPCT005, BPPCT012, BPPCT037, BPPCT039, pchcms4, PS12A02, UDP98-022
    (Dirlewanger et al., 2002, Sosinski et al., 2000, Testolin et al., 2000, Yamamoto et al., 2002)
  - Replication : 6
  - Number of participating laboratory : 11



Reproducibility of SSR marker performance

Marker : MA020a
Cultivar : Satonishiki



Reproducibility of SSR marker performance

Lab B			Lab F		
160.17	/	164.19	161.84	/	166.05
160.17	/	164.18	161.76	/	166.02
160.00	/	164.04	161.59	/	165.50
160.09	/	164.16	161.75	/	165.99
160.09	/	164.18	161.73	/	166.03
159.92	/	164.05	161.76	/	166.02

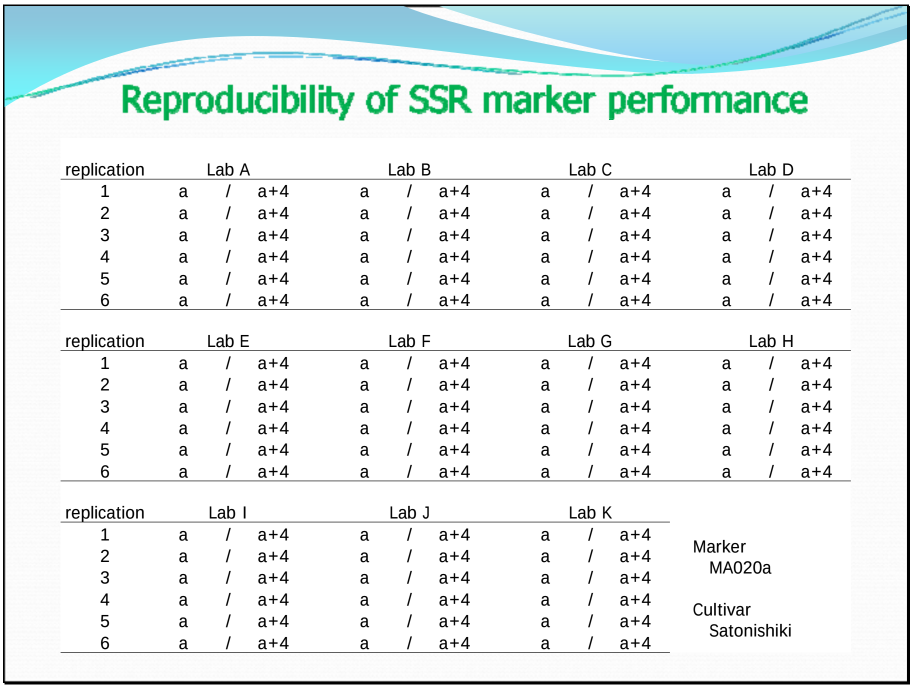
165.36
165.48
165.44
165.38
165.35
165.33

165.33
165.33
165.27
165.21
165.29
165.27

1	160.12	/	164.22	160.12	/	164.25	160.32	/	164.44
2	160.24	/	164.38	160.24	/	164.26	160.32	/	164.45
3	160.24	/	164.29	160.35	/	164.35	160.39	/	164.49
4	160.24	/	164.27	160.35	/	164.37	160.47	/	164.49
5	160.12	/	164.28	160.24	/	164.28	160.40	/	164.50
6	160.12	/	164.31	160.24	/	164.38	160.17	/	164.25

Marker
MA020a

Cultivar
Satonishiki



Reproducibility of SSR marker performance

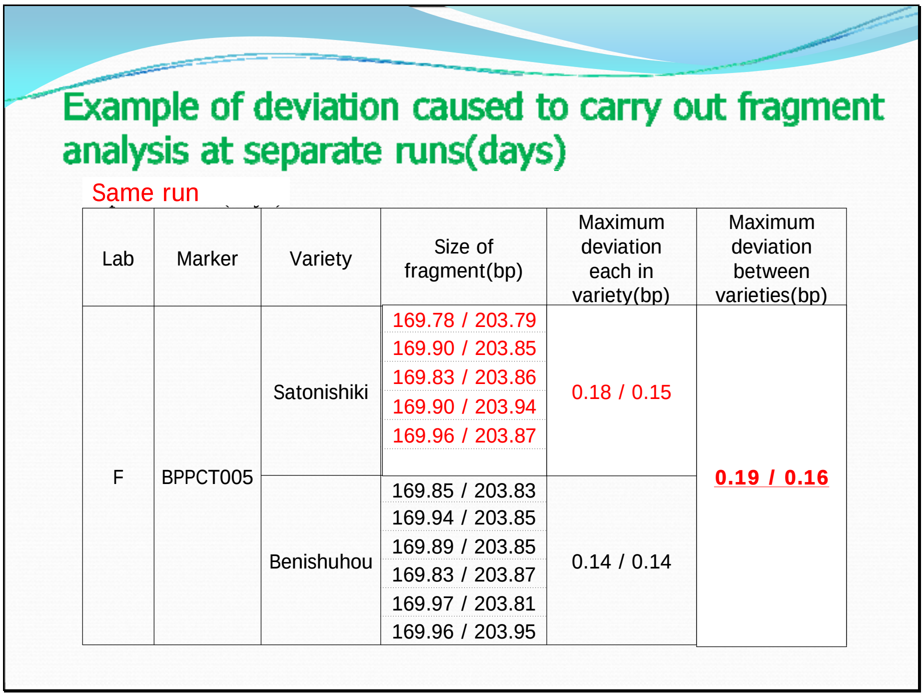
replication	Lab A			Lab B			Lab C			Lab D		
1	a	/	a+4	a	/	a+4	a	/	a+4	a	/	a+4
2	a	/	a+4	a	/	a+4	a	/	a+4	a	/	a+4
3	a	/	a+4	a	/	a+4	a	/	a+4	a	/	a+4
4	a	/	a+4	a	/	a+4	a	/	a+4	a	/	a+4
5	a	/	a+4	a	/	a+4	a	/	a+4	a	/	a+4
6	a	/	a+4	a	/	a+4	a	/	a+4	a	/	a+4

replication	Lab E			Lab F			Lab G			Lab H		
1	a	/	a+4	a	/	a+4	a	/	a+4	a	/	a+4
2	a	/	a+4	a	/	a+4	a	/	a+4	a	/	a+4
3	a	/	a+4	a	/	a+4	a	/	a+4	a	/	a+4
4	a	/	a+4	a	/	a+4	a	/	a+4	a	/	a+4
5	a	/	a+4	a	/	a+4	a	/	a+4	a	/	a+4
6	a	/	a+4	a	/	a+4	a	/	a+4	a	/	a+4

replication	Lab I			Lab J			Lab K		
1	a	/	a+4	a	/	a+4	a	/	a+4
2	a	/	a+4	a	/	a+4	a	/	a+4
3	a	/	a+4	a	/	a+4	a	/	a+4
4	a	/	a+4	a	/	a+4	a	/	a+4
5	a	/	a+4	a	/	a+4	a	/	a+4
6	a	/	a+4	a	/	a+4	a	/	a+4

Marker
MA020a

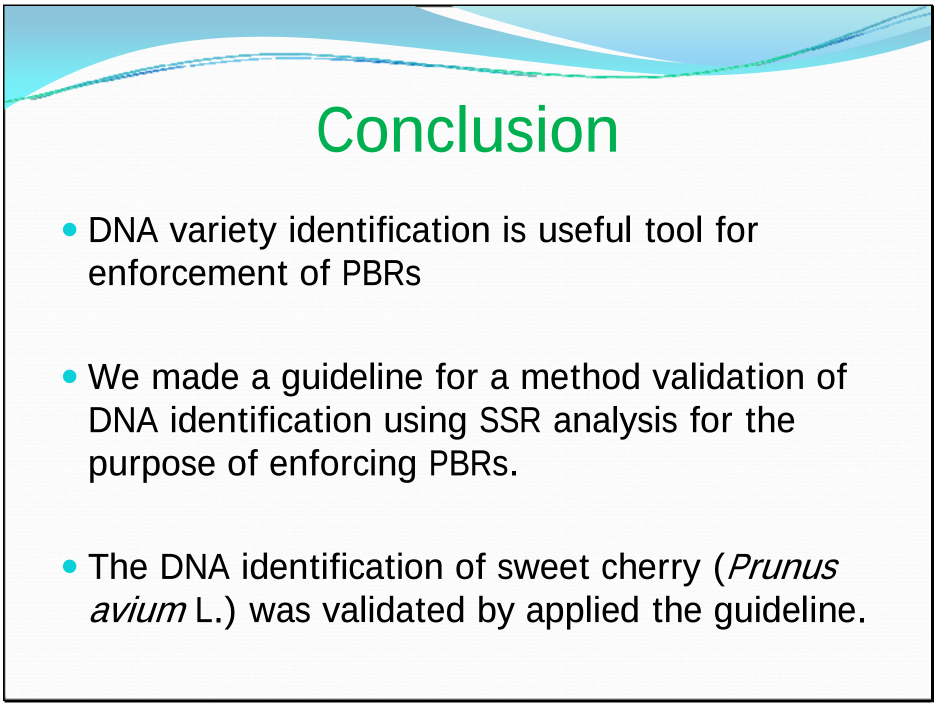
Cultivar
Satonishiki



Example of deviation caused to carry out fragment analysis at separate runs(days)

Same run

Lab	Marker	Variety	Size of fragment(bp)	Maximum deviation each in variety(bp)	Maximum deviation between varieties(bp)
F	BPPCT005	Satonishiki	169.78 / 203.79	0.18 / 0.15	**0.19 / 0.16**
			169.90 / 203.85		
			169.83 / 203.86		
			169.90 / 203.94		
			169.96 / 203.87		
		Benishuhou	169.85 / 203.83	0.14 / 0.14	
			169.94 / 203.85		
			169.89 / 203.85		
			169.83 / 203.87		
			169.97 / 203.81		
			169.96 / 203.95		



Conclusion

- DNA variety identification is useful tool for enforcement of PBRs
- We made a guideline for a method validation of DNA identification using SSR analysis for the purpose of enforcing PBRs.
- The DNA identification of sweet cherry (*Prunus avium* L.) was validated by applied the guideline.

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