



Kazusa DNA Res. Inst.

# Large-Scale Genotyping in Strawberry Genomic Selection

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2022/06/22

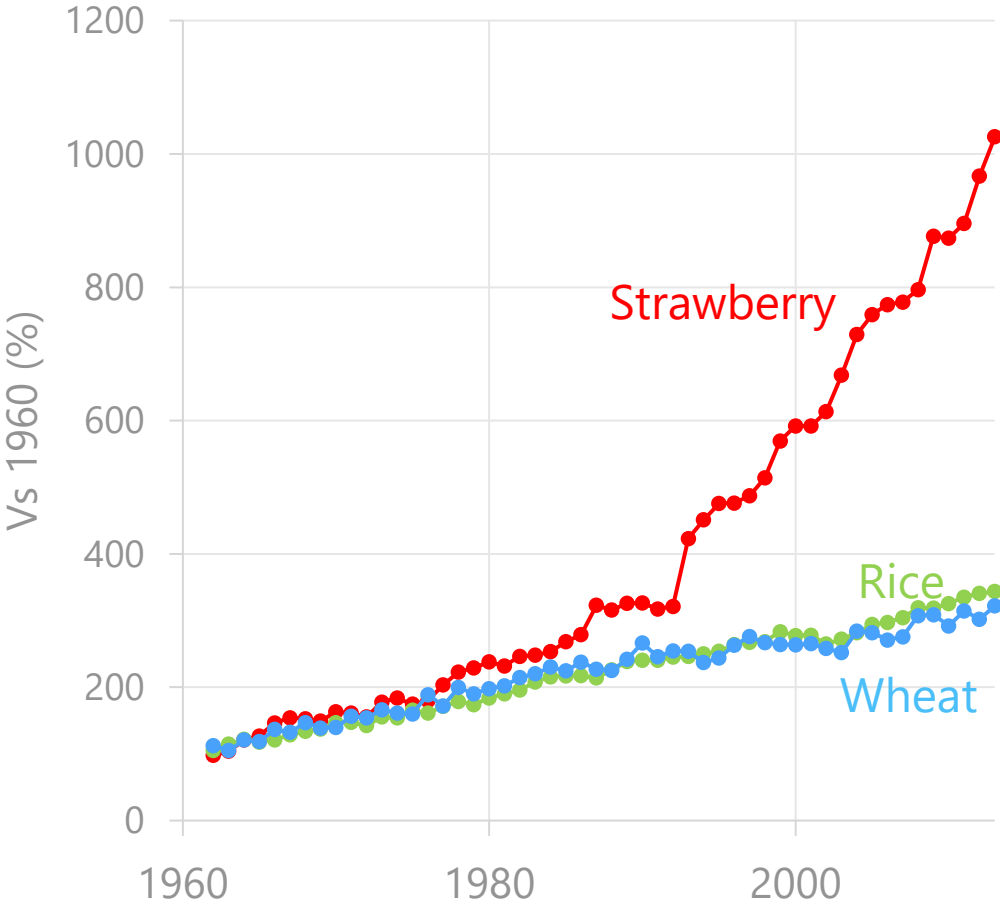
Sachiko Isobe and Kenta Shirasawa

Lab of Plant Genetics and Genomics

# Strawberry

More than 30 strawberry breeding station in Japan

World production vs 1960(%)



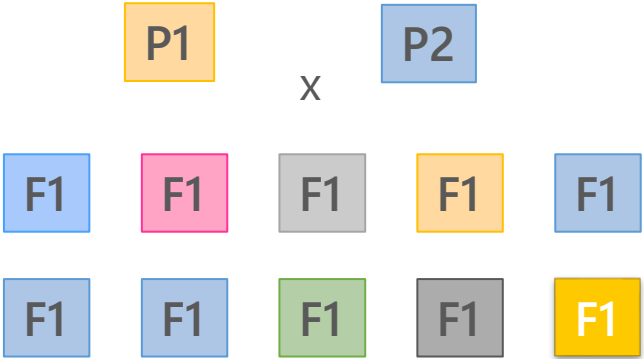
# Conventional Breeding strategy



Introduced from Europe to Japan in the late 19 century

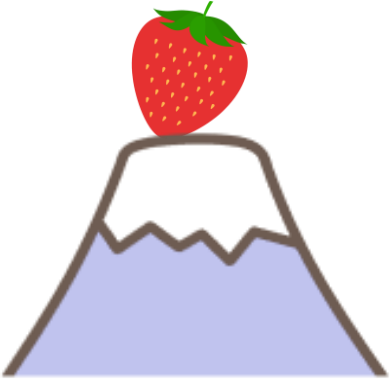
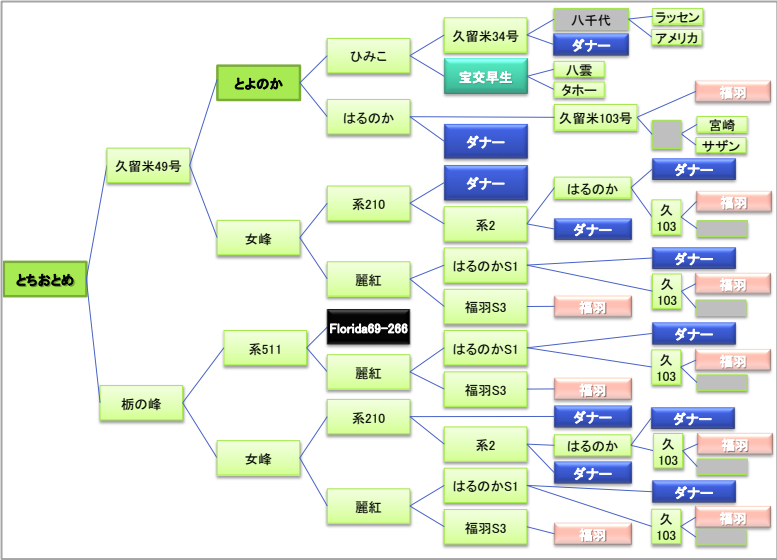
Less Genetic Diversity

## Breeding Strategy



- Clonal propagation
- Trait investigation in multiple years/locations
- Registration

cv. 'Tochiotome' Pedigree Tree ~ 1889 NIVTS, Dr. Noguchi



# "Climb Every Mountain....Till you find your dream"

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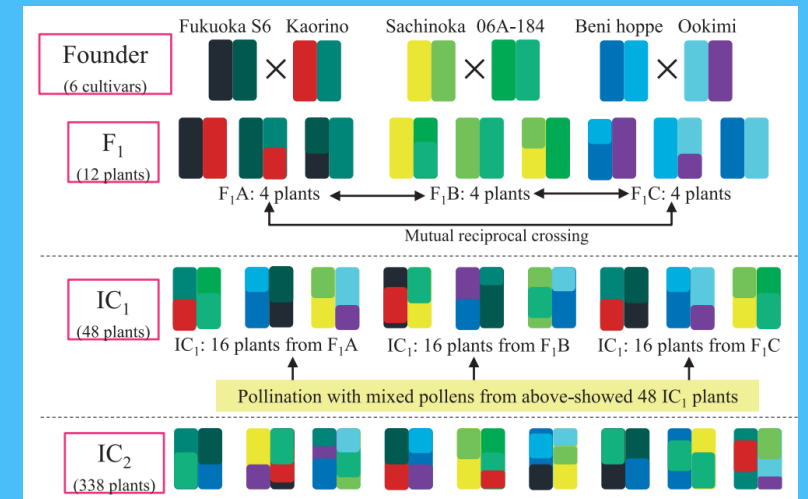
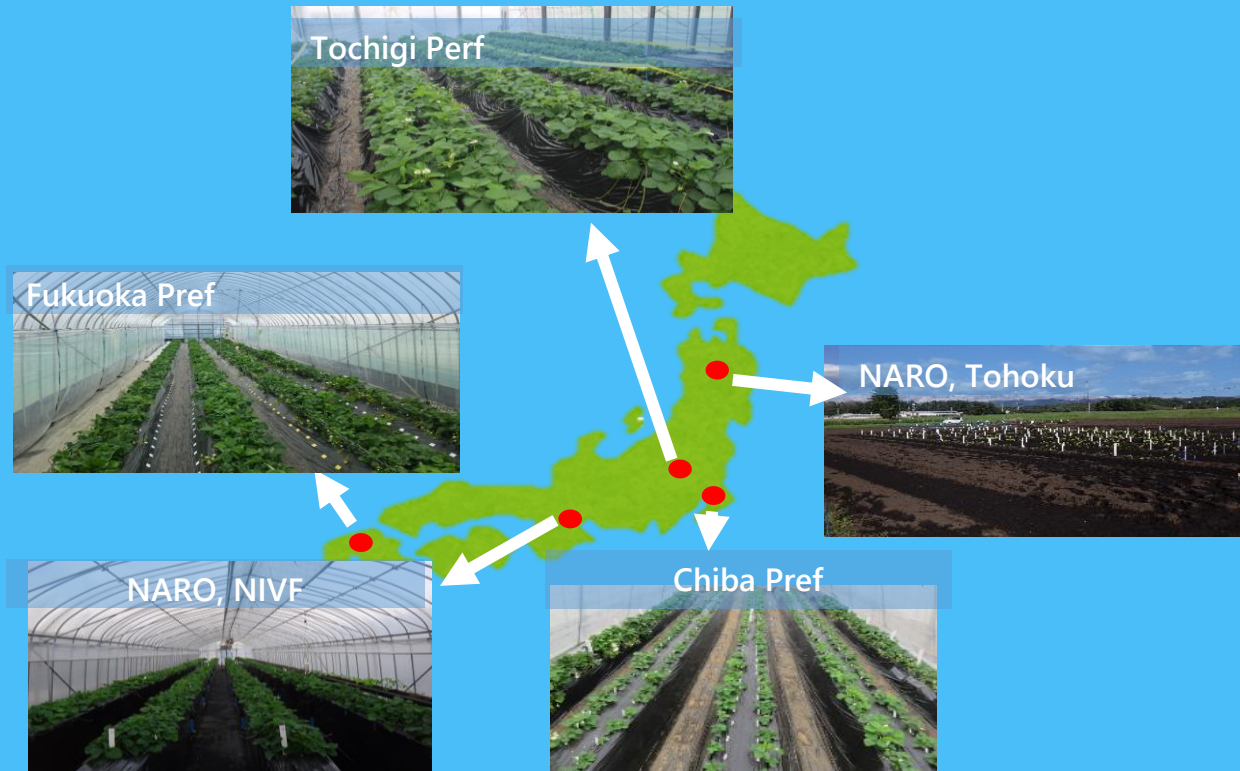
-The Sounds of Music-



It is hard without no guide.

# Strawberry Genomic Selection Project Since 2015

Use populations, which genomes were shuffled (ex. MAGIC population)



The five Breeding Stations in Japan

Breeding Science Preview  
doi:10.1270/jsbbs.17009

This article is an Advance Online Publication of the authors' corrected proof.  
Note that minor changes may be made before final version publication.

## Research Paper

### Development and characterization of a strawberry MAGIC population derived from crosses with six strawberry cultivars

Takuya Wada<sup>\*1)</sup>, Koichiro Oku<sup>1)</sup>, Soichiro Nagano<sup>2)</sup>, Sachiko Isobe<sup>2)</sup>, Hideyuki Suzuki<sup>2)</sup>, Miyuki Mori<sup>1)</sup>, Kinuko Takata<sup>1)</sup>, Chiharu Hirata<sup>1)</sup>, Katsumi Shimomura<sup>1)</sup>, Masao Tsubone<sup>1)</sup>, Takao Katayama<sup>1-3)</sup>, Keita Hirashima<sup>1)</sup>, Yosuke Uchimura<sup>1-4)</sup>, Hidetoshi Ikegami<sup>1-5)</sup>, Takayuki Sueyoshi<sup>1)</sup>, Ko-ichi Obu<sup>1-6)</sup>, Tatsuya Hayashida<sup>1)</sup> and Yasushi Shibato<sup>1-5)</sup>

# Selection Strategy

Seedlings



Crossing



Line	Accession	Genotype	Year	Observation	Image	Notes
1	5-79717	0	Year 18-400	Yield 0462	5-17	
2	5-79725	0	Year 18-299	Yield 0255	4-26	
2	5-79753	0	Year 18-276	Yield 0218	4-21	
4	5-79774	0	Year 18-297	Yield 0297	2-24	
5	5-79713	0	Year 18-260	Yield 0796	1-25	
6	5-79752	0	Year 18-298	Yield 0218	4-21	
7	5-79752	0	Year 18-291	Yield 0204	4-21	
8	5-79710	0	Year 18-283	Yield 0781	2-24	
8	5-79784	0	Year 18-280	Yield 0785	1-25	



Select parents

Modeling

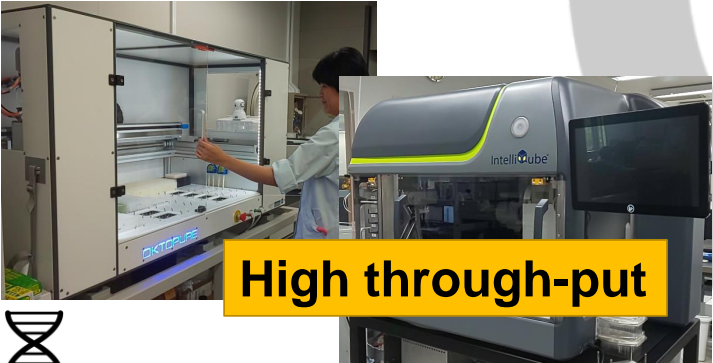


Sampling

2 selection / year



GW genotyping by RAD-Seq



High through-put



DNA extraction/ genotyping



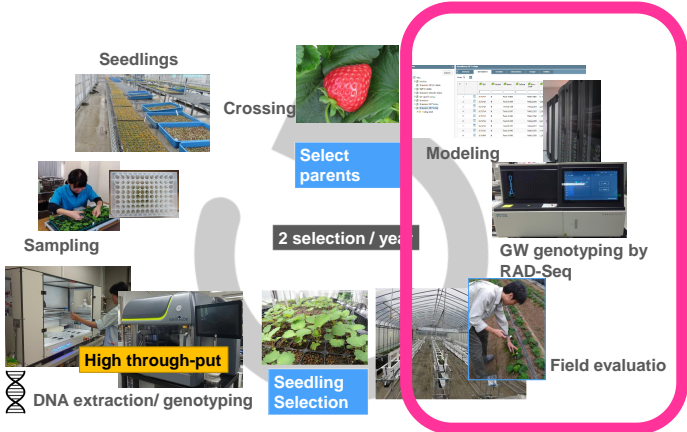
Seedling Selection



Field evaluation

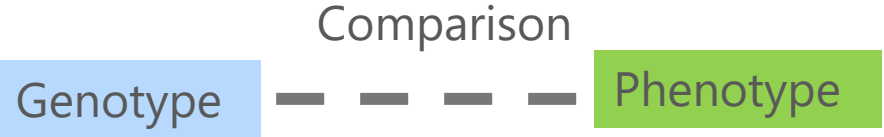
# Step1 Traits evaluation in field and construct prediction model

From Oct to April



Genotype: dd-RAD-Seq  
Phenotype: Field evaluation

Analyzed in each generation  
(=every year)  
Because the genetic composition  
of the population varies with  
selection



three approaches

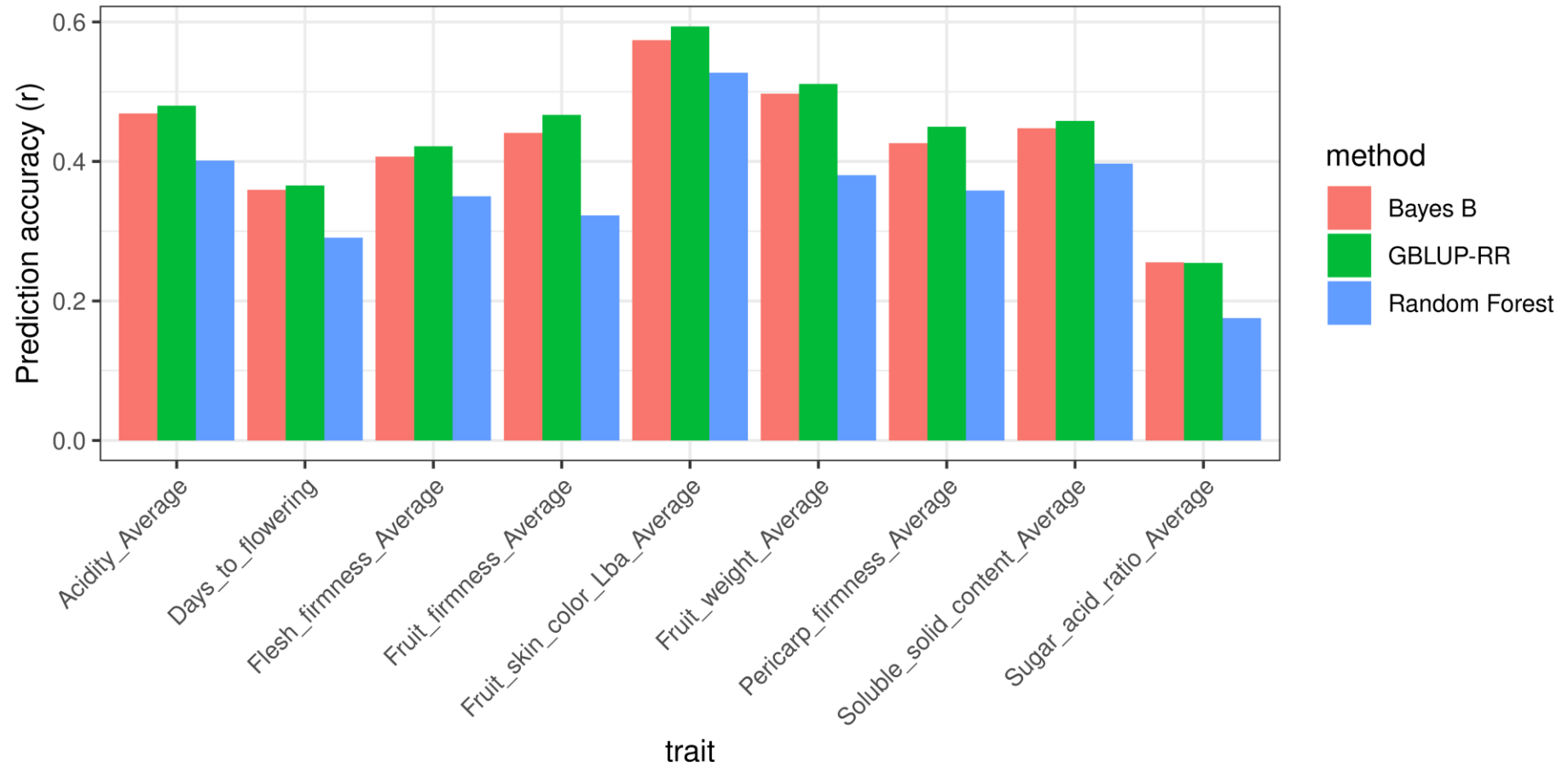
① Construction of prediction model: GBLUP-RR, Bayes B, Random Forest

② Identification of SNPs associate with traits : GWAS法

③ Select SNPs for selection of individuals with target trait values (EGGS)  
(Bootstrap based approach)

# Result Example: Correlation between predicted and observed values (Fukuoka, 5<sup>th</sup> generation)

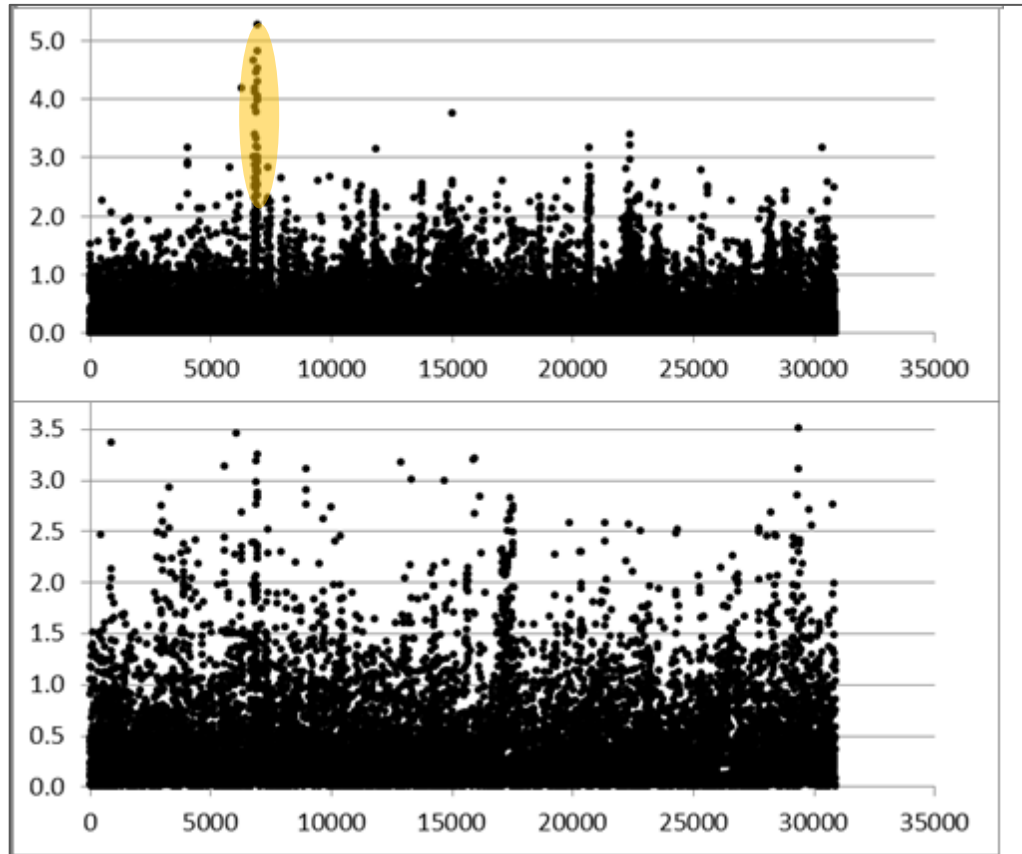
Effectiveness of prediction model varies by trait and population





# Result Example: GWAS Result in different generations (Chiba 1<sup>st</sup> and 2<sup>nd</sup> generation)

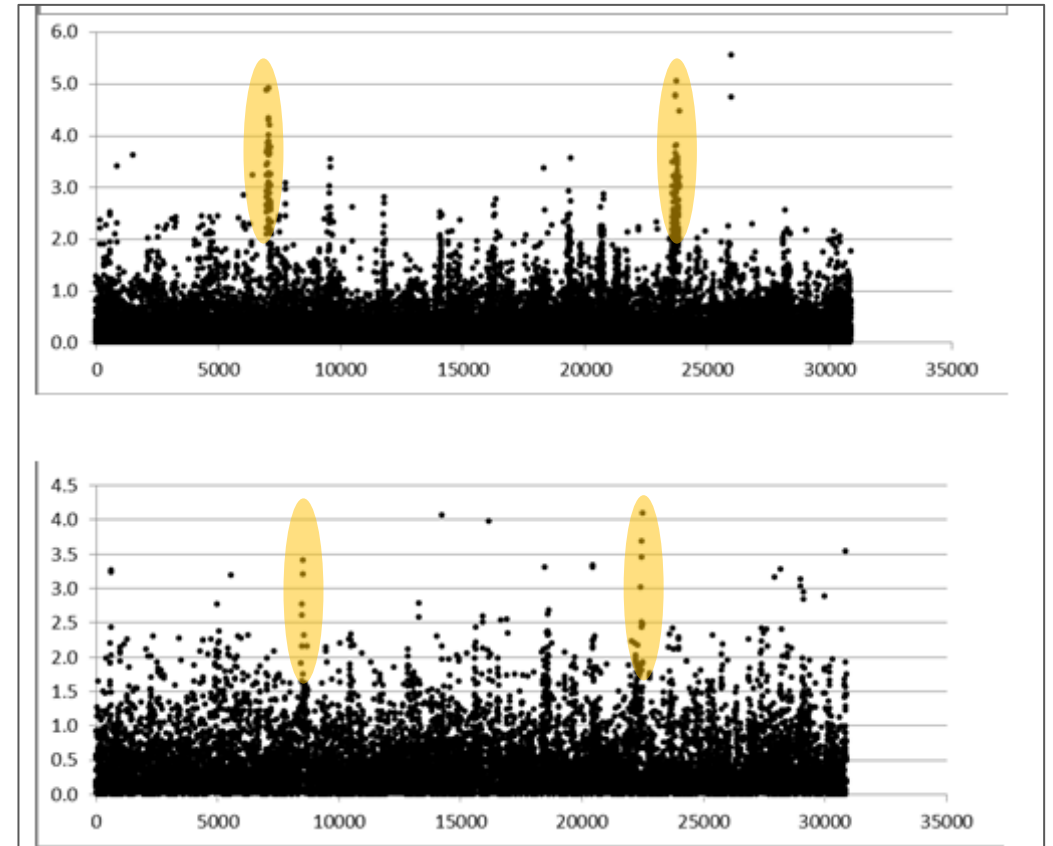
## Fruit hardness



2018/2019

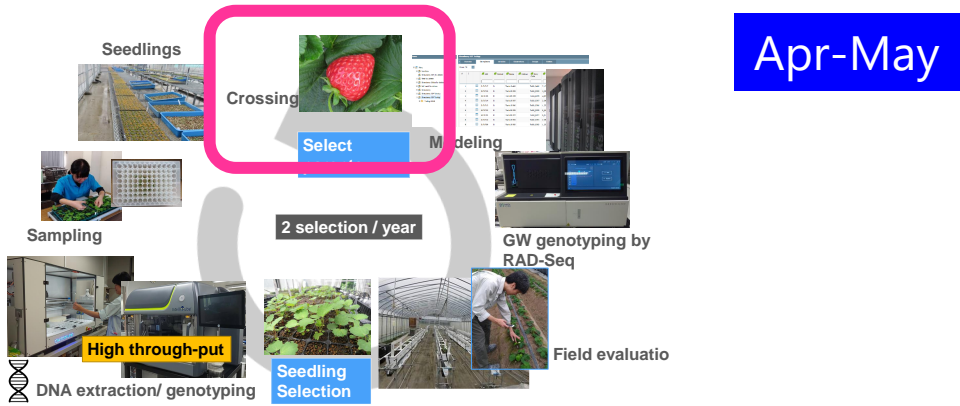
2019/2020

## Sugar contents



Target alleles were selected in the generation, and the alleles often did not show significant association in the next generation.

# Step 2 Select parental plants and crossing



Number of selected plants  
10~50 plants / population

Select individuals showed favorable traits in predicted and/or observed values  
Should consider balance between the multiple target traits

Example in Tochigi

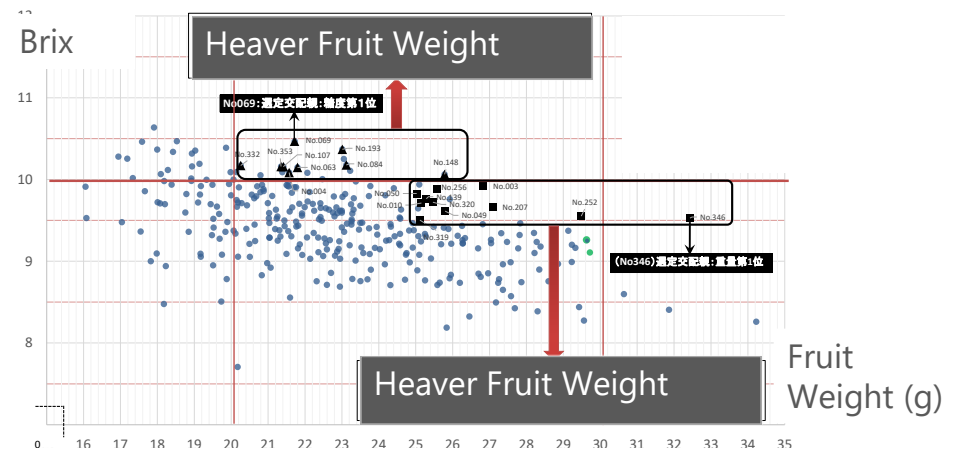


- ✓ Excellent transportability
- ✓ High Sugar Contents
- ✓ Large Fruit size

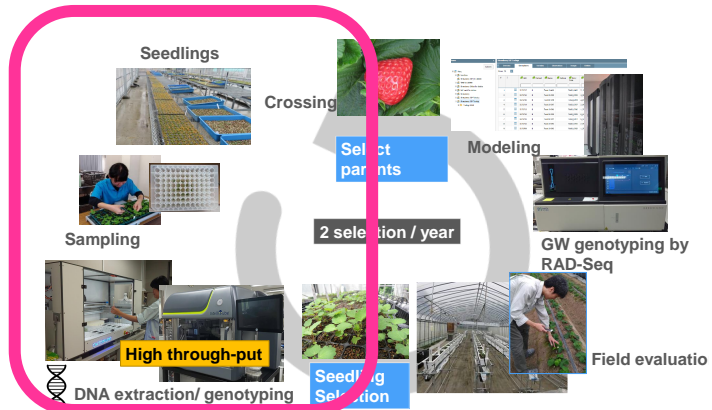
1sr criteria  
Fruit hardness  
(Prediction Values)  
 $(N/\varphi 2mm : 0.85 < < 1.3$



- ① Higher sugar contents : 9plants  
Brix >10 Fruit weight >20g
- ② Heaver Fruit weight : 11plants  
Fruit Weight >25g, Brix >9.5以上



# Step3 Seeding Selection and transplant to the field



May to Sep

Decision markers used in seedling selection

Significant association identified by GWAS or EGGs  
Should consider the balance of target traits

The number of used markers per population: ca.30  
Ideally, as many as possible, for example whole genome resequencing  
Should consider the balance of cost and periods

The number of seedling tested : 1000~2500 / population  
Genotyping period: 4 weeks

Need High-Throughput and low cost system!

# At the beginning of the project

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○

SSR



Accept crude DNA  
Lower cost for extraction

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SNP



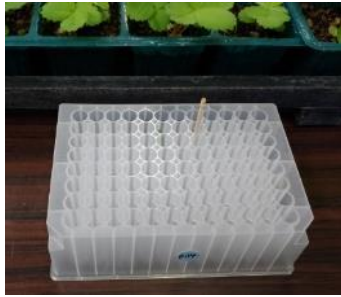
Require pure DNA  
Higher cost for extraction

# Introduce oKtopure for High quality, low cost and high-throughput DNA extraction

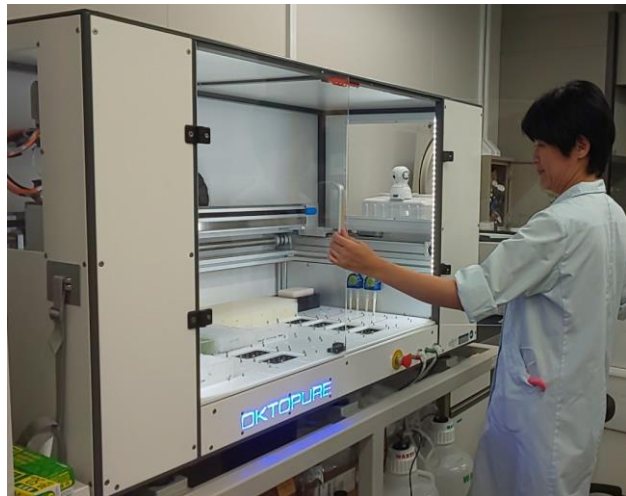
Sampling



96 plate



Freeze Dry



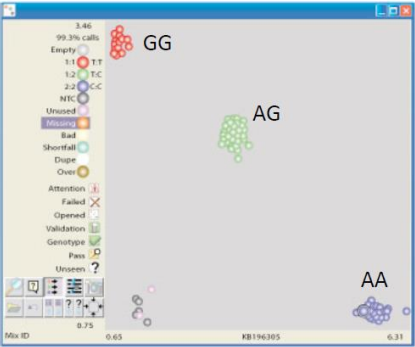
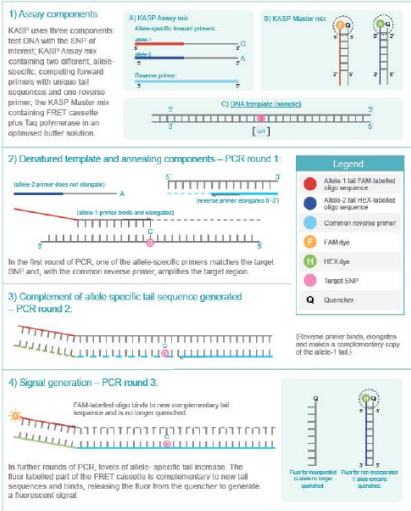
Ca. 40 plant species were extracted DNAs in our lab



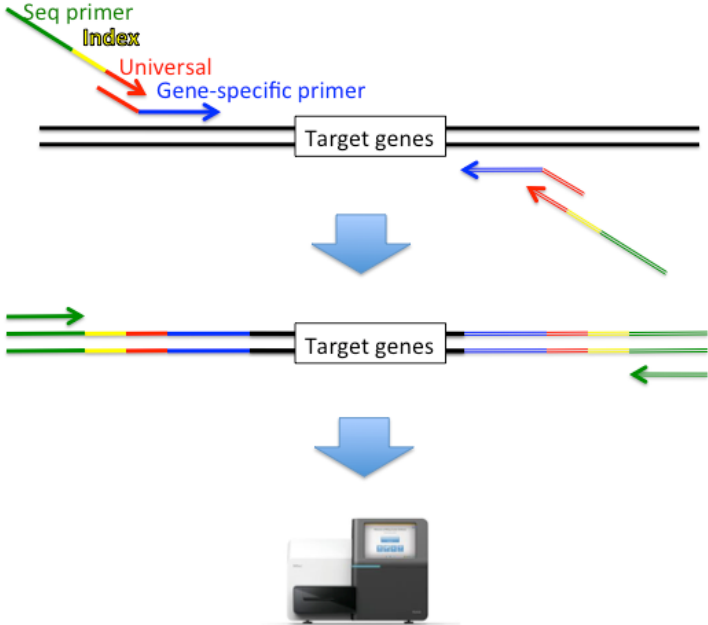
# SNP Genotyping

Two technologies have been used

## KASP



## TAS (Target Amplicon Sequencing)



**Accuracy: KASP > TAS**

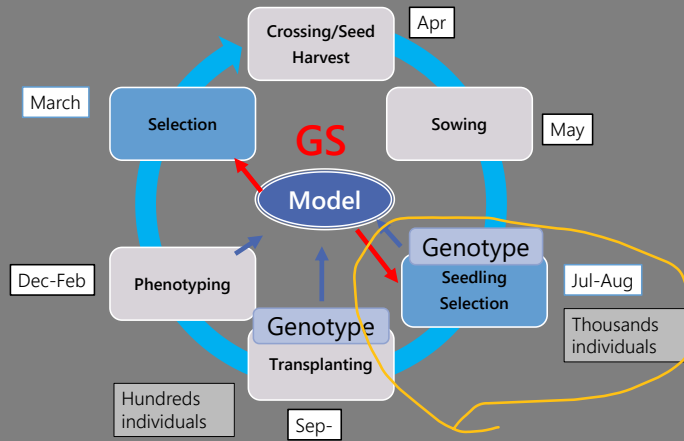
(The results of TAS sometimes changed depending on used bioinformatic tools)

**High throughput: KASP ≐ TAS**

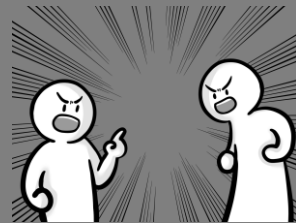
When we used typical real-time PCR thermal cyclers

# High throughput SNP Genotyping by IntelliQube

The Seedling genotyping is mainly done in July and August

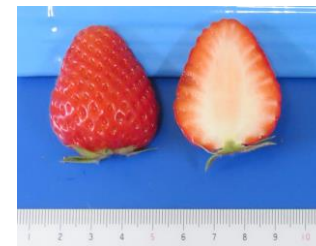
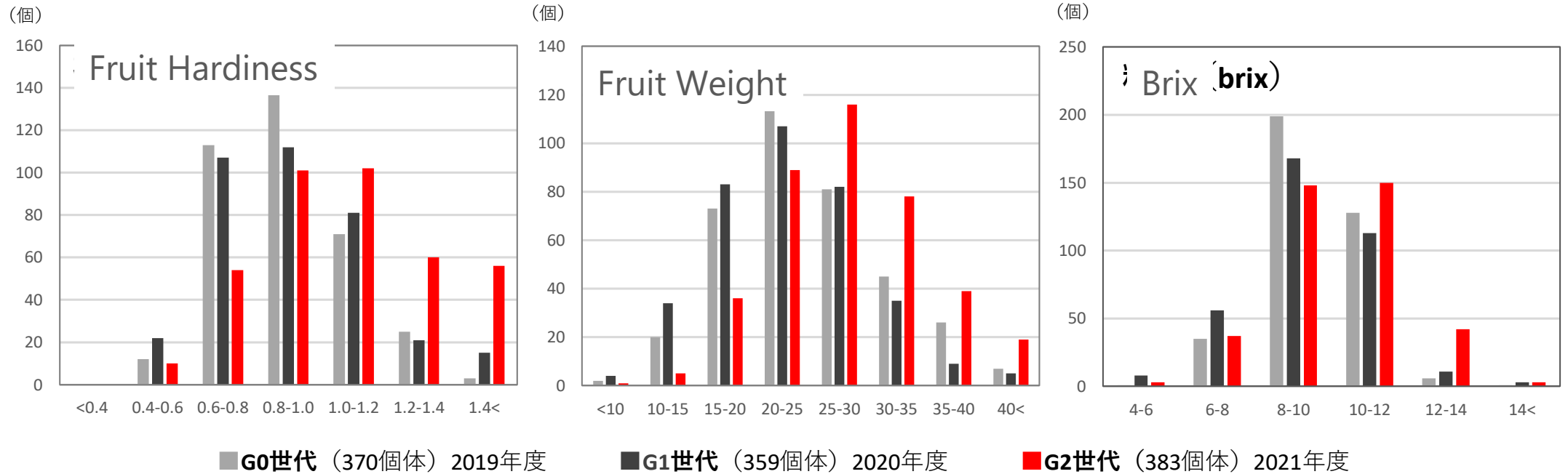


Approx. 300,000 reactions should be done in the period, and this is not only our lab works.....



We do 384 x 8 plate reaction / day  
Reaction cost is also decreased because of the small reaction volume

# Progress of our GS project





# Thoughts on genomic selection based on the experience

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- ✓ Genomic selection is a method of selecting promising individuals based on large-scale data, and the use of appropriate data can dramatically increase the accuracy of selection compared to conventional breeding methods.
- ✓ Although it was initially difficult to realize, the introduction of automated analysis robots, such as oKtopure and IntelliQube has made it possible.
- ✓ Though the large scale genotype data show useful information to breeders, the final decision is made by breeders. By utilizing new technologies, the possibilities for breeding can be further expanded.

# Acknowledgement

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