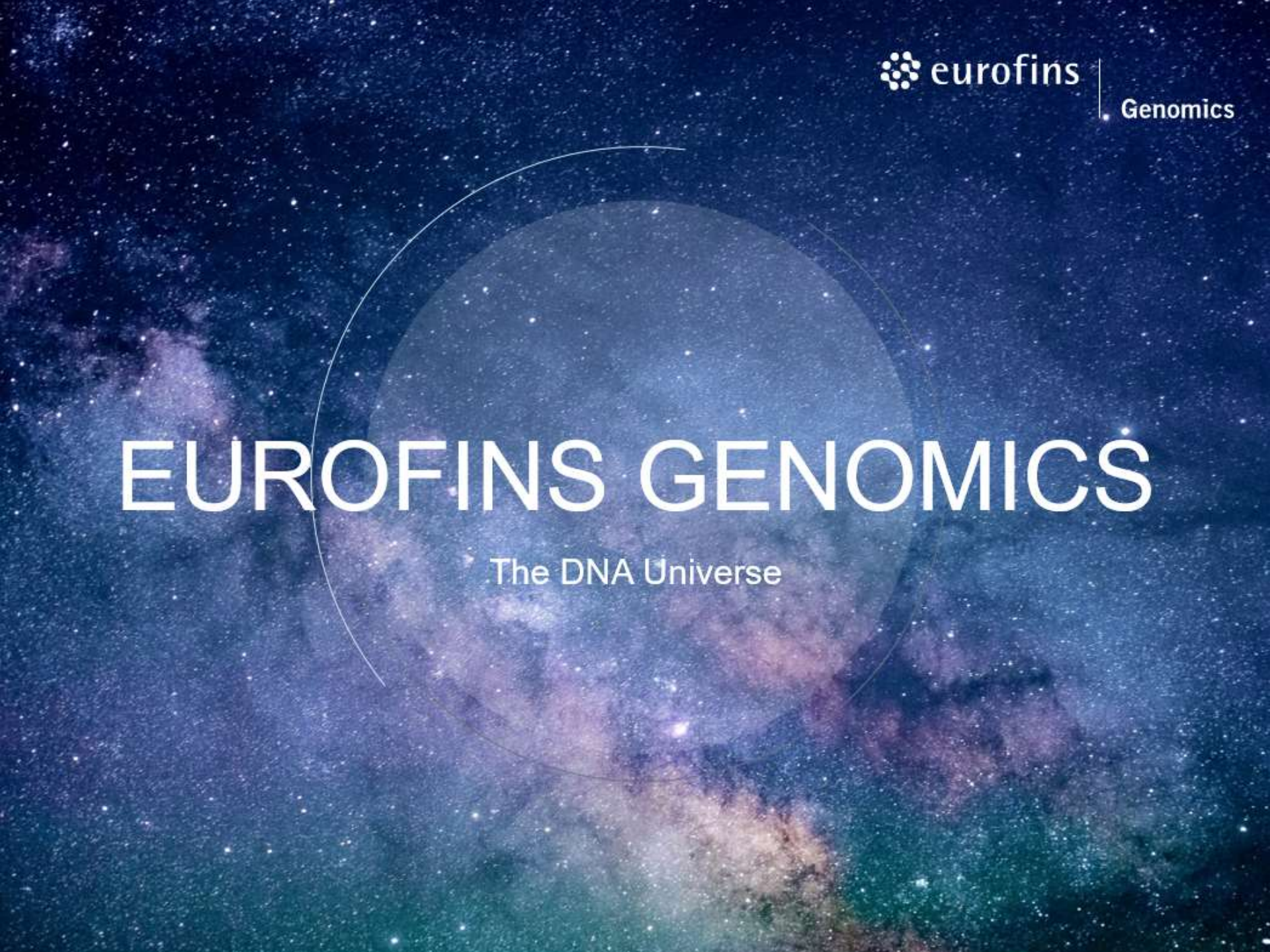
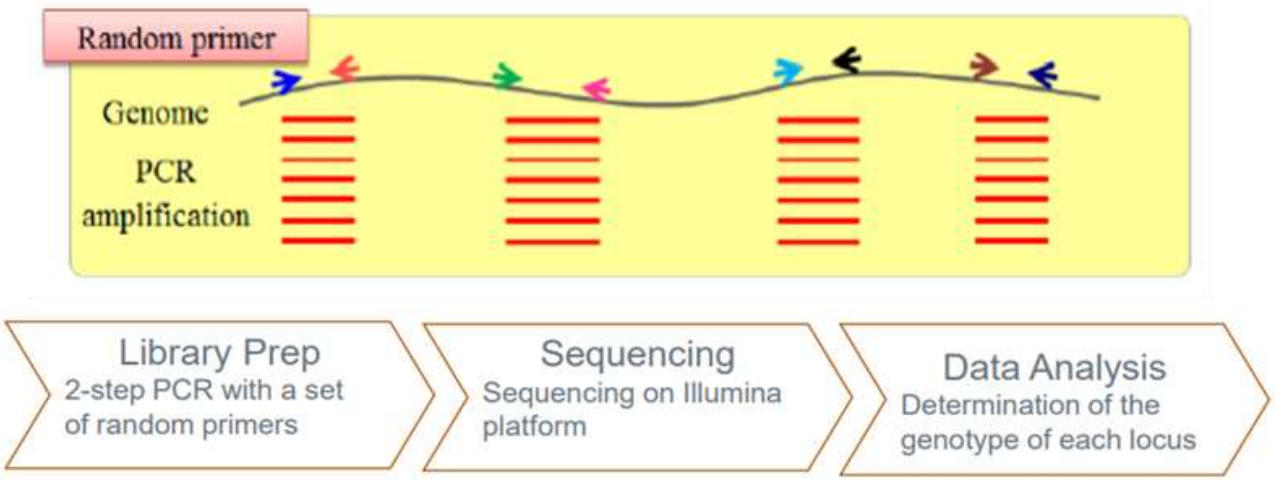

eurofins
Genomics
EUROFINS GENOMICS
The DNA Universe

GRAS-Di® for Marker discovery & Genotyping

- ***GRAS-Di***® (**G**enotyping by **R**andom **A**mplicon **S**equencing-**Di**rect) was developed by **TOYOTA Motor Corporation**
- *GRAS-Di*® amplifies the random regions throughout the genome for NextGeneration Sequencing.
- Skim based sequencing of whole genome covered by the amplicons through 2 step PCR with random primers, Non-targeted PCR-based GBS.
- Sequencing data is analyzed for presence / absence of amplicon markers and optionally SNPs calling.



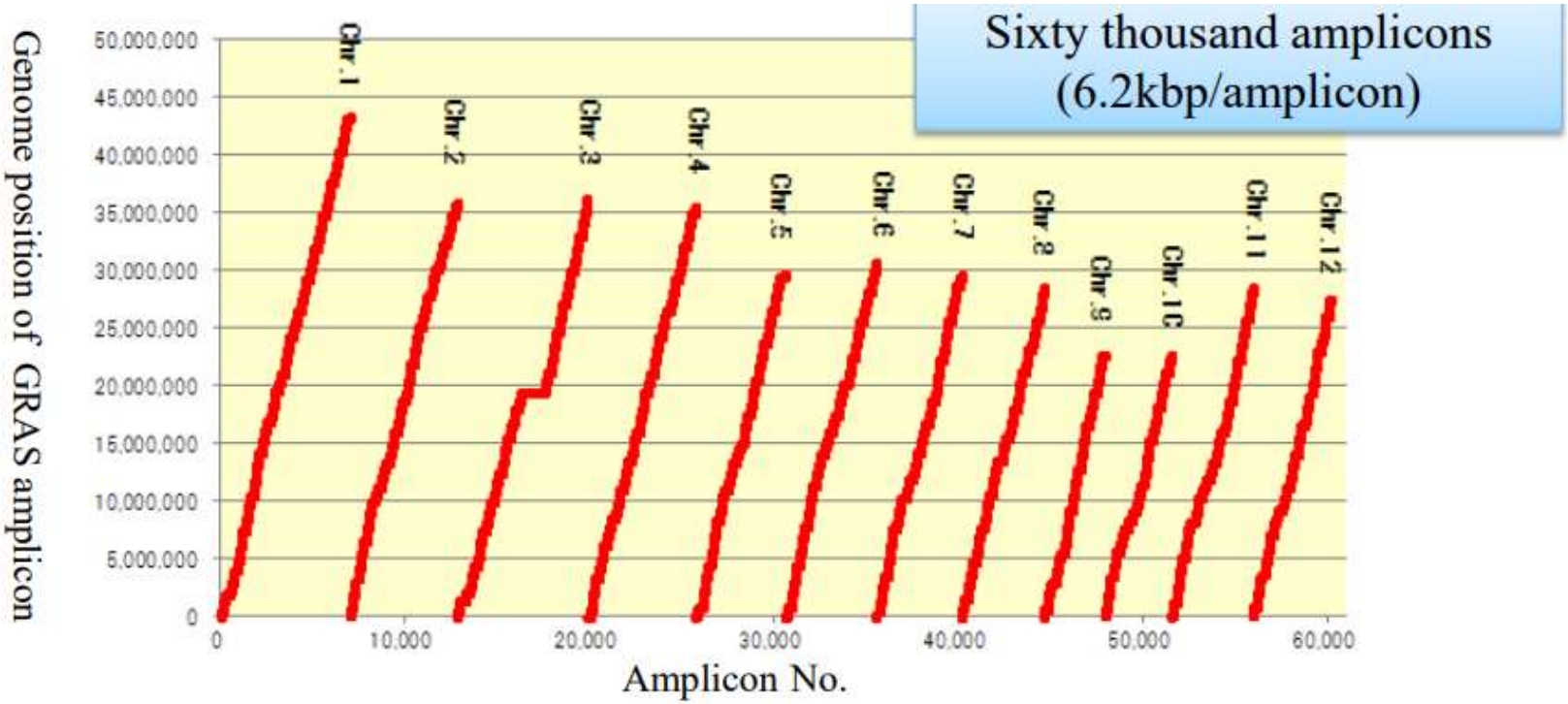
GRAS-Di® vs Whole Genome Sequence(WGS)

- ***GRAS-Di***® : Sequence reads are uniformly mapped to the genome **at intervals**.
- **WGS**: Sequence reads are uniformly mapped to the genome **without gaps**.

Amplicons covering on the chromosomes

eurofins | Genomics

- **Mapping of Amplicons onto the reference sequence shows the uniform distribution of Markers over the genome**

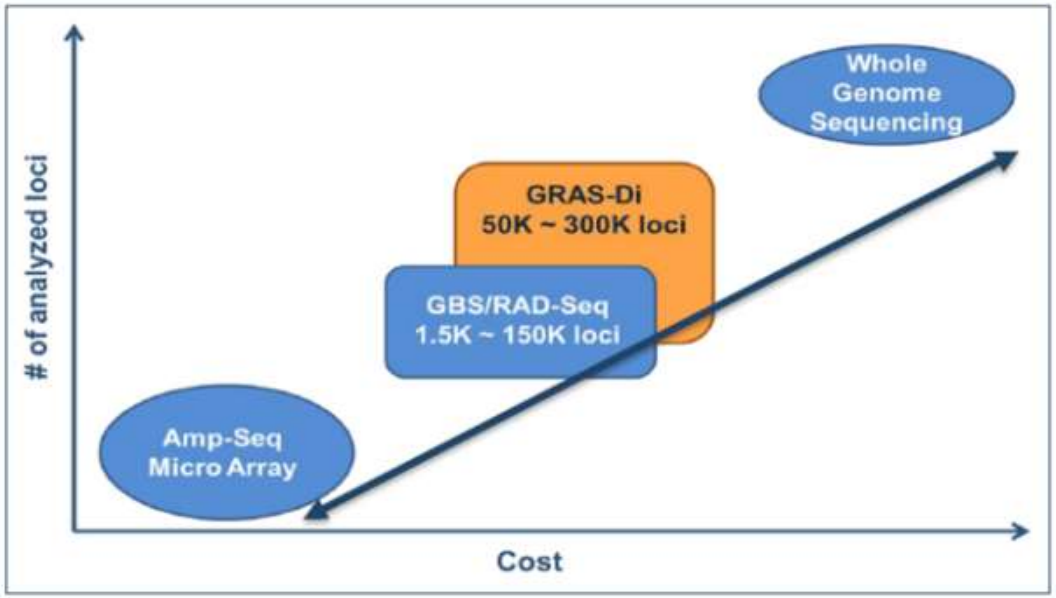


GRAS amplicons were distributed over genome, uniformly.

GRAS-Di® - Advantages

eurofins | Genomics

- More cost effective than Whole Genome deep Sequencing



- Economical & Ecological, simple method that tolerates low quality DNA or slightly fragmented DNA (>100ng)
- Applicable to both natural and segregated populations, all without the need for a reference genome
- Obtains fewer missing data than RAD-seq

GRAS-Di® - Applications

Applicable for Genetic diversity studies, Polymorphism detections (SNPs or InDels or Haplotypes). **Increased usage in the field of Phylogenetic and population structure studies**.

SNP Discovery

- Develop SNP markers for translation to a preferred genotyping system
- High quality alternative to GBS/WGS, RNA-Seq, RAD-Seq, ddRAD-Seq (GBS)

High Throughput Genotyping

- Discover and genotype massive amounts of genetic variants for Marker Selection
- High-density Linkage Mapping & QTL Mapping, Chromosome Mapping, Phylogenic & Population structure studies

Others

- Potential of large number of polymorphisms used as molecular markers for genetic analysis
- Possible to obtain genome-wide co-dominant markers Identify many markers covering all chromosomes even in a population with small genetic variation

GRAS-Di® Analysis Service - Experiences

eurofins | Genomics

GRAS-Di® has been implemented successfully in over 120 different species including crops, forestry, livestock and aquaculture.

The technology is also applicable to highly polyploid species.
Please refer the Bibliography for further details.

Agriculture/Plant

Forestry/Fruit tree

Livestock/Animal

Fishery/Aquaculture

Rice, Wheat, Corn, Sugar cane, Soy been, Groundnut, Green pea, Tomato, Eggplant, Green pepper, Paprika, Potato, Sweet potato, Cabbage, Lettuce, Chinese cabbage, Japanese white radish, Broccoli, Cauliflower, Green onions, Onions, Cucumber, Pumpkin, Spinach, Carrot, Burdock, Strawberry, Melon, Watermelon, Sub-clover, Horse gram, *Lotus japonicus*, Apple, Peach, Pear, Orange, Persimmon, Grapes, Fig, Kiwi, *Citrus junos*, *Citrus sudachi*, Prune, *Chrysanthemum*, Cedar, Cherry tree, Human, Cattle, Pigs, Chicken, Horse, Rabbit, Hamster, Guinea pigs, Rat, Tuna, Shiitake mushroom, Hen of the Woods

GRAS-Di® Analysis Service - Deliverables

eurofins | Genomics

- **Raw sequence data in FASTQ format**
- **Genotyping data by TOYOTA software with Dominant markers**

Analysis	Methodology	Deliverables	Requirements
Toyota software	Genotyping based on presence or absence of amplicon reads	GRAS-Di_RESULT.csv	Information about population (natural population or segregating population (including parent and progeny samples))
		GRAS-Di_RESULT_codominant.csv	Segregating population, segregation ratio of the genotypes in the segregating population (eg. F2 population 1:2:1), diploid, homozygous parents
		GRAS-Di_RESULT_mapping.csv	reference genome
SNP calling	Mapping and calling SNPs	*.bam	reference genome
		*.vcf	reference genome

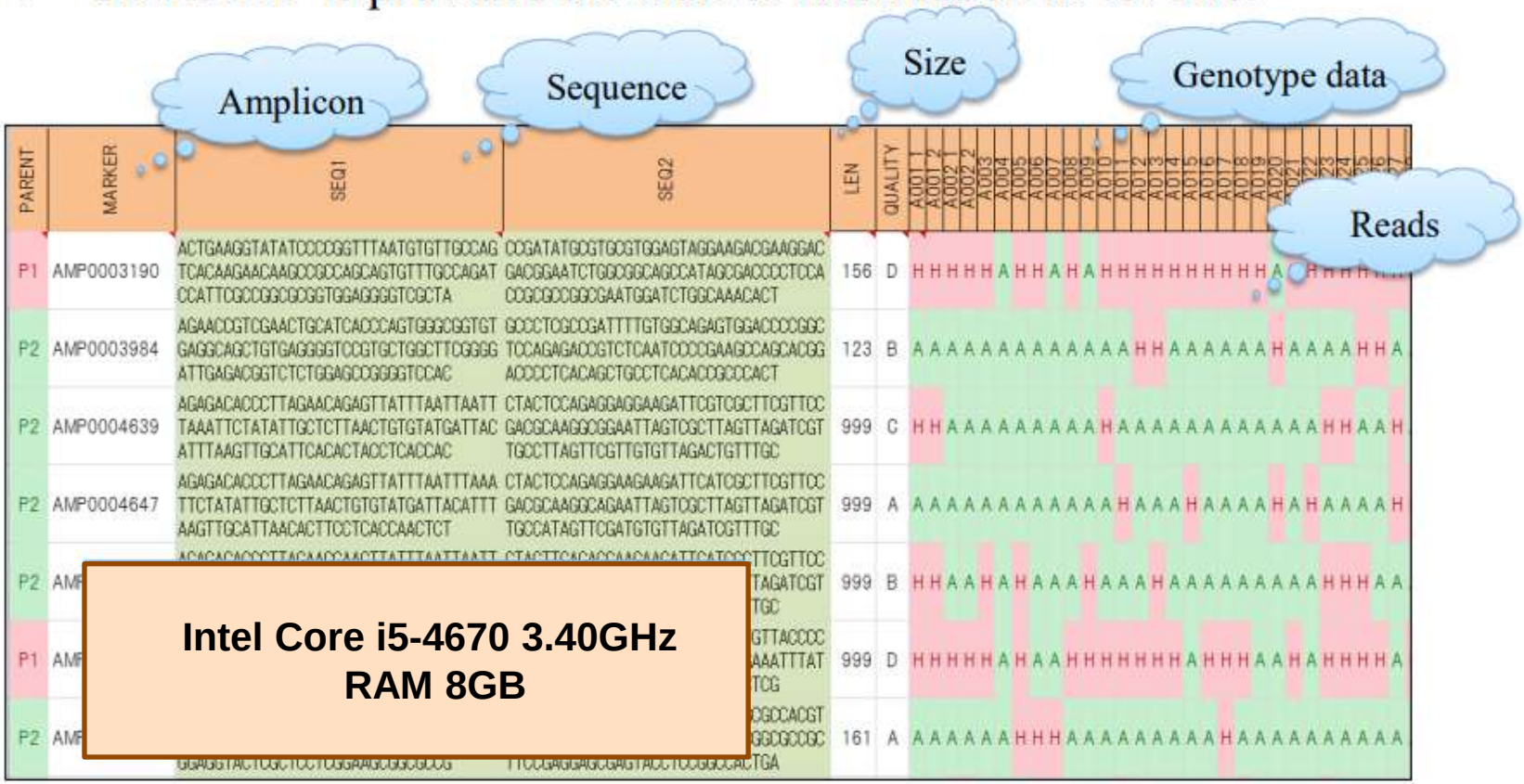
(Optionally)

- Genotyping result with Co-dominant markers
  * Available only in case of diploids, segregated population & homozygous parents, more than 40 samples of F2 generation.
- Mapping result of dominant markers to the reference genome
  * Available only for the species whose reference genome is available
- Mapping & SNP call result
  (1) Mapping result (BAM format)
  (2) SNP call result (VCF format)

Appendix; Result of TOYOTA software

- Genotyping through “ Amplicons Presence or absence”.
- GRAS-Di” SNP markers are proviced in csv file.

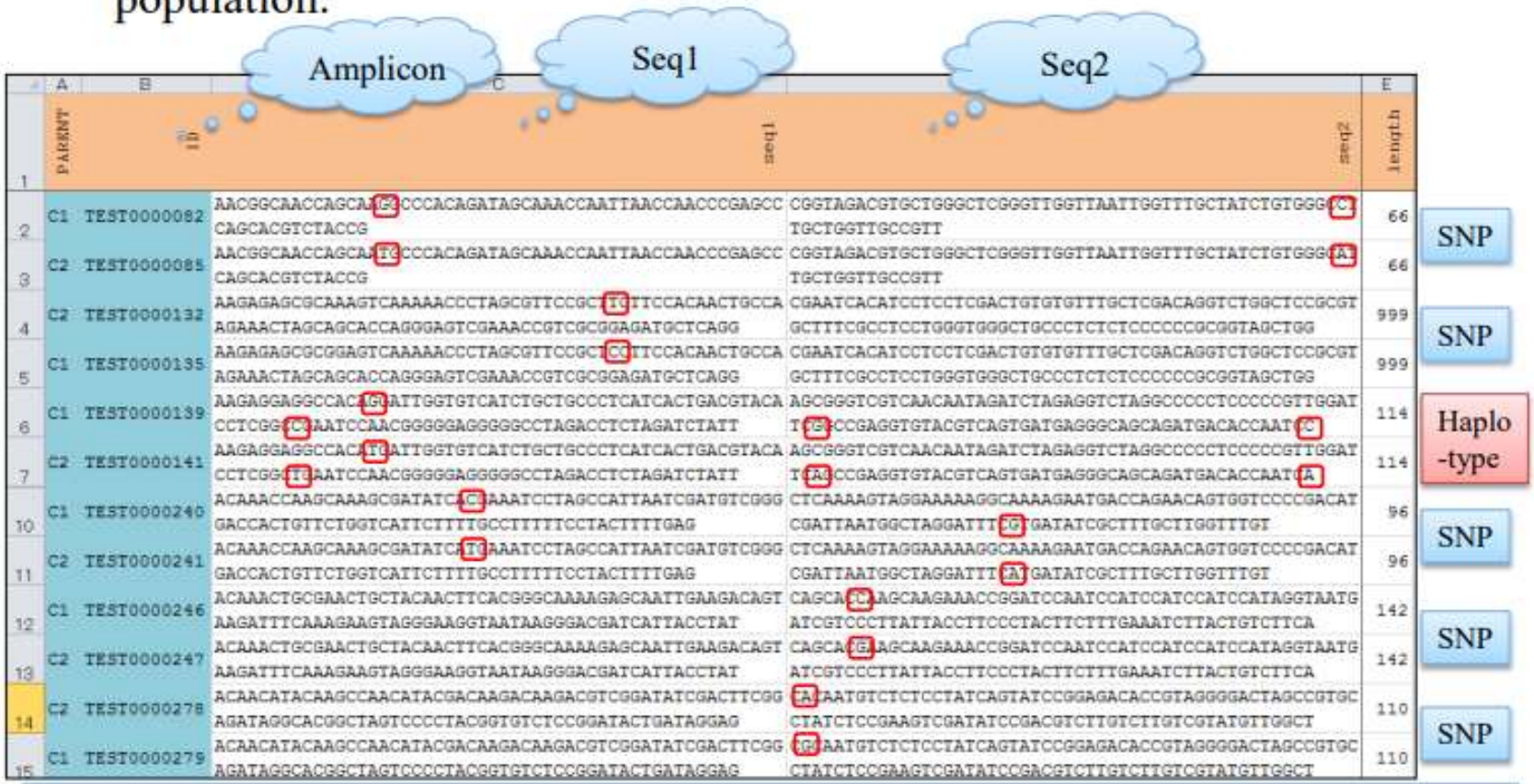
▽ “GRAS-Di” is provided the marker information in csv file.



Appendix; Result of TOYOTA software

eurofins | Genomics

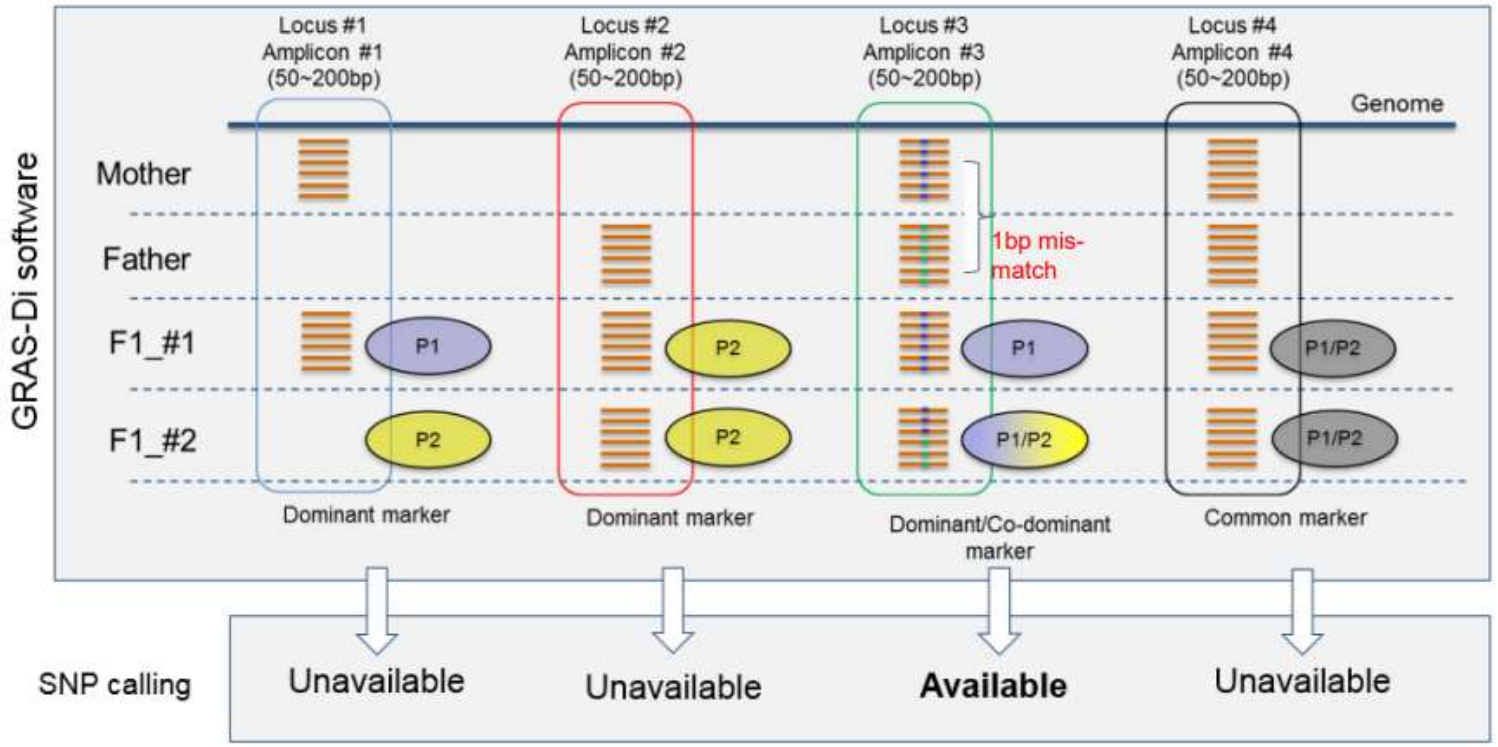
▽ "GRAS-Di" is provided the co-dominant marker in diploid segregation population.



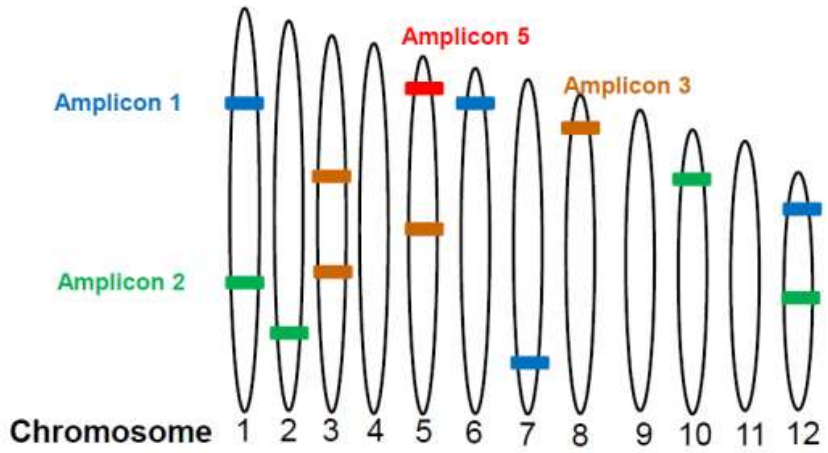
"GRAS-Di" could be identified markers by all sequence information.

Appendix; Marker detection strategy vs SNPs

- Genotypes of each locus are determined by GRAS-Di software based on the presence or absence of amplicons (amplicon is considered as locus).
- When amplicon sequences are different between parents, and a reference genome is available, then the amplicons are available for SNP calling.



Appendix; Amplicon Mapping on Chromosome



id	Seq1	Seq2	ref ID	pos	num mitakes	ref ID	pos	num mitakes	ref ID	Pos	num mitakes	ref ID	pos	num mitakes
Amplicon 1	ATCGATGTCTCCCGACGGGAATAATACTGTCAATG AACGGCAATAATCGCTCGTACATTTCCGTCGAGT CCAGCAATCGGGGCGCCCGACTGGGCCGACAAC	GACCGGTTTGTTGTCGGCCCAGTCGGGCGCCGA TTGCTGGACTCGAGCGGAAATGTACGAGCGATTATT GCCGTTCATTGACAGTATTATTCCCGTCGGG	Chr 01	43,216,785	4	Chr 07	334,562	4	Chr 12	16,339,644	4	Chr 06	19,715,592	5
Amplicon 2	ATCGGTAGTTAGCTTGGGCCTTTGGTGCACGTAC AAGCGCGACTATAATAATGCTTTTGCCCTTACGG GGATATAGATCGCTCGTACGCTCATCATCTGC	CGAATCTATGCACTTTTGTTCTCACGTTCCCGCGC GTGCTAAGTGTACGTTTGTAGCGCTATAAGGCCC ACGTGTTCCGATGTGTACACTGAAACGGGG	Chr 02	1,155,876	1	Chr 12	25,516,783	2	Chr 10	14,005,983	2	Chr 01	26,208,751	2
Amplicon 3	ACAGGGTCCACAAGGTGAGAGCGAGTTAGATAC GACCTATATAGTCTAGGGGCTATTTGCCATAGTA AAATGCTCAGCACGCAGGTT	TCATAGCATACGTTGCTTGATGGACGCCATCAAAG GTCACTGTAGATCCCACGATTCGAGCCGATTAAAA GGGTTACTCGGTAACCTCGCATGAGTCCCC	Chr 03	613,758	1	Chr 03	616,789	1	Chr 03	617,946	2	Chr 05	11,003,782	3
Amplicon 4	ACAGGGTCCACAAGGTGAGAGCGAGTTAGATAC GACCTATATAGTCTGGGGGCTATTTGCCATAGTA AAATGCTCAGCACGCAGGTT	TCATAGCATACGTTGCTTGATGGACGCCATCAAAG GTCACTGTAGATCCCACGATTCGAGCCGATTAAAA GGGTTACTCGGTAACCTCGCATGAGTCCCC	–											
Amplicon 5	GCCTAGTTCGTCCGATTGTGCAAGTTCGTTCAGG TTTTTTAATAGTGCCTAACCAGTTACTAAATGAG TGTATGAACGTTAAAGCCCGGCCTATGCCCCC	ACTCTCTGCTTTAGCTCACAGATTCAGCATTGCTGCC ATGGGGGGATAGGCCCGGGCTTTAACGTTCATACAC TCATTTAGTAACTGGTTAGGCACTATTAAAAA	Chr 05	9,994,255	0									

Multiple candidates of mapped positions for each amplicon are displayed

- ref ID: Chromosome name
- pos: coordinate position on the chromosome
- num mistakes: Number of mismatch bases with reference genomic sequence

GRAS-Di® Analysis Service

eurofins | Genomics

Please Contact to nearest Eurofins Genomics

For APAC Region

Eurofins Genomics K.K., Japan

E-mail:
ngs-jp@eurofins.com

APAC

For EU Region

Eurofins Genomics Germany GmbH

E-mail:
projectsales-eu@genomics.eurofinseu.com

EU