	Title	Citation		Species	Goal of the study
1	Random PCR-based genotyping by sequencing technology GRAS-Di (genotyping by random amplicon sequencing, direct) reveals genetic structure of mangrove fishes	Mol Ecol Resour. 19:1153-1163. (2019)	**	F. amboinensis L. fulvus Z. dunckeri	GRAS-Di method is applied to ecological studies where a large number of SNPs is required, such as population structure analysis of marine fish, while high quality DNA samples are not always available
2	Genetic Diversity and Genetic Structure of the Wild Tsushima Leopard Cat from Genome-Wide Analysis	Animals (Basel). 10:1375. (2020)		Wild Tsushima Leopard Cat	The identification of individuals in the Tsushima leopard population is difficult using microsatellite marker analysis due to the population's low genetic diversity, so more DNA markers must be developed to design an appropriate conservation program. To identify SNPs using genome-wide analysis with the GRAS-Di approach that would clarify the genetic diversity and structure of the wild Tsushima leopard cat.
3	East Asian origin of the widespread alpine snow-bed herb, Primula cuneifolia (Primulaceae), in the northern Pacific region	J Biogeogr. 00: 1–13. (2020)		East Asian origin of the widespread alpine snow-bed herb, Primula cuneifolia (Primulaceae)(P. cuneifolia and its sister species)	To explore the molecular evidence for the recent northward migration of alpine snow-bed species in the northern Pacific region.
4	Divergence and introgression in small apes, the genus Hylobates, revealed by reduced representation sequencing	Heredity (Edinb). 127:312-322. (2021)	erc	Gibbons in the genus Hylobates	To uncover the divergence history and the contributions of hybridisation and introgression to the evolution of Hylobates
5	GRAS-Di system facilitates high-density genetic map construction and QTL identification in recombinant inbred lines of the wheat progenitor <i>Aegilops tauschii</i>	Scientific Reports (2020)10:21455		Recombinant inbred lines of the wheat progenitor Aegilops tauschii	Developped a high-density linkage map and identify quantitative trait loci (QTLs) for recombinant inbred lines of <i>Aegilops tauschii</i> , a D-genome donor of bread wheat, using the recently developed genotyping by Random Amplicon Sequencing-Direct (GRAS-Di) system, which facilitates skimming of the large and complicated genome and generates a large number of genetic markers. The deduced linkage groups based on the GRAS-Di genetic markers corresponded to the chromosome number of <i>Ae. tauschii</i> .
6	Genetic Dissection of a Precocious Phenotype in Male Tiger Pufferfish (<i>Takifugu rubripes</i>) using Genotyping by Random Amplicon Sequencing, Direct (GRAS-Di)	Marine Biotechnology. 23:177-188 (2021)	O ++	Male Tiger Pufferfish (<i>Takifugu rubripes</i>)	In this study, we tested the utility of GRAS-Di for genetic analysis in a cultured population of the tiger pufferfish Takifugu rubripes. The genetic analyses included family structure analysis, genetic map construction, and quantitative trait locus (QTL) analysis for the male precocious phenotype using a population consisting of four full-sib families derived from a genetically precocious line.

7	A Major and Stable Quantitative Trait Locus <i>qSS2</i> for Seed Size and Shape Traits in a Soybean RIL Population	Front. Genet., 16 April 2021, Volume 12 - 2021	88	Soybean RIL Population	Seed size and shape traits are important determinants of seed yield and appearance quality in soybean [Glycine max (L.) Merr.]. Understanding the genetic architecture of these traits is important to enable their genetic improvement through efficient and targeted selection in soybean breeding, and for the identification of underlying causal genes.
8	QTL analysis of crown gall disease resistance in apple: first plant R gene candidates effective against Rhizobium rhizogenes (Ti)	Tree Genetics & Genomes volume 17, Article number: 25. (2021)		Apple tree crown gall	Identifing quantitative trait loci (QTLs) that control resistance to tumour-inducing Rhizobium isolates found in apple production areas in Japan. Fine mapping <i>Rrr1</i> , and determining the nuculeotide sequnence of its candidate region, and predicting candidate gene.
9	Biological and molecular characterization of linalool-mediated field resistance against <i>Xanthomonas citri</i> subsp. citri in citrus trees.	Tree Physiology, Volume 41, Issue 11, November 2021, Pages 2171–2188,		F1 population of the resistant Ponkan mandarin and susceptible 'Harehime' ('E-647' × 'Miyagawa-wase') cultivar	Quantitative trait locus (QTL) analysis revealed that linalool accumulation and Xcc (citrus canker disease, caused by the bacterial pathogen Xanthomonas citri subsp. Citri) susceptibility QTLs overlapped.
10	Analysis of the possible cytogenetic mechanism for overcoming hybrid lethality in an interspecific cross between <i>Nicotiana suaveolens</i> and <i>Nicotiana tabacum</i> .	Scientific Reports volume 11, Article number: 7812 (2021)		The seedlings of hybrid parents (N. suaveolens and N. tabacum), two lethal hybrid seedlings, and one hybrid seedling that overcame lethality.	Finding hybrids that overcome lethality, either spontaneously or by artificial culture, lack the distal part of the Q chromosome, a region that includes the gene responsible for lethality.
11	Detection of a major QTL related to smut disease resistance inherited from a Japanese wild sugarcane using GRAS-Di technology.	Breeding Science. 2021 Volume 71 Issue 3 Pages 365-374		Japanese wild sugarcane	Detection of an identical QTL for indices of smut disease incidence with a markedly high LOD score at the end of linkage group 8 of 'Yaenoushie'. This QTL could be used for marker-assisted selection (MAS) to significantly improve smut disease resistance without negative effects on other agronomic traits.
12	Effect of Three Types of Ion Beam Irradiation on Gerbera (<i>Gerbera hybrida</i>) In Vitro Shoots with Mutagenesis Efficiency	Plants. 10:1480. (2021)		Gerbera	The genomic mutation in the flower color, petal shape, and male sterile mutants were confirmed by GRAS-Di technology. As a result, 1 marker for flower color mutants, 6 markers for petal slender mutants, and 31 markers for male sterile mutants were identified.
13	Artificial Control of the <i>Prunus</i> Self-incompatibility System Using Antisense Oligonucleotides against Pollen Genes	The Horticulture Journal/Volume 91 (2022) Issue 4, Pages 437-447	×	Prunus	The objective was to develop an artificial method for overcoming self-incompatibility (SI) via the knockdown of SFB (S haplotype–specific F-box) or MGST(M-locus encoded glutathione S-transferase-like) genes in pollen. After the self-pollination, GRAS-Di analyses were performed to determine kinship relationships.
14	Genomic Evidence for Speciation with Gene Flow in Broadcast Spawning Marine Invertebrates	Mol Biol Evol. 38:4683-4699.		Abalone (H. discus, H. madaka, and H. gigantea)	To address how far speciation has proceeded along the speciation continuum, the patterns of genomic divergence in the Western Pacific abalones were examined. using nuclear SNP loci obtained with GRAS-Di and whole-genome sequencing, as well as with mitochondrial genome sequencing.

15	A Y-linked anti-Müllerian hormone type-II receptor is the sex-determining gene in ayu, <i>Plecoglossus altivelis</i>	PLoS Genet. 17: e1009705.	*	Ayu (Plecoglossus altivelis)	To validate genome-wide association scan for sex determining locus of Ayu, linkage analysis of genetic sex was performed, using Ayu F1 full-sib families captured from a wild population in the Nagaragawa River. Two F1 full-sib family (88 siblings, two male parents and two female parents) were used for linkage analysis using GRAS-Di analysis.
16	Genomic prediction of 10 metamorphic traits of captive-bred Japanese eels (<i>Anguilla japonica</i>) using the GRAS-Di genotyping method	Aquaculture. 548: 737671. (2022)	2	Japanese eel (Anguilla japonica)	To investigate the potential value of genomic selection for 10 traits related to the timing of metamorphosis and the body size at that time, 1689 glass eels were used. To identify and genotype SNP markers, which were used to assign parentage and as a basis for genomic selection, GRAS-Di sequencing was performed.
17	Repeated translocation of a supergene underlying rapid sex chromosome turnover in <i>Takifugu</i> pufferfish	Proc Natl Acad Sci U S A. 119: e2121469119. (2022)	€	Takifugu snyderi	To obtain the genome-wide genotype data from 98 full-sibling progenies in T. snyderi family, GRAS-Di technology was used. Then, linkage map construction and mapping of the sex-determining locus in T. snyderi were performed.
18	Comparison of the Genetic Diversity of the Captive and Wild Populations of the Tsushima Leopard Cat Using a GRAS-Di Analysis	Animals. 12:1464. (2022)		Tsushima leopard cat (Prionailurus bengalensis euptilurus)	In order to assess the genetic diversity of the captive population of Tsushima Leopard Cat (TLC), the authors modified the draft genome using short-read and long-read sequencing data and compared the genetic diversity of captive and wild populations of TLC by GRAS-Di analysis. The results showed that the captive population had almost the same genetic diversity as the wild population and managed to remain in good condition.
19	Ancient rivers shaped the current genetic diversity of the wood mouse (<i>Apodemus speciosus</i>) on the islands of the Seto Inland Sea, Japan	Zoological Lett. 8:9 (2022).	*	Wood mouse (Apodemus speciosus)	To examine genetic variations among wood mice inhabiting the islands of the Seto Inland Sea, the autohrs used the GRAS-Di method. They tested the hypothesis that ancient rivers could explain the genetic relationships among the wood mice on these islands.
20	Genetic relationship and diversity of cultivars and breeding lines of tetraploid Italian ryegrass (<i>Lolium multiflorum Lam.</i>) and its hybrids with <i>Lolium-Festuca</i> complex based on genome-wide allele frequency	Grassland Science. 69:65-78. (2023)		Italian ryegrass (Lolium multiflorum Lam.)	To reveal genetic relatedness and diversity of 62 cultivars and breeding lines of tetraploid Italian ryegrass (Lolium multiflorum Lam.; 39 accessions) and its interspecific hybrids, Festulolium (18 accessions), and hybrid ryegrass (Lolium × hybridum Hausskn.; 5 accessions), 2,824 genome-wide allele frequencies were obtained by GRAS-Di method using bulk genomic DNA testing.
21	A Survey of Genome-Wide Genetic Characterizations of Crossbred Dairy Cattle in Local Farms in Cambodia	Animals. 12:2072. (2022)		Cattle/Cow	In order to reveal the genetic variation and the milk production in Cambodian crossbred dairy cattle and provide information for improving the efficiency of future cattle crossbreeding strategies to establish an adequate dairy strain, the autors conducted a genome-wide survey of two dairy farm populations, using GRAS-Di analysis, in the suburb area of Phnom Penh city, Cambodia. Then, they performed STRUCTURE analyses of the two farm populations to understand their genetic characteristics, in comparison with a pure Japanese Holstein-Friesian cow, as a reference.

22	Genome-wide molecular phylogenetic analyses and mating experiments which reveal the evolutionary history and an intermediate stage of speciation of a giant water bug	Mol Ecol. 30:5179-5195. (2021)		Giant water bug (Appasus japonicus)	To present an example of a species in an intermediate stage of speciation, the giant water bug Appasus japonicus (Heteroptera, Belostomatidae) was investigated using mating experiments and phylogenetic analyses of the mtDNA COI and 16S rRNA regions, and nuclear DNA SSR and its genomewide SNPs (11,241 SNPs). The results of our phylogenetic analyses based on their mtDNA data set and the genome-wide SNPs data set strongly supported the paraphyly of the Japanese populations. suggesting that their ancestral lineage which being distributed in the Japanese Archipelago subsequently migrated to the Eurasian Continent.
23	Genetic basis of maturity time is independent from that of flowering time and contributes to ecotype differentiation in common buckwheat (<i>Fagopyrum esculentum</i> Moench).	BMC Plant Biol. 22:353. (2022)	00	Common buckwheat (Fagopyrum esculentum Moench)	To investigate the genetic loci associated with maturity time as a trait that defines the ecotype under natural linkage disequilibrium (LD) conditions. Using F2 segregating populations, the authors developed a high-density genetic linkage map by GRAS-Di analysis via re-estimation of co-dominant marker genotypes. Using this map, they detected major QTLs for maturity time. These QTLs were located at loci different from those of the flowering time QTLs, suggesting that the photoperiod responses of maturity time and flowering time would have different mechanisms.
24	Genetic diversity and structure of diploid Italian ryegrass (<i>Lolium multiflorum</i> Lam.) cultivars and breeding materials in Japan based on genome-wide allele frequency	Grassland Science. 68:263-276 (2022)		Italian ryegrass (Lolium multiflorum Lam.)	In order to reveal the genetic diversity of cultivars and breeding materials of diploid Italian ryegrass in Japan, the authors investigated genetic diversity among and within 89 accessions by using genome-wide allele frequencies obtained by GRAS-Di.
25	Chromosome-scale genome assembly of <i>Eustoma</i> grandiflorum, the first complete genome sequence in the genus <i>Eustoma</i>	G3 Genes Genomes Genetics, 13: jkac329 (2023)		Eustoma grandiflorum	To establish a chromosome-scale de novo assembly of E. grandiflorum genome sequences, the authors performed four genomic and genetic approaches: (1) PacBio Sequel deep sequencing, (2) error correction of the assembly by Illumina short reads, (3) scaffolding by chromatin conformation capture sequencing (Hi-C), and (4) genetic linkage maps derived from an F2 mapping population. To assess the genome structure divergence and phylogenetic relations, the assembled genome and predicted genes were compared with those of other species, including those in the order Gentianales, This is the first report on the construction of reference genome sequences in E. grandiflorum as well as in the genus Eustoma, leading to insights into the genome structure of the genus Eustoma and the family Gentianaceae.
26	Efficient Production of Doubled Haploids as Isogenic Line Founders via Double Heat Shock Treatment in Willow Gudgeon (<i>Gnathopogon caerulescens</i>)	Aquaculture Research. 2023:9783381 (2023)	~	Willow Gudgeon (Gnathopogon caerulescens)	To confirm the zygosity of some hatchlings treated with Double Heat Shock, GRAS-Di was used.

2	Phylogeography of Kisaura Ross (Trichoptera: Philopotamidae) of the Japanese Archipelago and the character displacement evolution observed in a secondary contact area between genetically differentiated intra-specific lineages	Zoological Journal of the Linnean Society, 197:176–188. (2023)	>	Kisaura Ross (Trichoptera: Philopotamidae)	To present evidence of an ongoing speciation event in the Japanese Archipelago, the authors conducted molecular phylogenetic analyses based on the mtDNA COI region (658 bp) and genome-wide nDNA single nucleotide polymorphisms (SNPs) (16 254 loci) using the K. nozakii species complex and a sister-species, Kisaura borealis. It was revealed that the Kisaura nozakii species complex is composed of multiple allopatrically differentiated genetic lineages.
2	Chromosome-Scale Genome Assembly and Transcriptome Assembly of Kawakawa <i>Euthynnus</i> <i>affinis</i> ; A Tuna-Like Species	Front Genet. 2021 12:739781		Euthynnus affinis	The authors report the chromosome-level genome assembly of kawakawa E. affinis, the first available chromosome-level assembly within the Pelagiaria clade. Two types of linkage evidence: diploid, based on full-sib family linkage analyses, and haploid, based on linkage analyses of interspecific hybrids of E. affinis female and T. orientalis male, were obtained. Based on a genome coordinate of each marker in the linkage maps using GRAS-Di, they anchored and oriented scaffolds into pseudochromosomes.
2	GRAS-Di SNP-based molecular characterization and fingerprinting of a Turkish <i>Corylus avellana</i> core set provide insights into the cultivation and breeding of hazelnut in Turkey	Mol Genet Genomics. 298:413–426 (2023)		Hazelnut (Corylus avellana L.)	GRAS-Di was used to identify SNPs in a panel of 96 individuals representing the Turkish national hazelnut collection. The resulting 7609 high-quality SNPs were physically mapped to the Tombul cultivar reference genome and used for population structure and diversity analyses. These analyses revealed that cultivars are not less diverse than wild accessions and that 44% of the panel had admixed ancestry.
3	High-Density Linkage Maps from Japanese Rice <i>japonica</i> Recombinant Inbred Lines Using Genotyping by Random Amplicon Sequencing-Direct (GRAS-Di)	Plants. 12:929. (2023)		Rice	To unveil potential polymorphism between closely related Japanese rice cultivars, the authors used a GRAS-Di method to develop genetic linkage maps. The average genetic distance between markers showed a clear decrease in the four RIL populations (190 for RIL71, 96 for RIL98, 95 for RIL16, and 94 for RIL91) when markers of both linkage maps (maps by GRAS-Di and those by GoldenGate SNP assay) were integrated. Genetic studies using these markers successfully localized five QTLs associated with heading date on chromosomes 3, 6, and 7, and which previously were identified as Hd1, Hd2, Hd6, Hd16, and Hd17.
3	Identification of Novel Quantitative Trait Loci for Culm Thickness of Rice Derived from Strong-Culm Landrace in Japan, Omachi	<u>Rice 16:4 (2023)</u>		Rice	To identify the genetic factors of culm strength of Omachi using RILs between Omachi and Koshihikari, QTL analysis were performed. Using 1,904 DNA markers obtained from a GoldenGate® Assay and GRAS-Di® analysis, three QTLs for the culm diameter of the 5th internode on chromosomes 3 and 7 (qCD3, qCD7-1, qCD7-2) were identified. The results suggest that the Omachi alleles of qCD3, qCD7-1 and qCD7-2 are useful for breeding to increase lodging resistance and yield.