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# Transcriptome-based single nucleotide polymorphism markers for genome mapping in Japanese pear (*Pyrus pyrifolia* Nakai)

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Abstract The development of single nucleotide polymorphism (SNP) markers in Japanese pear (Pyrus pyrifolia Nakai) offers the opportunity to use DNA markers for marker-assisted selection in breeding programs because of their high abundance, codominant inheritance, and potential for automated high-throughput analysis. We developed a 1,536-SNP bead array without a reference genome sequence from more than 44,000 base changes on the basis of a largescale expressed sequence tag (EST) analysis combined with 454 genome sequencing data of Japanese pear 'Housui'. Among the 1,536 SNPs on the array, 756 SNPs were genotyped, and 609 SNP loci were mapped to linkage groups on a genetic linkage map of 'Housui', based on progeny of an interspecific cross between European pear (Pyrus communis L.) 'Bartlett' and 'Housui'. The newly constructed genetic linkage map consists of 951 loci, comprising 609 new SNPs, 110 pear genomic simple sequence repeats (SSRs), 25 pear EST-SSRs, 127 apple SSRs, 61 pear SNPs identified by the "potential intron polymorphism" method, and 19 other loci. The map covers 22 linkage groups spanning 1341.9 cM with an average distance of 1.41 cM between markers and is anchored to

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K. Kurita · H. Kanamori · Y. Katayose National Institute of Agrobiological Sciences, Tsukuba, Ibaraki 305-8602, Japan reference genetic linkage maps of European pears and apples. A total of 514 contigs containing mapped SNP loci showed significant similarity to known proteins by functional annotation analysis.

Keywords Genetic linkage map  $\cdot$  Genome-wide SNPs  $\cdot$  GoldenGate  $\cdot$  Housui  $\cdot$  Linkage map

## Introduction

High-density genetic linkage maps constructed with genomewide molecular markers are useful for fruit tree breeding programs, as the implementation of marker-assisted selection (MAS) can accelerate the selection process and reduce the progeny size and the cost of raising individuals to maturity in the field (Luby and Shaw 2001). Attempts to use MAS, however, are just beginning and remain limited to the selection of a few simply inherited traits, especially in fruit trees, because marker development for MAS via bi-parental quantitative trait locus (QTL) mapping is hindered by the same complications as phenotyping for traditional breeding. New genomic-based strategies using genome-wide molecular markers such as genome-wide association study (GWAS) and genomic selection (GS) (Meuwissen et al. 2001) have now emerged as powerful tools (Grattapaglia and Resende 2011; Kumar et al. 2012; Kumar et al. 2013).

The family Rosaceae is a medium-sized family of flowering plants, including about 3,000 species in 100 genera. Many economically important products are derived from the Rosaceae, including many edible fruits (apples, apricots, plums, cherries, peaches, pears, raspberries, and strawberries) and ornamental trees and shrubs (Hummer and Janick 2009). Draft genome sequences for apple (*Malus×domestica* Borkh., Velasco et al. 2010), the woodland strawberry (*Fragaria vesca*, Shulaev et al. 2011), and peach (*Prunus persica* (L.)

Batsch, http://www.rosaceae.org/) were the first to be reported. Recently, genome sequences have become available of Chinese pear (*Pyrus bretschneideri* Rehd.) (Wu et al. 2013).

Pears (*Pyrus* spp.) have been one of the most important fruit trees in Europe, East Asia, and North America for up to 3,000 years and are commercially grown in around 50 temperate-climate countries (Bell et al. 1996). Pear, like the other pipfruit species apple, belongs to the subfamily Spiraeoideae, tribe Pyreae, sharing a basic chromosome number of x=17 which indicates a polyploid origin. It was shown that a relatively recent (>50 million years ago) genome-wide duplication has resulted in the transition from 9 ancestral chromosomes to 17 chromosomes in the Pyreae and that the ancestral paleohexaploidy of eudicots was supported (Velasco et al. 2010). Pear genotypes are highly heterozygous, because of the self-incompatibility character (Kikuchi 1929).

Owing to these advances in the sequencing of plant genomes, single nucleotide polymorphisms (SNPs) have become a practical choice in genetic analysis and are routinely used as markers in many genetic applications, including the detection of genotype-phenotype associations, the construction of genetic maps, and marker-assisted breeding (Gupta et al. 2001). SNPs are abundant throughout the genome and suited to automated detection (Chen and Sullivan 2003), and represent codominant markers with a simple, well-defined mutation model (Brookes 1999). SNPs have a low mutation rate and fewer detection or evaluation errors than simple sequence repeats (SSRs; Yu et al. 2011) and are often transferable across species within a genus (Grattapaglia et al. 2011). They are far more prevalent than SSRs and therefore may provide a high density of markers near loci of interest. SNPs are now considered useful markers in ecological and evolutionary studies of non-model species (Morin et al. 2004).

Large numbers of SNP markers are currently available for many plant species, including *Arabidopsis thaliana* (Schmid et al. 2003), maize (*Zea mays* L.) (Tenaillon et al. 2001), rice (*Oryza sativa* L.) (Feltus et al. 2004), and soybean (*Glycine max* Merr.) (Zhu et al. 2003). Among Rosaceae fruit trees, the 8K SNP array developed in apple (Chagné et al. 2012) also includes 1K pear SNPs (Montanari et al. 2013) and there is a 9K SNP array in peach (Verde et al. 2012) and a 6K array in cherry (Peace et al. 2012). However, although pears (*Pyrus* spp.) are among the most important fruits worldwide, the number of SNP markers developed in pears is still low.

In this study, we developed 1,300 SNPs from expressed sequence tags (ESTs) and 236 SNPs from genome sequences for a GoldenGate assay of Japanese pear 'Housui'. More than 600 SNPs were used to construct a genetic linkage map of 'Housui'. Future applications of the new genome-wide SNP markers are discussed.

#### Materials and methods

Plant materials and extraction of nucleic acids

Japanese pear 'Housui' (syn. 'Hosui') was used both for RNA sequencing (EST analysis) and genome sequencing. We constructed 11 cDNA libraries, representing leaf bud, leaf, flower bud, flower before opening, and flower at full bloom, as well as fruitlets at three developmental stages: immature fruit, fruit at optimum maturity for eating, and overripe fruit (Nishitani et al. 2009a). Total RNA was extracted by the hot borate method (Wan and Wilkins 1994), and  $poly(A)^+$  RNA was purified with a FastTrack 2.0 kit (Invitrogen, USA).

Genomic DNA was isolated from young leaves with a genomic DNA buffer set and Genomic-tip 20/G anion-exchange columns (Qiagen, Germany) as described in Yamamoto et al. (2006).

The progenies of an interspecific cross between European pear (*Pyrus communis* L.) 'Bartlett' and 'Housui' ( $63 F_1$  plants) were used to construct the genetic linkage map of 'Housui' (Terakami et al. 2009). All plant materials were maintained at the NARO Institute of Fruit Tree Science (NIFTS, Ibaraki, Japan).

#### Pyrosequencing of cDNA and genomic DNA

Approximately 3  $\mu$ g of adaptor-ligated complementary DNAs (cDNAs) from each of the 11 libraries was sheared via nebulization into small fragments, and then, fragments ranging from 400 to 1,000 bp in length were recovered. cDNAs were sequenced on a Roche/454 Genome Sequencer (GS)-FLX Titanium platform (Roche Diagnostics, Germany; Margulies et al. 2005). Total genomic DNA of 'Housui' was sheared by nebulization (600 to 900 bp in length), amplified by emulsion PCR, and pyrosequenced on the Roche/454 GS-FLX Titanium platform as described in Kim et al. (2012).

#### SNP discovery and bead array construction

The Roche library adaptors were screened and masked with the CROSS\_MATCH utility (Ewing and Green 1998; http:// www.phrap.org/), using the parameters -minmatch 12 -penalty -2 -minscore 20. All sequences were screened for contaminants and trimmed by the SeqClean script (http://compbio. dfci.harvard.edu/tgi/software/) using the Japanese pear chloroplast genome (Terakami et al. 2012, NCBI accession AP012207), mitochondrial gene sequences of tobacco (*Nicotiana tabacum*, BA000042), and the Univec database (http://www.ncbi.nlm.nih.gov/VecScreen/UniVec.html) with default parameters. Invalid sequences shorter than 40 bp were removed. After screening and trimming, the EST sequences were assembled using the MIRA v. 3.0.5 sequence assembler (Chevreux et al. 2004, http://sourceforge.net/apps/ mediawiki/mira-assembler) with the commands -job= denovo,est,normal,454 -fasta -noclipping -notraceinfo -LR:eq=none -AS:mrl=40 -SK:pr=98 -AL:mo=40 -AL:mrs=95. The genome was assembled by the PCAP.REP assembler (Huang et al. 2006) with default parameters. Contigs of <100 bases in length, singletons, and any unassembled or "debris" sequences were excluded from further analyses. Bi-allelic SNPs identified from contigs using GigaBayes software (Marth et al. 1999) were filtered according to quality value (QV >20), position on the contigs (>100 bp apart), distance from contig ends (>100 bp), read depth of coverage (>6), and allele frequency (0.33–0.67).

To choose SNPs in non-exon regions for an alternative approach, we excluded contigs of genome sequences matching EST contigs. The obtained contig sequences harboring SNPs obtained from both exon and non-exon regions were then submitted to the online Assay Design Tool (ADT; Illumina, USA) for analysis. SNPs with an ADT score below 0.6 were discarded, and 2,786 SNPs on exon and 1,406 SNPs on non-exon regions remained. Finally, a total of 1,536 SNPs with an ADT score of >0.6 were used to design a 1,536-plex custom oligo pool assay (Illumina) (Fig. 1). Of the 1,536 SNPs, 1,300 were positioned in gene exons and the other 236 in non-exon regions. These newly developed SNPs are denoted "JPsnpHou." All of the pyrosequence data (.sff files) were deposited in DDBJ Sequence Read Archive (DRA001738).

## SNP genotyping

SNPs were genotyped using an Illumina GoldenGate Genotyping Assay. The scanned data were analyzed by the Genotyping module (v. 1.9.4) of Illumina GenomeStudio (v. 2011.1) software to generate genotype data for individuals. Clustering of SNPs was adjusted by eye when necessary. Acceptable SNPs had scores of "GenTrain score" $\geq$ 0.5, "call freq" $\geq$ 0.85, "P-P-C errors"=0, and "minor freq" $\geq$ 0.01.

#### Genome mapping of SNP markers

Molecular markers used for construction of the genetic linkage map of 'Housui'—pear SNPs, pear SSRs, apple SSRs, and other markers—are listed in Table 1 as well as the 609 newly designed markers. They include 61 SNP markers developed by the potential intron polymorphism (PIP) method, designed from putative intron information of apple ESTs (Terakami et al. 2013). AFLP markers, which were denoted "*Eco*R I primer/*Mse* I primer-fragment size" in the previous linkage map of 'Housui' (Terakami et al. 2009), were excluded.

The new 'Housui' genetic linkage map was constructed using JoinMap v. 4.0 software (Van Ooijen 2006), with a pseudo-testcross strategy (Grattapaglia and Sederoff 1994). An independence logarithm of odds (LOD) score of 5.0 was used to define linkage groups (LGs). The regression mapping algorithm was used to build the linkage maps, and map distances were calculated according to Kosambi's mapping function (Kosambi 1944). The linkage map was drawn in MapChart 2.2 software (Voorrips 2002).

The contigs that generated SNPs on the genetic linkage map were functionally annotated by similarity searches against the NCBI non-redundant protein (Nr) database (http://www.ncbi.nlm.nih.gov) using the BLASTX algorithm with an E-value cutoff of 1E–15. A BLASTN analysis against the draft genome of the Chinese pear (*P. bretschneideri* Rehd., BioProject; PRJNA157875, Pbr\_v1.0) was also performed to search for a top hit scaffold associated with the contigs.

### Results

De novo assembly of pyrosequenced ESTs and genome DNA

We obtained 497,325 sequences from the 11 cDNA libraries, with an average sequence length of 373.5 bases (range, 40–1,196). They yielded a total sequenced length of 185 Mb. After poly(A/T) and adapter sequences were removed, 484,361 EST sequences (97.4 %) remained for assembly. This generated 46,606 contigs consisting of 359,959 sequences. Contig lengths averaged 573.6 bp (range, 100–2,258). The *N50* size was 595 bp. A total of 854 contig sequences (1.8 %) were longer than 1,000 bp. The average depth of contigs was 7.72, and 7,545 contigs (16.2 %) were assembled from more than 10 sequences.

We generated 1,267,079 sequences from the genomic DNA, with an average sequence length of 417.4 bases, yielding a total sequenced length of 529 Mb, which is equivalent to the haploid genome of *P. bretschneideri* (Wu et al. 2013). Chloroplast and mitochondrial genomes, present at the respective ratios of 2.6 and 1.1 %, were excluded from further analysis. Assembly generated 83,787 contigs consisting of 316,084 sequences. Contig lengths averaged 667.3 bp (range, 100–3,596). The average depth of contigs was 3.77, and 2,753 contigs (3.3 %) were assembled from more than 10 sequences.

### SNP discovery and detection

The assembly of cDNA sequences revealed 24,031 base changes in putative heterozygous alleles of 'Housui' derived from 8,777 EST contigs. Similarly, the assembly of genome sequences revealed 20,882 base changes in putative

Fig. 1 Flowchart for designing the 1,536-plex custom oligo pool assay (OPA). Raw reads obtained by the GS-FLX Titanium platform were treated to mask library adaptors and to exclude low-QV and short reads. The resulting clean reads were assembled with the MIRA or PCAP.REP sequence assembler. Bi-allelic SNPs were identified from assembled contigs using GigaBayes software. Candidate SNPs were filtered according to several criteria



heterozygous alleles of 'Housui' derived from 7,365 contigs. After filtering, 5,215 candidate SNPs remained.

Optimization resulted in 2,786 SNPs from exon regions (ESTs) and 1,406 SNPs from non-exon regions (genome sequences). From these, 1,536 SNPs were selected (ADT score >0.60; mean, 0.86). Each SNP was chosen from a unique contig. Of the 1,536 SNPs, 1,300 were developed from exon regions and 236 from non-exon regions.

## SNP genotyping and mapping

A total of 756 SNPs from the 1,536-SNP GoldenGate bead array were successfully genotyped and exhibited a heterozygous genotype. Among them, 617 were heterozygous for 'Housui' and homozygous for 'Bartlett', and showed clear segregation within the mapping population. The remaining SNPs were unmapped or ungenotyped. Linkage analysis allowed 609 of these markers to be located in 'Housui' LGs. The remaining eight markers were not mapped to LGs. Detailed information on the mapped SNPs is given in supplementary materials (Table S1).

The new genetic linkage map of 'Housui' consists of 951 loci, comprising 609 new SNPs (denoted as JPsnpHou), 110 pear genomic SSRs, 25 pear EST-SSRs, 127 apple SSRs, 61 pear SNPs determined by the PIP method, and 19 other loci (Fig. 2). The map covers 22 LGs spanning 1,341.9 cM with an average distance of 1.41 cM between markers (Table 2). Twenty LGs are anchored to the reference genetic linkage maps of European pear (Yamamoto et al. 2007) and apple (Celton et al. 2009a; Liebhard et al. 2003; Silfverberg-Dilworth et al. 2006). Subgroups of three LGs (Ho2, Ho5, and Ho12), with an independence LOD score of 3.0, are not grouped. Two LGs (uk-1 and uk-2) are not anchored to any LGs of reference maps. The mapped SNP markers seem to be distributed among all LGs without bias. Segregation of 127 loci was distorted (P < 0.05), and many molecular marker loci in LGs Ho2-1 and Ho2-2 were distorted.

 Table 1
 Designations for markers mapped in the genetic linkage map of Housui

Marker type	Marker designation	Reference
Pear SNPs	JPsnpHou	This study
	TsuSNP	Terakami et al. 2013
Pear EST-SSRs	TsuENH	Nishitani et al. 2009b
Pear genomic-SSRs	TsuGNH	Yamamoto et al. 2013
	BG, HG, KA, NB, NH, RLG	Yamamoto et al. 2002
	EMPc	Fernandez-Fernandez et al. 2006
	IPPN	Inoue et al. 2007
Apple SSRs	CH, MS	Liebhard et al. 2002
	AF, AJ, AT, AU, U, Hi	Silfverberg-Dilworth et al. 2006
	NZ	Guilford et al. 1997
	NZms	Celton et al. 2009
	MEST	Moriya et al. 2012
Others	TsuRTP	Kim et al. 2012
	SIMS18, Slocus, leafc	Yamamoto et al. 2002

#### Discussion

High-throughput systems for screening genetic markers are essential for genetic studies and plant breeding. In the Rosaceae, the International RosBREED SNP Consortium developed an 8K genome-wide SNP array for apple (Chagné et al. 2012), selecting 27 apple cultivars to represent worldwide breeding germplasm and re-sequencing them at low coverage. Alignment of these sequences with the whole genome sequence of 'Golden Delicious' (Velasco et al. 2010) enabled the consortium to establish 7,867 apple SNPs (Chagné et al. 2012). Khan et al. (2012) developed a highthroughput 1,536-EST-derived SNP GoldenGate genotyping platform in apple, containing 1.411 genic SNPs and 125 genomic SNPs. The International Peach SNP Consortium resequenced the whole genome of 56 peach breeding accessions through the use of next-generation sequencing platforms (Verde et al. 2012) and developed a 9K SNP array (Verde et al. 2012). Martínez-García et al. (2013) evaluated a set of 1,536 SNPs through a GoldenGate genotyping assay of peach (P. persica (L.) Batsch), which was developed from the whole genome sequences of three cultivars. The RosBREED Consortium also developed a 6K SNP array for diploid sweet cherry (Prunus avium) and allotetraploid sour cherry (Prunus cerasus) (Peace et al. 2012). Our large-scale transcriptomebased analysis of Japanese pear in conjunction with genome sequencing data allowed us to develop a 1,536-SNP array without a reference genome sequence. It will be interesting to integrate our transcriptome-based analysis and Chinese pear genome information. Montanari et al. (2013) reported that 1,096 SNPs were developed from three European pear cultivars by using new-generation sequencing technology and that SNPs obtained showed high transferability across several *Pyrus* spp. Our 609 new SNP markers will complement the 829 SNP markers reported in Montanari et al. (2013) to segregate in interspecific *Pyrus* progenies, which enable the discovery of associations between marker loci and traits, the identification of the genetic architecture of quantitative traits, investigation of genetic variation, and GS in pear.

Of the 1,536 SNP loci, 609 (39.6 %) were successfully validated for genome mapping, resulting in the development of a high-density genetic linkage map comprising 951 loci. The remaining SNPs were ungenotyped or unmapped, resulting from several reasons, i.e., the unexpected homozygous allele combination for 'Housui' (27.6 %), the heterozygous genotypes for both parents of 'Housui' and 'Bartlett' (9.0 %), unassigned SNPs to 17 LGs (0.5 %), ambiguous clustering (4.5 %), and the low signal for scoring (18.7 %). A rather low success ratio of SNP mapping (39.6 %) in our study may be improved by the use of transcriptome and genome sequencing for plural cultivars. Furthermore, only 8 out of 256 SNPs (3.1 %) developed from non-exon regions that could be positioned on the 'Housui' map, compared with 601 out of 1,300 SNPs (46.2 %) that showed successful genome mapping from exon regions. This may be consistent with the report that the randomly distributed SNPs tended to show a lower success rate than SNPs located within coding regions in the apple 8K SNP array (Chagné et al. 2012). Mapping of new SNP markers enables the newly constructed map to increase about threefold in marker loci, decrease to two fifths in average distance between markers, and expand 168 cM in map distance, compared with the previous map (Terakami et al. 2009). In our previous report, three particular genomic regions (LGs 4, 5, and 12) were identified showing short length of map distance, which may be due to homozygous regions (Terakami et al. 2009). The new map could expand the three LGs, from 9.7 to 18.5 cM in LG 4, from 23.2 to 48.9 cM in LG 5, and from 0 to 27.5 cM in LG 12, even if LGs 5 and 12 were divided into two parts. The GoldenGate assay has also been used to evaluate SNPs for the creation of high-density genetic linkage maps in other crops: Khan et al. (2012) mapped 569 new SNPs of 1,536 GoldenGate genotyping platform along with 447 other markers, constructing of new genetic maps for 'Co-op 16' and 'Co-op 17' of apple. Khan et al. (2012) also reported that markers common across five apple genetic maps resulted in successful positioning of 2,875 markers, consisting of 2,033

**Fig. 2** Genetic linkage map of Japanese pear Housui. The *number to the* left of each marker indicates genetic distance (cM). Markers designated in green are developed from pear and markers in red from apple. New markers developed in this study are denoted by JPsnpHou. Distorted segregation is indicated by a significant P value of the  $\chi^2$  test: \*P=0.05, \*\*P=0.01, \*\*\*P=0.005

Ho1	
Ho1	

0.0		- TsuRTP004
8.4 10.0 13.3 16.6	7	⊣ JP snpH ou00098 JP snpH ou00062 <sup>∽</sup> <b>NH013a</b> ⊣ JP snpH ou00442 JP snpH ou00117 ⊣ <mark>CH03g12-m3</mark> JP snpH ou00057
21.6	+	TsuGNH064 TsuGNH102 JP snpHou00536 JP snpHou00422 T suSNP1134
24.9	1	<ul> <li>JP snpH ou00521</li> <li>JP snpH ou00629*</li> </ul>
29.7	Æ	- JP snpHou00511
33.6	++	– JP snpH ou00477
38.7		JPsnpHou00273 JPsnpHou00514
43.8	1	MEST120
47.2	A	T suSNP100219 T suSNP10011 SMS18 JP snpH ou00441 JP snpH ou00217 JP snpH ou00649 TsuGNH095
5Z.Z		Tel CNH219 MEST160
57.3		MEST193
64.0 65.6	$\downarrow$	TSUGNHOO6 JP snpH ou00408 TSUGNH250 JP snpH ou00639 TSUSNP1113
67.3	A	KA4b JP snpHou00376
67.9	//T	JP snpHou00308
68.8 72 0	ŰЦ	IP snpH ou00621 I suSNP1009
77.5	///	JP snpH ou00628
80.1	11	<ul> <li>JP snpH ou00608</li> <li>JP snpH ou00066</li> <li>JP snpH ou00016</li> </ul>
04.9 88.1		
89.8	-0	- JP snpH ou00132

Ho5	
Ho5-	1

0.0 —	JP snpH ou00389**
11.0 12.7	JP snpH ou00251 JP snpH ou00053
20.7 21.9 23.6 26.2	TsuENH086 JP snpH ou00510 JP snpH ou00444 HE04d02-m1 JP snpH ou00104
32.7 —	TSUSNP1076 TSUSNP1138
37.6 — 40.9 —	UP snpH ou00247 JP snpH ou00439 <b>TsuGNH187</b> <b>CH04f04</b>

## Ho5-2

0.0 —	JP snpH ou00413
3.2 —	MEST109 JP snpH ou00641
8.0 —	JP snpH ou00305*

## Ho6 Ho6

Ho2

0.0 4.9 6.5 9.8

14.7 -16.4 ~

18.0 -21.3 23.5

0.0 ~

3.3 ~ 6.6 -10.0 -117 -15.0 16.7 21.7 -25.0 -

26.7

36.7 40.1

589 59.1 60.1 62.5

Ho2-2

JP snpH ou00310\*\*\*

Ho2-1

JP snpH ou00154\*\* JP snpH ou00652\*\* T suSNP 1030\*\* C+Ode120\*\* JP snpH ou00214\*\* CNSs1493SR\*\*\* JF snpH ou00378\*\*\* JP snpH ou00171\*\*\* JP snpH ou00378\*\*\* JP snpH ou00494\*\*\* MEST87\*\*\*

 MEST28\*\*\*
 JP snpH ou00357\*\*\*
 JP snpH ou00374\*\*\*

 JP snpH ou00357\*\*\*
 JP snpH ou00357\*\*\*
 JP snpH ou00387\*\*\*

 JP snpH ou00591\*\*\*
 JP snpH ou00288\*\*\*
 JP snpH ou00540\*\*\*

JP snpH ou00310\*\*\*
 BGT 23b\*\*\* H24f04\*\*\*
 JP snpH ou00110\*\*\*
 JP snpH ou00627\*\*\*
 JP snpH ou0075\*\*\*
 JP snpH ou00565\*\*\*
 JP snpH ou00565\*\*\*
 JP snpH ou00565\*\*\*
 JP snpH ou0029\*\*\*

- <mark>NZnsEB149908\*\*\*</mark> JPsnpHou00388\*\* <mark>CH03d10\*\*</mark> H<mark>01c11-m1</mark>\*\* TsuSNP1168\*\* JPsnpHou00275\*\* CH02b10\*\*

JP snpH ou00437 JP snpH ou00532 JP snpH ou00312 JP snpH ou00524 TSUENH087 JP snpH ou00101 JP snpH ou00382

JP snpH ou00614 **TsuGNH239** JP snpH ou00336 JP snpH ou00427 **NZnsEB107305-m1** JP snpH ou00458 JP snpH ou00150 JP snpH ou00575

0.0	✓ JP snpH ou00585 J JP snpH ou00302 JP snpH ou00358 JP snpH ou00236
9.0 11.2 12.8 14.4 16.1 19.4	<ul> <li>JP snpH ou00337</li> <li>JP snpH ou00535</li> <li>JP snpH ou00518 TsuGNHD43</li> <li>TsuGNH097 TsuGNH213</li> <li>JP snpH ou00199</li> <li>JP snpH ou00482</li> </ul>
29.8         33.9           35.8         39.5           34.6         46.3           49.7         52.8           56.1         57.8           61.1         57.8           61.1         57.8           61.1         57.8           61.1         57.8           77.0         77.0           78.1         79.7	TsuSNH225 ml           UP snpHou00316           UP snpHou00315           UP snpHou00315           UP snpHou00315           UP snpHou00315           UP snpHou00323           UP snpHou00423           UP snpHou00423           UP snpHou00423           UP snpHou00423           UP snpHou00418           UP snpHou00418           UP snpHou00418           UP snpHou00418           UP snpHou00283           UP snpHou0083           UP snpHou0083           UP snpHou0083           UP snpHou00505           UP snpHou00505           UP snpHou00502           UP snpHou00502

Ho	3
00 1.6 4.9 100 11.6 15.0 16.6 19.9 21.6 23.2 24.9 26.5 28.2 29.8	Turner 1120 JP snpH ou00179 JP snpH ou00205 JP snpH ou00261 JP snpH ou00261 JP snpH ou0085 JP snpH ou00353 JP snpH ou003531 JP snpH ou00142 JP snpH ou00355 JP snpH ou000355 JP snpH ou000182 JP snpH ou00146 JP snpH ou00355 JP snpH ou00640 JP snpH ou00465 JP snpH ou00650 JP snpH ou00465 JP snpH ou00650 JP snpH ou00465 JP snpH ou00550 JP snpH ou00274 JP snpH ou0092 JP snpH ou00272 JP snpH ou00095 JP snpH ou00275 JP snpH ou00469 JP snpH ou00556 CH02408
36.6 38.3 40.0 41.6 45.0	JP snpHou00204 NH023a JP snpHou00292 JP snpHou00645 TsuGN+1153 JP snpH ou00142 JP snpHou00231 JP snpH ou00653 JP snpHou00866 TsuENH074 TsuGN+220
58.4	NB113a JP snpH ou005 /0 NH203a T suR TP 021 T suGNH225 T suGNH244- m1 MESTED
60.5 / 69.6 / 71.3 / 73.0 / 76.3 / 79.6 / 81.2 / 82.9 / 84.5 / 86.2 / 87.8 / 91.1 / 92.8 / 95.9 / 101.1 /	JP snpH ou00215* JP snpH ou00648 JP snpH ou00630* JP snpH ou00630* JP snpH ou00630* JP snpH ou00630* JP snpH ou00682 JP snpH ou00684 JP snpH ou00430 JP snpH ou00432 JP snpH ou00432 JP snpH ou00432 JP snpH ou00432 JP snpH ou00432 JP snpH ou0055 JP snpH ou00551 JP snpH ou00551 JP snpH ou00551 JP snpH ou00551 JP snpH ou00551 JP snpH ou00551 JP snpH ou0051 JP s
102.8 104.4 106.1	U snipi ou00117 JP snipi ou00317 JP snipi ou00306 JP snipi ou00356 JP snipi ou00301 CH03012- m4 JP snipi ou00387 JP snipi ou00097

Ho3

## Ho7 Ho7

0.0 JP snpH ou00513 3.0 JP snpH ou00544 4.0 JP snpH ou00560 JP snpH ou00623 5.5 JS	
23.8 JP snpH ou00167	
27.1 JP snpH ou00122	
43.2 JP snpH ou00615	
44.7 TSUGNH090 NZmcCN943067	
46.4 JP snpHou00428	
51.8 JP snpH ou00468	
MEST37	
54.4 JP snpH ou00379 JP snpH ou00605	
57.1 JP snpHou00350 TsuGNH159	
58.7	
60.1 JEMPc111 JP snpH ou00497	
70.8 JP snpHou00631* 71.4 JP snpHou00293* NZmcEB137749	*
72.1 JP snpH ou00109*	
74.0 JP snpH ou00049 JP snpH ou00500	
79.8 JP snpH ou00520 JP snpH ou00042	
86.9 JP snpH ou00644	
87.8 / V JP snpH ou 00525	

## Ho4 Ho4

0.0	/ JP snpH ou00501
20 \ /	TsuSNP1171 JPsnpHou00508
2.0 \A	TsuSNP1131 JPsnpHou00253
4.2	JP snpH ou00158
7.5	TsuENH014*
07.	JP snpH ou00550* JP snpH ou00129*
9.1 V	JP snpH ou00072*
11.2	MEST91-m1 JP snpH ou00085
12.7	JP snpH ou00450*
127/4	NB131a CH02c02h
13.7 1	Tsi GNH078 Hi23d11b-m1
16.9 // \	JP snpH ou00579
18.5	TsuGNH076

## Ho8 Ho8

0.0	/ JP snpH ou00106
\ .	/ JP snpH ou00237 JP snpH ou00555
3.3 \/_/	JP snpH ou00625 JP snpH ou00578
	/ JP snpH ou00484
4.9	JP snpH ou00111
/T	JP snpH ou00646 JP snpH ou00606
5.5 /	JP snpH ou00617 JP snpH ou00233
A	NH036b
10.0 //日	JP snpH ou0006/* EMPc116*
100 11	I JP shph ou00360*
10.9	Demail autobic
12.5	ID smpH ou00200*
140	Dependence of the second secon
174	
10.0	
19.0	I I Dann Hau 00220 I Dann Hau 001E0
20.7	JP SIDE OU00323 JP SIDE OU00133
224	IP sppH ou0000
24.0	
24.0	IP snpH ou00244
25.5	IP snpH ou00244
27.2	IP snpH ou00221
501 /H	of onprodoczer
50 1 2/1 1/	LEOOLOO
44.9	Hi20b03
44.9	Hi20b03 NH022a NH005b MEST62, pt
44.9	Hi20b03 NH022a NH005b MEST62-m1 JP snpH ou00068
44.9 46.6 49.9	Hi20b03 NH022a NH005b MEST62-m1 JP snpHou00068 JP snpHou00162 Hi01c11-m2
44.9 46.6 49.9	<ul> <li>H20b03</li> <li>NH022a</li> <li>NH005b MEST62-m1.</li> <li>JP snpH ou00068</li> <li>JP snpH ou00162 H01c11-m2.</li> <li>JP snpH ou00267 JP snpH ou00512.</li> </ul>
44.9 46.6 49.9 53.1	H20b03 NH005b MEST62-m1 JP snpH ou00068 JP snpH ou00162 H <u>601c11-m2</u> JP snpH ou00267 JP snpH ou00512 JP snpH ou002553 JP snpH ou00410
44.9 46.6 49.9 53.1	H20003 NH022a NH005b MEST62-m1 UP snpHou00082 JP snpHou00182 H01c11-m2 JP snpHou00267 JP snpHou00512 UP snpHou00595 JP snpHou00512 T suSNP1179
44.9 46.6 49.9 53.1 53.3	H2003 NH022A NH0025 MEST62-m1 JP snpH ou0068 JP snpH ou0068 JP snpH ou00675 H0162 H01612 JP snpH ou00595 JP snpH ou00512 JP snpH ou00595 JP snpH ou00410 IT suSNP 1179 JP snpH ou00456
44.9 46.6 49.9 53.1 53.3 54.9	H2003 NH025 MEST62-mL UP snpHou0062 H01c11-m2 UP snpHou00162 H01c11-m2 UP snpHou00162 H01c11-m2 UP snpHou00267 JP snpHou00512 UP snpHou00595 JP snpHou00410 T suSNP1179 UP snpHou00456 T suR1P005
36.1 44.9 46.6 49.9 53.1 53.3 54.9 56.5	H2003 NH0225 MEST62-mL UP snpt1ou0068 UP snpt1ou0068 UP snpt1ou0067 UP snpt1ou0067 JP snpt1ou00512 UP snpt1ou0057 JP snpt1ou00512 UP snpt1ou00595 JP snpt1ou00410 UP snpt1ou00456 Fault 1P 005 UP snpt1ou00456
36.1 44.9 46.6 49.9 53.1 53.3 54.9 56.5	H2023           NH025         MEST62-m1           JP snpH ou00068         JP snpH ou00162           JP snpH ou00267         JP snpH ou00267           JP snpH ou00559         JP snpH ou00410           T suB NP 1179         JP snpH ou00456           JP snpH ou00456         T suB NP 1179           JP snpH ou00456         JP snpH ou00450           JP snpH ou00456         JP snpH ou00456           JP snpH ou00456         JP snpH ou00456           JP snpH ou00456         JP snpH ou00456
36.1 44.9 46.6 49.9 53.1 53.3 54.9 56.5 58.1	H2023           NH0025         MEST62-ml.           VH0025         MEST62-ml.           VB-snP1000068         UP-snP10000612           UP-snP1000067         UP-snP10000512           UP-snP1000057         UP-snP10000512           UP-snP1000595         UP-snP10000512           UP-snP1000595         UP-snP10000512           UP-snP1000595         UP-snP10000512           UP-snP1000507         UP-snP10000207           UP-snP10000507         UP-snP10000207           UP-snP1000038         UP-snP10000207           UP-snP1000038         UP-snP10000507
36.1 44.9 46.6 49.9 53.1 53.3 54.9 56.5 58.1	H2003           NH025         MEST62-m1.           JP snpH ou0068         JP snpH ou0068           JP snpH ou0059         JP snpH ou00512           JP snpH ou00595         JP snpH ou00216           JP snpH ou00595         JP snpH ou00252           JP snpH ou00507         JP snpH ou00207           JP snpH ou00507         JP snpH ou00282           JP snpH ou00507         JP snpH ou00506           MEST128         JP snpH ou00506
36.1           44.9           46.6           49.9           53.1           53.3           54.9           56.5           58.1           59.8	H2003           NH0025         MEST62-m1.           VH0025         MEST62-m1.           VB-snP1 ou0068         UP snP1 ou0068           UP snP1 ou0067         UP snP1 ou00612           UP snP1 ou0057         UP snP1 ou00612           UP snP1 ou00565         UP snP1 ou00656           UP snP1 ou00566         UP snP1 ou00456           UP snP1 ou00560         UP snP1 ou00207           UP snP1 ou00580         UP snP1 ou00207           UP snP1 ou00398         UP snP1 ou00207           UP snP1 ou00398         UP snP1 ou00206           UP snP1 ou00398         UP snP1 ou00206           UP snP1 ou00398         UP snP1 ou00506
36.1           44.9           46.6           49.9           53.1           53.3           54.9           56.5           58.1           59.8           61.4	H2003           NH0025         MEST62-mt.           VH0025         MEST62-mt.           VB-sn04000068         UP snpHou00068           UP snpHou00162         H01c11-m2.           UP snpHou00267         JP snpHou00512           UP snpHou00265         JP snpHou00512           UP snpHou00267         JP snpHou00512           UP snpHou00267         JP snpHou002612           UP snpHou00266         JP snpHou00207           UP snpHou00169         JP snpHou00207           UP snpHou00169         JP snpHou00207           UP snpHou00388         JP snpHou002082           UP snpHou00575         JP snpHou005067           JP snpHou00576         JP snpHou005067           JP snpHou00576         JP snpHou00506           JP snpHou00576         JP snpHou00506           JP snpHou00576         JP snpHou00506           JP snpHou00576         JP snpHou00576
36.1           44.9           46.6           49.9           53.1           53.3           54.9           56.5           58.1           59.8           61.6	H2003           NH0025         MEST62-m1.           VP snpH ou0068         JP snpH ou00762           JP snpH ou00767         JP snpH ou00671           JP snpH ou00267         JP snpH ou000512           JP snpH ou00267         JP snpH ou000512           JP snpH ou00267         JP snpH ou00410           TsuSNP1 19         SnpH ou00456           JP snpH ou00519         JP snpH ou00270           JP snpH ou00507         JP snpH ou00202           JP snpH ou00398         JP snpH ou002082           JP snpH ou002475         JP snpH ou002475           JP snpH ou002475         JP snpH ou002475
36.1           44.9           46.6           49.9           53.1           53.3           54.9           56.5           58.1           59.8           61.4           71.4	H2003           NH0025         MEST62-m1.           VP8070         VB snpt000068           UP snpt1000068         UP snpt10000612           UP snpt1000267         UP snpt10000512           UP snpt1000267         UP snpt10000512           UP snpt1000267         UP snpt10000512           UP snpt1000267         UP snpt10000512           UP snpt1000267         UP snpt10000261           UP snpt10000169         UP snpt10000207           UP snpt10000382         UP snpt10000282           UP snpt1000388         UP snpt10000506           MEST128         UP snpt10000163           UP snpt1000715         TELGNH057           UP snpt10000388         UP snpt10000510           UP snpt1000238         UP snpt1000515           UP snpt1000336         UP snpt1000336
30.1 44.9 46.6 49.9 53.1 53.3 54.9 56.5 58.1 59.8 61.4 61.6 61.4 61.4 771.4 74.9	H2003           NH0025         MEST62-m1.           VP snpH ou0066         JP snpH ou0067           JP snpH ou0067         JP snpH ou00612           JP snpH ou0057         JP snpH ou00612           JP snpH ou0067         JP snpH ou0027           JP snpH ou0067         JP snpH ou00287           JP snpH ou0067         JP snpH ou00282           JP snpH ou0067         JP snpH ou00282           JP snpH ou00475         TsuCR PN19           JP snpH ou00475         TsuCR PN19           JP snpH ou00282         JP snpH ou00284           JP snpH ou00284         JP snpH ou00284           JP snpH ou00284         JP snpH ou00284           JP snpH ou00284         JP snpH ou00284
30.1 44.9 49.9 53.1 53.1 53.3 54.9 56.5 58.1 58.1 59.8 61.4 61.6 74.9 80.0	H2003           NH0025         MEST62-m1.           VP snpH ou00162         H01211-m2.           VP snpH ou00267         VP snpH ou00261           VP snpH ou00456         T sult TP 005           VP snpH ou00308         VP snpH ou00207           VP snpH ou00388         VP snpH ou00282           VP snpH ou00388         VP snpH ou00282           VP snpH ou00475         T sult TP 305           VP snpH ou00475         T sult NP 305           VP snpH ou00475         T sult NP 305           VP snpH ou00472         VP snpH ou00524           VP snpH ou00224         VP snpH ou00380           CH01100         SnpH ou00359
30.1         44.9           44.9         53.1           53.3         54.9           56.5         58.1           59.8         61.4           61.4         61.4           71.4         74.9           80.0         85.0	H2003           NH0025         MEST62-m1.           VP snpH ox0066         JP snpH ox0067           JP snpH ox0067         JP snpH ox00612           JP snpH ox0057         JP snpH ox0067           JP snpH ox0067         JP snpH ox00207           JP snpH ox0057         JP snpH ox002082           JP snpH ox00224         JP snpH ox0027           JP snpH ox0027         JP snpH ox0027           JP snpH ox0027         JP snpH ox0027           JP snpH ox00224         JP snpH ox00227           JP snpH ox00224         JP snpH ox00226           JP snpH ox00224         JP snpH ox00227           JP snpH ox00226         JP snpH ox00226           JP snpH ox00227         JP snpH ox00226           JP snpH ox00266         JP snpH ox00259
30.1 44.9 45.4 49.9 53.1 53.1 53.3 54.9 56.5 58.1 58.1 59.8 61.4 61.6 61.4 61.6 71.4 71.4 71.9 85.0 91.2	H2003           NH0025         MEST62-m1.           VP snpH ou00162         H01c11-n2.           VP snpH ou0068         VP snpH ou00612           VP snpH ou0067         JP snpH ou00612           VP snpH ou0067         JP snpH ou00612           VP snpH ou00595         JP snpH ou00612           VP snpH ou00596         JP snpH ou0027           VP snpH ou00456         TsuR TP 005           VP snpH ou00389         JP snpH ou00207           VP snpH ou00389         JP snpH ou00282           VP snpH ou00389         JP snpH ou00506           MEST128         PPNH P           VP snpH ou00274         VP snpH ou00274           VP snpH ou00274         VP snpH ou00380           VP snpH ou00274         VP snpH ou00328           VP snpH ou00274         VP snpH ou00326           VP snpH ou00274         VP snpH ou00326           VP snpH ou00476         VP snpH ou00359           VP snpH ou00476         VP snpH ou00475           VP snpH ou00466         VP snpH ou00475



## Ho13

0.0	∫ JP snpH ou00118***
2.9	TsuGNH174 JP snpH ou00202 TsuGNH170 T suSNP 1013
16	JP snpH ou00417
7.0	JP snpH ou00526* JP snpH ou00210*
1.0	U// JP snpH ou00206*
9.3	U/JP snpH ou00145* JP snpH ou00419*
10.9	CH05h05-m1**
12.5	JP snpH ou00077
12.7 -	CH05-06- m2** TsuSNP1156**
14.4	JP snpHou00113**
	NZmsEB111793* CH02q01*
160	CHO2eO2* T suSNP 1086*
10.0	TsuSNP022* JPsnpHou00584*
	I suSNP1149* JP snpH ou00564*
	→ JP snpH ou00270*
17.5 -	JP snpH ou00554**
19.0	JP snpH ou00616*
19./ 1	IP snpH ou00216***
25.5	- JP snpHou00548
27.2	JP snpH ou00084 JP snpH ou00307
28.8	JP snpH ou00264
33.8 1	T suSNP 1184 .IP snpH ou00108
35.5	JP snpH ou00303 JP snpH ou00517
38.7	JP snpH ou00364
40.3	MEST3 MEST168
45.4	H JP snpH ou00504 JP snpH ou00163
46.9	JP snpH ou00637
48.5	JP snpH ou00060
55.8	JP snpH ou00033
58.8	MEST153
64.1	JPsnpHou00055 TsuGNH212
65.7 <sup>J</sup>	JP snpH ou00334

## Ho14

Ho10

(NH020a-m2 NH206a UP snpH ou00043 NH017a UP snpH ou0043 UP snpH ou00313 UP snpH ou00313 UP snpH ou00481 JP snpH ou00285

I suSNP10/4 NHD455\_CH02-11 UP snpHou00339 UP snpHou00433 UP snpHou00453 JP snpHou00327 UP snpHou00636 JP snpHou00239 UP snpHou002630 JP snpHou002537 UP snpHou002450 JP snpHou00537

□ μ<sup>o</sup> snph ou00536 JP snph ou00239 JP snph ou00537 **Ts EN103** JP snph ou0045 JP snph ou00537 JP snph ou00259 Ts EN103 JP snph ou00259 Ts EN103 JP snph ou00242 **CH03d11** EN105 EGT24 TsUSNP 1082 JP snph ou00430 JP snph ou00431 JP snph ou00073 JP snph ou00211 JP snph ou00073 JP snph ou0051 JP snph ou00521 JP snph ou0052 JP snph ou00510 JP snph ou00230 JP snph ou00230 JP snph ou00230 JP snph ou00215 JP snph ou00191 JP snph ou00115 JP snph ou00215 JP snph ou00215 JP snph ou00215 JP snph ou00191 JP snph ou00115 JP snph ou00216 JP snph ou00216 JP snph ou00216 JP snph ou00215 JP snph ou0025 JP snph ou0025

 UP srphou00363

 The srph cov Trailer M cov

 UP srph cov 288 JP snpH cu00377

 JP snph cu0286 JP snpH cu00599

 JP snph cu0242

 JP snph cu00561

 Mest Troza

 JP snph cu00383

 JP snph cu00296

 JP snph cu00228

 JP snph cu00228

 JP snph cu0228

 JP snph cu0227

Hi03e04-m1. MEST038

EMPc114 Hi03f06

CH01f12 JP snpH ou00581 T suSNP 1074

0.0 6.8 8.5 10.2 12.8 16.0

16.3

18.0

22.9 24.6 25.2 29.8

31.3

34.6

35.8

364

38.0

39.6

40.5

41.4

42.4 57.3

59.0

60.7

62.3 65.7

67.4 72.3 74.0 75.6

77.3 78.9 82.2 83.7 85.2

0.0 4.5 7.7 10.9 12.9 14.2 14.6 18.1	JP snpHou00249* TsuEN+058* JP snpHou002474 TsuEN+1058* PPPN01** MESTO41** IsuSR1003* JP snpHou00597* JP snpHou00563* JP snpHou00564* JP snpHou00564* JP snpHou00564* JP snpHou00564*
19.6 20.9 24.0 25.1	T suSNP 1043 TsuGNH025 JP snpH ou00519* JP snpH ou00593* JP snpH ou00134
33.6 35.2 36.8 41.6	CHOICOS TSUGNH029- m1. T suR TP010 CHO302 UP snpH ou00381 JP snpH ou00392 UP snpH ou00381 JP snpH ou00287 TSUGNH022 CHO406 JS suR TP002 JP snpH ou00463
49.3 50.9 52.4 53.9 56.7	JP srpHou00103 - JP srpHou00431 - JP srpHou00258 - JP srpHou00256 - JP srpHou00265 JP srpHou00480 - JP srpHou00409 - MS01a05
69.7 —	JP snpH ou00284 CH03g06 JP snpH ou00366

001	JP snpH ou00046
23	JP snpH ou00304
3.8	MEST070 CH04b02
5.5	JP snpH ou00498
7.1 🔬	JP snpH ou00569
8.8 1/1	IPPN02
137.	JP snpH ou00326 JP snpH ou00165
10.7 WE	IJP snpH ou00596 JP snpH ou00454
15.4	∭ JPsnpHou00078*
17.0	JP snpH ou005 / 1*
18.7	JP snpH ou00402* 1 suS NP 1093*
20.4 -	I JP SnpH ou00592 JP SnpH ou00187
23.7 🚛	IP shph ou00270 JP shph ou00056
\\ <del>\</del>	IP snpH ou00131 IP snpH ou00323
28.7 \\_	Por sipirououror or sipirououzoz
30 4 🖑	JP snpH ou00562 JP snpH ou00451
32.0	JP snpH ou00140 JP snpH ou00455
· · · · ·	JP snpH ou00390 NB111a-mB
Y	JP snpH ou00459 T suSNP 1025
35.3 🔨	JP snpH ou00052 JP snpH ou00262
	JP snpH ou00566 MEST44
A_	I JP snpH ou00340
37.0 //_	// JP snpH ou00195
37.6 -	JP SnpH ou00123
40.2 🕼 –	ID ann H au 00201
41 o ///=	IP snpH ou00201
1.3 //	
436 ///	IPsnnHou00178 CHOEcOD
43.6 // 46 9 //	JP snpH ou00178 CH05c02 TsuR TP 001
43.6	JPsnpHou00178 CH05c02 TsuRTP001 TsuRTP011 TsuRTP018
43.6 46.9 48.6	UPsnpHou00178 CH05c02 TsuRTP001 TsuRTP011 TsuRTP018 H04c11 TPPN14
43.6 46.9 48.6	UPsnpHou00178 CH05c02 TsuRTP001 TsuRTP011 TsuRTP018 H04g11 JPPN14 TsuGNH164 TsuSNP1050
43.6 46.9 48.6 50.3	UP snpHou00178 CH05c02 TsuRTP001 TsuRTP011 TsuRTP018 H04g11 JPPN14 TsuGNH164 TsuSNP1050 TsuGNH229 JP snpHou00151
43.6 46.9 48.6 50.3	I JP snpHou00178 CH05c02 TsuR TP001 TsuR TP011 TsuR TP018 H04g11 JPPN14 TsuGNH164 I suSNP 1050 TsuGNH129 JP snpH ou00151 TsuGNH229 JP snpH ou00151
43.6 46.9 48.6 50.3	JP enpHou00178 CH05C02 TsuRTP001 TsuRTP011 TsuRTP018 H04g11 PP014 TsuCRH64 IsuSNP1050 TsuCRH229 JP snpHou00151 TsuERH044 JP snpHou00373 JP snpHou00373
43.6 46.9 50.3 55.2 56.8	M JP snpHou00178 CH05c02 TsuRTPO1 TsuRTPO11 TsuRTP018 H04g11 PPN14 TsuGNH154 JBSNP1050 TsuGNH229 JP snpHou00151 TsuGNH229 JP snpHou00351 JP snpHou00333 JP snpHou00346 JP snpHou00333 JP snpHou00346
43.6 46.9 50.3 55.2 56.8 57.5	(JP smpHou00178 CHOSCO2) I suRT PO01 T suRT PO11 T suRT PO18 HO4211 PPN14 T suGNH229 UP smpHou00151 T suGNH229 UP smpHou00151 T suGNH229 UP smpHou00151 JP smpHou00353 JP smpHou00346 UP smpHou00478 JP smpHou00478
43.6 46.9 50.3 55.2 56.8 57.5	M JP snphou00178 CH05c02 T suR TP001 T suR TP011 T suR TP018 H04c11 PP014 T suCH1220 JPsnphou0051 T suCH1220 JPsnphou0051 T suCH1220 JPsnphou00351 JP snphou00353 JP snphou003478 JP snphou00458 JP snphou00457 JP snphou00478 JP snphou00457
43.6 46.9 50.3 55.2 56.8 57.5 58.5	M JP smpHou00178 CH05c02 T suRTP011 T suRTP018 H04211 PPN14 T suGNH24 I suSNP 1050 T suGNH229 JP snpHou00151 T suGNH229 JP snpHou00353 JP snpHou00353 JP snpHou00346 JP snpHou00456 JP snpHou00466 JP snpHou00466 JP snpHou00466
43.6 46.9 50.3 55.2 56.8 57.5 58.5	JP smpHou00178 CH05c02     TsuRTP01     TsuRTP011 TsuRTP018     H04c11 PP014     TsuCAHL04 ISLNP1050     TsuCAHL02 JPSnpHou00151     TsuCAHL02 JPSnpHou00373     JP smpHou00373     JP smpHou00353 JP smpHou00448     JP smpHou00153 JP smpHou00478     JP smpHou00457 JP smpHou00468     JP smpHou00257 JP smpHou00063     JP smpHou00158 JP smpHou00054     JP smpHou00158 JP smpHou00054     JP smpHou00158     JP smpHou00158     JP smpHou00157     JP smpHou00157     JP smpHou00157     JP smpHou00157     JP smpHou00157     JP smpHou00157     JP smpHou00057     JP smpHou00157     JP smpHou0015     JP smpHou00057     JP smpHou00057     JP smpHou00054     JP smpHou0015     JP smpHou0015     JP smpHou00054     JP smpHou0015     JP s
43.6 46.9 50.3 55.2 56.8 57.5 58.5 58.5 61.8	(JP smpHou00178 CHOSCO2 T surk PPO1 T surk PPO1 T surk PPO11 T surk PPO14 T surk PH14 T surk PH14 T surk PH14 T surk PH14 (JP smpHou0037 JP smpHou0037 JP smpHou0037 JP smpHou0037 JP smpHou0037 T surk PO2 P smpHou0038 JP smpHou0038 JP smpHou00068 JP smpHou00054 Mescri 17 Surk PD2 SmpHou0054 JP smpHou00054 JP smpHou00054 JP smpHou00054 JP smpHou00054 JP smpHou00054 JP smpHou00054 JP smpHou0054 JP
43.6 440.9 50.3 55.2 56.8 57.5 58.5 61.8 63.1	JP serpHou00178 CHOSC02     TauR TP01     TauR TP018     TauR TP018     TauR TP018     TauR TP018     TauR TP018     TauR TP014     TauR TP019     TauR TP019     TauR TP019     TauR TP019     TauR TP01     JP serpHou00373     JP serpHou00373     JP serpHou00373     JP serpHou00153     JP serpHou00153     JP serpHou00153     JP serpHou00153     JP serpHou00153     JP serpHou00257     JP serpHou00154     JP serpHou00154     JP serpHou00154     JP serpHou00154     JP serpHou00164     JP serpHou00154     JP serpHou0154     JP serpHou0154     JP serpHou0154     JP serpHou0154     JP serpHou0154
43.6 446.9 50.3 55.2 56.8 57.5 58.5 58.5 61.8 63.1 63.1	(JP smpHou00178 CHOSCO2 T surk PPO1 T surk PPO11 T surk PPO11 T surk PPO11 T surk PPO15 T surk PP15 T surk PP15 T surk PP15 JP smpHou00353 JP smpHou00353 JP smpHou00353 JP smpHou00446 JP smpHou00446 JP smpHou00446 JP smpHou0063 RLC-mB JP smpHou0063 JP smpHou00054 MESTIT 7 surk PP 1017 JP smpHou00330 NHD24P m1
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JP snpH ou00212 TsuENH040 JP snpH ou00138 JP snpH ou00107 CH0200 PMEST102 NB102a JP snpH ou00143 JP snpH ou00328 TsuENH124 JP snpH ou00405 TsuENH016

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Ho11

## Ho12-1

## Ho12-2



## Ho16

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16.4	JP snpH ou00234 JP snpH ou00368 TsuENH022 JP snpH ou00059
19.6	- JP snpH ou00228
22.9	TsuENH036 CH05c06-m8 JP snpH ou00294 JP snpH ou00173 JP snpH ou00166
39.1	JP snpH ou00324
43.7	JP snpH ou00112 TsuGNH120
44.0	JP snpH ou00225 JP snpH ou00530
45.3	JP snpH ou00280 JP snpH ou00099
61.9	JP snpH ou00528
63.5	JP snpH ou00070 JP snpH ou00155 JP snpH ou00189
77.2	NB116b
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859

#### Ho17 JP snpH ou00472 JP snpH ou00489 JP snpH ou00509 JP snpH ou00271 CH05g03 JP snpH ou00341 0.0 1.6 NB125a TsuENH033 27 AJ0016815SR N2715MDAJ1681 TsuENH002 UP snpH ou00557 JP snpH ou00315 JP snpH ou00516 AT0001745SR JP snpH ou00281 JP snpH ou00365 T suSNP 1118 40 5.4 AF527800SSR MEST69 TSUENH026 JP snpH ou00438 SEE663955 JP snpH ou00144 6.2 7.0 7.6 8.5 10.1 CH01h01 JP snpH ou00246 NH015a JP snpH ou00393 CH04c10 TsuGNH061 CH04C10 TsuGNH061 TsuGNH226 JP snpH ou00183 JP snpH ou00485 CH04c06-m1 JP snpH ou00503 JP snpH ou00088 10.2 11.6 14.3 16.0 19.1 HGT6 MEST20 NB126 JP snpH ou00200 JP snpH ou00487 JP snpH ou00069 JP snpH ou00318 JP snpH ou00391 20.8 24.3 32.1 JP snpH ou00256 367 TsuGNH045 JP snpH ou00213 T suSNP 009 40.1 Hi07e08-m1 TsuSNP1100 JPsnpHou00546 TsuRTP003 TsuRTP016 JPsnpHou00319 41.8 45.1 47.2 48.7 50.3 51.0 leafc MEST29 JP snpH ou00580 JP snpH ou00479 JP snpH ou00483 JP snphotu0452 T suSNP 1180 JP snpHou00452 JP snpHou00613 T suSNP 1073 JP snpHou00116 JP snpHou00311 JP snpHou00515 JP snpHou00372 NH014a JP snpHou00243 52.0 52.8 53.5 55.1 IsuENH028 56.7 TsuGNH245 JP snpH ou00640 JP snpH ou00440 58.3 JP snpH ou0022 JP snpH ou0012 59.1 JP snpH ou00125 **TsuGNH179** JP snpH ou00533 JP snpH ou00626 JP snpH ou00486 JP snpH ou00590 JP snpH ou00124 59.9 JP snph ou00105 JP snph ou00105 S bcus JP snph ou00632 JP snph ou0059 CN444542SSR JP snph ou00572 JP snph ou00149 JP snph ou00547 60.4 61.4 63.1 64.7 66.4 68.0 NZmEB137525 JP snpH ou00574 JP snpH ou00240 **(EST096**

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uk-2

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Fig. 2 (continued)

SNPs and 843 SSRs, and that the total length of the consensus map was 1,991 cM. Martínez-García et al. (2013) identified 738 of 1,037 SNP markers on the Pop-DF map of peach. Shirasawa et al. (2010) mapped 1,137 markers, including 793 genotyped SNPs, in two mapping populations of tomato. Hyten et al. (2008) designed a custom 384-SNP assay through the re-sequencing of five soybean accessions, evaluated it with three mapping populations of recombinant inbred lines, and genotyped 89 % of the SNP loci in the complex soybean genome. Jones et al. (2009) used a 768-marker multiplex assay on the GoldenGate platform to create a high-resolution genetic map in maize.

SNPs have several advantages over other molecular markers, including their high abundance in the genome, codominant mode of inheritance, and high integrity. In the previous study, we applied apple PIP marker information (Wu et al. 2007) to develop SNPs in Japanese pear (Terakami et al. 2013), since apple and pear, which belong to the same tribe, Pyreae, showed genome-wide synteny (Yamamoto et al. 2001; Silfverberg-Dilworth et al. 2006;

Marker type	Linka	ge grot	dn																			
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ear SNPs (this study)	29	12	22	59	10	10	с. С	36 3	5 4	7 19	) 51	57	2	11	40	25	55	24	54	5	9	609
Pear SNPs (PIP method)	5	1	1	3	2	2	0	5	ŝ	4	9	5	0	3	9	2	9	1	5	0	0	11
Pear EST-SSRs	1	0	1	1	1	1	0	-	0	3	Э	-	0	0	1	1	7	2	4	0	0	5
Pear genomic-SSRs	8	1	2	13	3	1	0	6	8	7	6	8	0	0	4	9	13	4	12	0	0	10
Apple SSRs	4	5	9	7	3	5	-	~	-	5	15	6	-	Ц	6	7	14	З	16	0	0	27
Others	2	0	1	1	0	0	0	0	1	1	0	3	0	1	0	2	Э	0	4	0	0	6
fotal number of loci	49	19	33	84	19	16	4	55 4	8	9	) 84	83	3	16	09	43	93	34	95	2	9	151
Jenetic distance (cM)	89.8	23.5	62.5	106.1	18.5	40.9	8.0	3 1.67	9.4 9	1.2 10	01.1 85	.2 92.4	4 3.2	24.3	65.7	69.7	134.4	80.5	68.0	4.8	3.0	341.9
Marker density (markers/cM)	0.55	0.81	0.53	0.79	1.03	0.39	0.50	0.69 (	.54 0	.72 0.	39 0.9	)6 <sup>.</sup> 0 6(	0 0.94	0.66	0.91	0.62	0.69	0.42	1.40	0.42	2.00 (	0.71)
Average distance between markers (cM)	1.83	1.24	1.89	1.26	0.97	2.56	2.00	1.45 1	.86 1	.38 2.	59 1.(	1.1 10	1 1.07	1.52	1.10	1.62	1.45	2.37	0.72	2.40	0.50 (	1.41)

Summary of the genetic linkage map of Japanese pear Housui

Table 2

Celton et al. 2009b). The use of PIP markers designed from apple ESTs enabled the mapping of 55 markers on the genetic linkage map of 'Bartlett' and 61 in 'Housui'. However, in the present study, we established a more robust approach for developing the larger numbers of genome-wide SNP markers that will be needed for introducing GWAS and GS into Japanese pear breeding.

Kumar et al. (2012) evaluated the accuracy of GS in a population of seven full-sib families comprising 1,120 seedlings genotyped for 2,500 SNPs using the 8K apple SNP array and phenotyped for six fruit quality-related traits (fruit firmness, soluble solids, russet, weighted cortex intensity, astringency, titratable acidity). They observed accuracies ranging from 0.67 (astringency) to 0.89 (soluble solids) for BLUPbased selection, demonstrating that GS is a credible alternative to conventional selection for fruit quality traits. It was suggested that a higher SNP density and a larger training population might capture more genetic variation with higher accuracy (Kumar et al. 2013). Kumar et al. (2013) tried GWAS using the same genotyped 1,200 apple seedlings, demonstrating that the genomic regions could be identified with significant effects for several fruit quality traits and pointing out the value of GWA-significant SNP-trait associations in a breeding population for MAS. Iwata et al. (2013) examined the potential of GWAS and GS using 76 Japanese pear cultivars genotyped with 162 DNA markers including 155 SSRs and phenotyped for nine agronomic traits, using multilocus Bayesian models for analysis in Japanese pear. Correlations between phenotypic values and predicted genotypic values were significantly detected for harvest time, resistance to black spot, and the number of spurs with the accuracies of 0.75, 0.38, and 0.61, respectively. Two associations to known loci for resistance to black spot and harvest time were correctly identified. It was noted that neither the number of markers nor genotypes used were sufficient to conduct full-scale GWAS and to train a prediction model for GS. The more than 600 SNP markers newly obtained are a valuable resource which will enable the implementation of MAS and will contribute to complete the further application of GWAS and GS in Japanese pear breeding programs.

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#### **Data submission**

All of the pyrosequence data (.sff files) were deposited in DDBJ Sequence Read Archive (DRA001738).