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Establishment of an optimized set of 406 microsatellite markers covering the whole genome for the Japanese population

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Abstract Microsatellites, an essential tool for genetic linkage analyses, are selected in genetic studies on the basis of both informativeness and their positions with respect to one another on the genetic map. In order to establish a microsatellite marker set useful for linkage studies in the Japanese population, we first genotyped 64 unrelated Japanese subjects, using 400 microsatellite markers from a commercially available set (ABI PRISM Linkage Mapping Set-MD10) and then determined the allelic frequencies and heterozygosities for these marker loci in the population. In order to optimize the set, we replaced 41 markers having a heterozygosity lower than 0.6 with as many informative markers in the corresponding loci, and newly added six markers in the set to minimize the several gaps found at intervals of over 20 cM. We finally established a set comprising 406 microsatellites with average intervals of 9 cM (maximum, 17 cM) and minimum heterozygosities of over 0.6 (mean, 0.76). All data generated in this study, including the specific polymerase chain reaction (PCR) primer sequences of the newly added markers, are freely available to all researchers at our web site. The genetic tool established here should facilitate genetic linkage studies of various hereditary diseases, especially in the Japanese.

Key words Microsatellite · Heterozygosity · Linkage analysis · Genotyping · Ethnic difference

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Introduction

Genetic mapping locates one gene locus relative to another, the position of which is known by the frequency of recombination for Mendelian traits, or the concordance or discordance of affected sib pairs for polygenic traits. Because microsatellites are distributed prolifically throughout the genome and are highly variable in repeat length and in polymorphisms, they have become powerful tools for the genetic mapping of disease susceptibility loci. Several screening sets of microsatellite markers have been described in the literature (Reed et al. 1994; Levitt et al. 1994; Dubovsky et al. 1995; Yuan et al. 1997), and such marker sets designed for genome-wide screening have recently become available commercially. Because the allelic frequency

Table 1. Distribution of heterozygosities of microsatellite markers in the original ABI PRISM Linkage Mapping Set MD-10 before optimization

	Frequency (%)	
Heterozygosity	Japanese	Caucasians
<0.5	5.5	0
0.5–0.6	5.3	0.5
0.6–0.7	18.9	8.7
0.7–0.8	35.4	38
>0.8	34.9	52.8
>0.6	89.2	99.5

Table 2. Comparison of the original marker set and our optimized marker set

	Original set	Optimized set
Quantity of markers	400	406
Intermarker distance of over 20 cM	Four positions	0
Maximum interval	26.1 cM	17.0 cM
Average heterozygosity	0.726	0.756
Markers with heterozygosity lower than 0.6	40 Markers	1 Marker
Observed heterozygosity	0.11–0.93	0.55–0.93

Table 3. Heterozygosities of tested markers

Locus	Het	cM
D1S468	0.71	6.2
D1S214	0.72	16.4
D1S450	0.85	22.9
D1S2667	0.82	26.9
D1S507	**0.83**	**36.2**
D1S2697	*0.53*	*39.9*
D1S199	0.77	47.7
D1S234	0.81	56.6
D1S496	**0.87**	**65.6**
D1S255	*0.27*	*66.6*
D1S2797	0.78	77.6
D1S2890	*?*	*87.7*
D1S2700	**0.83**	**89.3**
D1S230	0.61	97.4
D1S2841	0.83	108.8
D1S207	0.83	117.6
D1S2868	0.61	129.9
D1S206	0.78	137.6
D1S2726	0.72	149.0
D1S252	0.8	155.1
D1S498	0.73	160.7
D1S484	0.68	173.9
D1S2878	0.84	181.7
D1S196	0.72	186.4
D1S218	0.82	196.5
D1S238	0.81	206.7
D1S413	0.63	216.5
D1S249	0.67	225.1
D1S425	0.61	235.3
D1S213	0.85	246.2
D1S2800	0.71	256.1
D1S2785	0.85	269.7
D1S2842	0.72	277.3
D1S2836	0.68	290.1

Locus	Het	cM
D2S319	0.72	6.0
D2S2211	0.64	14.0
D2S162	0.79	21.3
D2S168	0.8	28.6
D2S305	0.77	40.7
D2S165	0.86	50.7
D2S367	0.87	58.3
D2S2259	0.62	67.4
D2S391	0.71	73.8
D2S337	0.84	84.1
D2S2368	0.83	89.2
D2S286	0.74	98.4
D2S2333	0.84	107.7
D2S2216	0.72	115.3
D2S160	0.71	127.4
D2S347	0.61	135.7
D2S112	0.6	145.8
D2S151	0.77	156.4
D2S142	0.73	166.3
D2S2330	0.84	175.5
D2S335	0.84	182.5
D2S364	0.78	192.9
D2S117	0.88	201.4
D2S325	0.79	210.9
D2S2382	*0.5*	*220.7*
D2S164	**0.65**	**222.0**
D2S126	0.8	228.8
D2S396	0.85	240.2
D2S206	0.8	248.3
D2S338	0.81	258.7
D2S125	0.81	269.5

Locus	Het	cM
D3S1297	0.76	2.5
D3S1304	0.79	16.5
D3S1263	0.89	30.4
D3S2338	0.73	36.3
D3S1266	0.66	46.9
D3S1277	0.69	56.1
D3S1289	0.81	69.1
D3S1300	0.81	79.0
D3S1285	0.76	91.0
D3S1566	0.84	97.2
D3S3681	0.82	108.8
D3S1271	0.6	117.7
D3S1278	0.7	131.8
D3S1267	0.65	141.1
D3S1292	0.89	148.7
D3S1569	0.79	162.0
D3S1279	0.62	173.0
D3S1614	0.67	183.1
D3S1565	0.8	193.0
D3S1262	0.72	207.2
D3S1580	0.84	213.7
D3S1601	0.79	220.4
D3S1311	0.73	230.7

Locus	Het	cM
D4S412	0.68	3.7
D4S2935	0.64	12.2
D4S3036	**0.78**	**23.1**
D4S403	*0.49*	*24.9*
D4S419	0.69	32.6
D4S391	0.78	43.2
D4S405	0.75	56.7
D4S1592	0.78	68.4
D4S392	0.82	77.9
D4S2964	0.7	87.1
D4S1534	0.8	93.5
D4S414	0.75	99.2
D4S1572	0.83	106.3
D4S406	0.7	115.8
D4S402	0.83	123.5
D4S1575	*0.11*	*131.9*
D4S3039	**0.82**	**131.9**
D4S424	0.76	143.8
D4S413	0.62	157.9
D4S1597	*0.35*	*169.1*
D4S2979	**0.65**	**170.9**
D4S2991	**0.81**	**179.6**
D4S1539	*0.39*	*181.2*
D4S415	0.73	185.0
D4S1535	0.76	198.5
D4S426	0.72	211.0

Locus	Het	cM
D5S1981	0.76	0.6
D5S406	0.73	10.7
D5S630	0.9	18.6
D5S416	0.64	27.9
D5S419	0.87	39.5
D5S426	0.78	51.6
D5S418	0.78	58.1
D5S407	0.86	65.0
D5S647	0.82	74.7
D5S424	0.68	82.8
D5S641	0.81	92.3
D5S428	0.68	95.4
D5S644	0.83	104.5
D5S433	0.75	112.2
D5S2027	0.59	118.9
D5S471	0.71	129.6
D5S2115	0.74	138.6
D5S436	0.75	147.2
D5S640	**0.79**	**152.8**
D5S410	*0.57*	*156.0*
D5S422	0.81	163.9
D5S400	0.88	174.3
D5S1960	**0.73**	**179.1**
D5S408	0.68	195.8

Locus	Het	cM
D6S1574	0.73	8.7
D6S309	0.76	13.6
D6S470	0.72	17.7
D6S289	0.81	29.6
D6S422	0.67	35.7
D6S276	0.73	44.9
D6S1610	0.78	53.9
D6S1575	**0.84**	**60.7**
D6S452	**0.85**	**72.2**
D6S257	0.88	80.0
D6S460	0.8	90.0
D6S462	*0.53*	*99.0*
D6S300	**0.71**	**103.5**
D6S434	0.78	109.2
D6S287	0.68	122.0
D6S262	0.77	129.8
D6S292	0.85	138.2
D6S308	0.65	145.5
D6S441	0.79	155.3
D6S1581	*0.43*	*165.0*
D6S305	**0.82**	**166.6**
D6S1719	**0.77**	**177.9**
D6S264	*0.4*	*179.1*
D6S446	0.55	188.4
D6S281	0.81	201.1

Locus	Het	cM
D7S531	0.77	4.8
D7S517	0.79	7.8
D7S513	0.9	17.7
D7S507	0.82	29.1
D7S493	0.73	35.0
D7S516	0.76	42.1
D7S484	0.79	55.6
D7S510	0.82	60.5
D7S519	0.74	70.5
D7S502	0.85	79.6
D7S669	0.83	90.9
D7S630	0.77	98.7
D7S657	0.77	105.2
D7S515	0.75	112.9
D7S486	0.76	125.3
D7S530	0.72	136.4
D7S640	0.85	139.7
D7S684	0.78	149.6
D7S661	0.84	157.5
D7S636	0.93	165.0
D7S798	0.75	171.3
D7S2465	0.77	182.1

Locus	Het	cM
D8S264	0.83	0.7
D8S277	0.81	8.4
D8S550	0.72	20.4
D8S549	*0.52*	*30.7*
D8S1731	**0.7**	**30.7**
D8S258	0.68	40.3
D8S1771	0.68	49.6
D8S505	0.77	60.0
D8S285	0.7	70.6
D8S260	0.76	78.8
D8S543	**0.73**	**86.7**
D8S1705	**0.75**	**94.3**
D8S270	0.7	102.1
D8S1784	0.65	116.8
D8S514	0.77	128.9
D8S284	0.8	142.7
D8S272	0.8	152.5

Locus	Het	cM
D9S288	0.81	8.8
D9S286	0.75	16.8
D9S285	0.62	27.9
D9S157	0.83	31.8
D9S171	*0.32*	*42.0*
D9S265	**0.63**	**42.0**
D9S161	*0.56*	*50.3*
D9S1678	**0.75**	**50.3**
D9S1817	0.86	57.9
D9S273	*0.54*	*64.5*
D9S166	**0.75**	**65.0**
D9S175	0.62	68.8
D9S167	0.84	82.4
D9S283	0.73	93.2
D9S287	0.64	103.3
D9S1690	0.78	106.5
D9S1677	0.87	117.8
D9S1776	0.76	124.2
D9S1682	0.64	132.9
D9S290	0.66	141.1
D9S164	0.79	148.1
D9S1826	0.82	160.2
D9S158	0.72	163.0

Locus	Het	cM
D10S249	0.82	0.0

Table 3. Continued

D10S591	*0.49*	*12.3*
D10S552	**0.76**	**13.0**
D10S189	0.72	17.3
D10S547	*0.47*	*28.1*
D10S570	**0.73**	**32.1**
D10S1653	0.75	38.8
D10S548	0.59	43.4
D10S197	0.72	50.5
D10S208	0.79	60.2
D10S196	0.7	72.5
D10S1652	0.7	83.3
D10S537	0.82	93.8
D10S1686	0.66	109.2
D10S185	0.78	123.3
D10S192	0.84	131.2
D10S597	*0.51*	*137.6*
D10S1269	**0.64**	**140.2**
D10S1693	0.82	146.1
D10S587	0.82	156.6
D10S217	0.82	167.2
D10S1651	0.64	178.3
D10S1711	**0.61**	**180.5**
D10S212	*0.37*	*180.7*
Locus	Het	cM
D11S4046	0.85	3.9
D11S1338	0.62	14.9
D11S902	0.84	24.7
D11S904	0.71	37.0
D11S935	0.72	49.6
D11S905	0.81	55.7
D11S4191	0.88	63.4
D11S987	0.84	67.5
D11S1314	0.79	77.5
D11S937	0.76	84.6
D11S901	0.68	89.8
D11S4175	0.84	96.3
D11S898	*0.41*	*103.1*
D11S1339	**0.7**	**104.8**
D11S908	*0.4*	*112.5*
D11S4111	**0.8**	**112.9**
D11S925	0.81	123.5
D11S4151	0.61	132.9
D11S910	**0.72**	**145.6**
D11S1320	*0.27*	*147.2*
D11S968	*0.46*	*152.8*
D11S4125	**0.74**	**152.8**
Locus	Het	cM
D12S352	0.68	0.0
D12S99	0.81	13.9
D12S336	0.74	21.0
D12S364	0.81	31.7
D12S310	0.64	36.1
D12S1617	0.84	45.1
D12S345	0.84	54.4
D12S85	0.8	62.7
D12S368	0.66	67.3
D12S83	0.81	76.5
D12S326	0.61	87.6
D12S351	0.74	97.1

D12S346	0.73	106.1
D12S78	0.79	113.3
D12S79	0.8	126.1
D12S86	0.68	135.1
D12S324	0.64	148.3
D12S1659	*0.57*	*157.2*
D12S367	**0.71**	**160.9**
D12S1723	0.79	165.7
Locus	Het	cM
D13S175	0.67	7.4
D13S217	0.67	19.1
D13S171	0.65	27.3
D13S218	0.6	35.3
D13S263	0.81	40.4
D13S153	0.89	47.5
D13S156	0.82	57.3
D13S170	0.83	65.4
D13S265	0.66	70.6
D13S159	0.73	81.5
D13S158	0.73	86.9
D13S173	0.63	95.9
D13S1265	0.83	101.7
D13S285	0.85	112.8
Locus	Het	cM
D14S261	0.59	0.0
D14S283	0.8	7.5
D14S275	0.6	21.9
D14S70	0.67	32.9
D14S288	0.87	39.1
D14S276	0.77	47.0
D14S63	0.76	59.0
D14S258	0.64	65.8
D14S74	0.8	76.4
D14S68	0.83	86.3
D14S280	0.68	95.5
D14S65	0.71	108.1
D14S985	0.72	117.1
D14S292	0.71	124.2
Locus	Het	cM
D15S128	0.85	6.1
D15S1002	0.76	14.5
D15S1048	**0.66**	**19.1**
D15S165	*0.16*	*20.2*
D15S1007	0.82	25.9
D15S1042	**0.78**	**32.3**
D15S1012	*0.56*	*35.3*
D15S994	0.76	40.0
D15S978	0.74	45.5
D15S117	0.74	50.8
D15S153	0.79	62.1
D15S131	0.75	70.7
D15S205	0.88	77.4
D15S127	0.83	84.8
D15S1004	**0.62**	**95.7**
D15S130	*?*	*98.0*
D15S120	0.79	109.6
Locus	Het	cM
D16S423	0.85	8.4
D16S404	0.69	16.7

D16S3075	0.8	21.8
D16S3103	*0.29*	*31.1*
D16S3017	**0.73**	**31.1**
D16S3046	0.65	39.3
D16S3068	0.73	46.6
D16S3136	0.65	60.0
D16S415	0.69	65.6
D16S503	0.66	81.8
D16S515	0.87	90.2
D16S516	0.72	98.3
D16S3091	0.83	109.1
D16S520	0.8	123.3
Locus	Het	cM
D17S849	0.74	0.6
D17S831	0.85	6.6
D17S938	0.82	14.8
D17S1852	0.8	23.2
D17S799	*0.58*	*32.8*
D17S947	**0.85**	**32.8**
D17S921	0.73	37.3
D17S1857	*0.46*	*44.1*
D17S925	**0.71**	**49.5**
D17S798	*0.53*	*53.9*
D17S1872	**0.9**	**58.3**
D17S1868	0.78	65.1
D17S787	0.83	75.7
D17S948	**0.7**	**84.1**
D17S944	*0.55*	*84.2*
D17S949	0.8	94.9
D17S785	0.7	104.7
D17S784	0.6	117.7
D17S928	0.83	128.7
Locus	Het	cM
D18S59	0.8	0.1
D18S63	0.71	7.9
D18S452	0.81	17.7
D18S464	*0.57*	*32.4*
D18S1153	**0.81**	**34.7**
D18S53	0.82	40.4
D18S478	0.65	52.3
D18S1102	0.68	61.7
D18S474	0.72	71.3
D18S64	0.82	83.0
D18S68	0.72	94.4
D18S61	0.81	102.8
D18S1161	0.74	112.0
D18S462	0.72	118.0
D18S70	0.75	123.8
Locus	Het	cM
D19S209	0.82	10.8
D19S894	**0.81**	**15.4**
D19S216	*0.49*	*19.1*
D19S884	0.84	26.0
D19S221	0.81	35.5
D19S226	0.86	41.7
D19S414	0.6	53.2
D19S220	0.87	61.4
D19S420	0.79	66.0
D19S902	0.79	76.2

D19S571	*0.48*	*87.7*
D19S921	**0.84**	**91.7**
D19S418	0.65	97.5
D19S210	0.67	104.9
Locus	Het	cM
D20S117	0.82	2.9
D20S889	0.78	11.0
D20S192	**0.76**	**18.5**
D20S115	*0.56*	*20.9*
D20S186	0.88	33.2
D20S112	0.73	39.3
D20S195	0.74	50.2
D20S107	0.71	54.9
D20S119	0.79	61.0
D20S178	0.77	65.5
D20S196	0.81	74.5
D20S100	0.71	83.4
D20S171	0.71	94.4
D20S173	0.64	96.5
Locus	Het	cM
D21S1256	0.82	8.6
D21S1914	0.81	23.0
D21S263	0.82	31.4
D21S1252	0.82	38.7
D21S266	0.82	49.9
Locus	Het	cM
D22S420	0.7	0.0
D22S539	*0.57*	*9.0*
D22S446	**0.65**	**9.0**
D22S315	0.8	16.2
D22S280	0.79	25.9
D22S283	0.75	33.4
D22S423	0.83	40.2
D22S274	0.83	45.5
Locus	Het	cM
DXS1060	0.85	10.1
DXS8051	0.82	15.7
DXS987	0.74	25.5
DXS1226	0.81	36.8
DXS1214	0.67	46.2
DXS1068	0.64	56.2
DXS993	0.66	66.1
DXS1055	**0.63**	**77.9**
DXS991	0.66	86.9
DXS986	0.87	95.9
DXS990	0.76	104.9
DXS1106	*0.22*	*115.1*
DXS1059	**0.72**	**121.0**
DXS8055	*0.18*	*126.8*
DXS8067	**0.67**	**136.5**
DXS1001	0.73	139.4
DXS1047	0.85	150.3
DXS1227	0.68	164.7
DXS8043	0.7	176.7
DXS8091	*0.5*	*186.3*
DXS1193	**0.73**	**187.7**
DXS1073	0.67	196.5

Het, Heterozygosity; cM, centi-morgans from telomere of p arm
Newly added markers are shown in bold
Markers with heterozygosity under 0.6 are shown in italics and shaded
The genetic positions were obtained from the 1996 Genethon map (Dib et al. 1996)

of each marker is readily available in public databases, the sets were prepared on the basis of information on Caucasians. Such allelic frequencies are known to vary widely among ethnic groups; indeed, Yamane-Tanaka et al. (1998) examined allelic frequencies and heterozygosities of one of the early commercial marker sets by genotyping 32 Japanese subjects, and found marked differences in both the allelic frequency and the heterozygosity of many markers between Caucasians and Japanese. In the present study, we compared the allelic frequencies and heterozygosities of all of the 400 microsatellite markers included in one of the latest marker set versions, and optimized a set for the Japanese population.

Subjects and methods

Genomic DNA was extracted from 64 unrelated Japanese subjects. An ABI PRISM Linkage Mapping Set-MD10 (Applied Biosystems, Foster City, CA, USA), which contains fluorescence-labeled primer pairs for 400 microsatellite markers, was used for genotyping. Polymerase chain reaction (PCR) amplification of each DNA segment of interest was performed in 96-well plates in a volume of 6μl, containing 5ng of genomic DNA, 0.2mM dNTPs, 1.5mM $MgCl_2$, 0.6μl 10 × PCR buffer, 2pmol of each primer, and 0.15U AmpliTaq Gold (Applied Biosystems). After a pre-PCR heating step for 12min at 95°C, 35 cycles of amplification (15s at 94°C for denaturing, 15s at 55°C for annealing, and 30s at 72°C for extension) were performed in GeneAmp 9700 thermal cyclers (Applied Biosystems). The PCR products were combined into pools, analyzed on ABI 377 DNA sequencers, and genotyped using GeneScan (version 3.1) and Genotyper (version 2.5) software (Applied Biosystems). New markers examined were obtained from the Genome Database (GDB, http://www.gdb.org), or one of the other commercial sets (ABI PRISM Linkage Mapping Set-HD5; Applied Biosystems). Genotypic data derived from Caucasians for each marker, which was used as a reference for the selection, was also obtained from the GDB. Primer sequences for the markers were modified to facilitate genotyping, as described (Brownstein et al. 1996).

Results and discussion

Among the 400 microsatellite markers in the original set (ABI PRISM Linkage Mapping Set MD-10), heterozygosity was different in a significant fraction of the markers in Japanese and Caucasians (Table 1). Differences in heterozygosity of over 0.2 between the groups were found in 37 markers (9.3%), most showing lower heterozygosity in Japanese than in Caucasians. The mean heterozygosity of the markers in the original set was 0.73 (range 0.11–0.93) in this study, and 40 markers (10.0%) were found to show heterozygosities lower than 0.6 (Table 2). To our surprise, 5.5% of the markers examined in Japanese showed heterozygosities below 0.5, insufficient for linkage analysis, while such low informativeness was not found in any of the markers in Caucasians. Because all markers included in the sets should be highly informative, the less informative markers were replaced by markers in the same locus with a heterozygosity of over 0.6, with the exception of D6S446 (heterozygosity of 0.55), for which no appropriate substitute could be found in the neighboring region. Because the intermarker interval at four positions was greater than 20 centi-Morgan (cM) (maximum, 26.1cM) in the original set, the interval was filled with six markers, resulting in a maximum interval of 17cM. For two markers (D1S2890, D15S130), sufficient PCR products could not be generated for analysis, possibly because of poor efficiency of amplification with the standard reaction conditions, so these two markers were replaced with alternatives (D1S2700, D15S1004) from the corresponding loci. Finally, a set of 406 microsatellite markers optimal for Japanese, covering the entire genome, with an average interval of 9cM (maximum, 17cM) and minimum heterozygosity of over 0.6 (mean, 0.76), was established (Tables 2 and 3).

The marker set optimized for Japanese described here should provide an informative framework for genome-wide screening for disease susceptibility loci. We have constructed a database of the allelic frequencies and heterozygosities of all of the 447 markers evaluated in this study, as well as the PCR primer sequences of the newly added markers, and have uploaded it to our web site (http://imcr.sb.gunma-u.ac.jp/lab/genetics/suppl.html), where the data are freely available to all researchers.

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