RESEARCH NOTES

Chicken QTL mapping by multiplex PCR*

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Abstract To facilitate rapid determination of the chromosomal location of quantitative trait loci, the current approaches to gene mapping are improved using a multiplex PCR technique. The high-throughput linkage analysis method described here allows selection of 178 from 328 microsatellite markers through the multiplex PCR method combined with the semi-automatic fluorescence-labeled DNA analysis technology. Those polymorphism markers are distributed on 23 autosomes and one sex chromosome (chromosome Z), covering 3080cM genetic distance. The average marker density is 18cM, dispersed into 30 different sets. These selected polymorphism microsatellite markers segregate with the family members, following the Mendel's heritage laws, and are very useful for chicken linkage map analysis as well as for the research on some important economic quantitative characters of chicken.

Keywords: chicken genomic scanning, multiplex PCR, microsatellite marker, genotype.

There are two major methods for locating the quantitative trait loci (QTL) presently. One is the candidate gene approach, which can deduce the possible genes responsible for the certain characters based on the known physiological and biochemical knowledge, then, checking the gene variance in the high-level phenotypic discrimination species or populations. Although this method is easy to manipulate, its effectiveness is limited by the known candidate gene, and it hardly finds new genes. The other one is genomic scanning method, which is to scan a whole genome by a large number of microsatellite markers to look for the polymorphic markers closely linked to the phenotype concerned and to locate these genes on the linkage map through the linkage analysis and QTL. Compared with the candidate gene approach, genomic scanning method requires detailed pedigree information on the family members and a large number of microsatellite markers; therefore, it is very expensive and laborious. Recently, many research groups have located some QTLs influencing meat traits, milk traits and reproduction traits[1] by this method and they have made great efforts to improve this mapping technique. In 1988, Chamberlain et al.[2,3] firstly reported of performing a multiplex PCR (M-PCR), in which several sequences fragments of a gene could be co-amplified in one reaction simultaneously. The key to M-PCR is optimization of amplification conditions. This technique has been widely applied to the research on gene's function in the Human Genome Project (HGP). Combining the M-PCR with semi- or full-automated fluorescent-labeled DNA analysis, Applied Biosystems has launched a series of genomic analysis kits, such as Cofiler™, Profiler Plus™, SGM Plus™ and Identifiler™, which provided useful tools for the evolution and forensic studies. In the research on animals, how to use the valuable information obtained from the HGP to facilitate QTL mapping in animals is the goal to achieve. In this study, we optimized the M-PCR conditions for simultaneously amplifying several sequences with 3 ~ 8 primer pairs in one reaction, and obtained 30 sets including 178 microsatellite markers. With these polymorphic markers, a large-scale chicken genomic scanning was conducted.

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1 Materials and methods

1.1 DNA samples

Genomic DNA was isolated by a Wizard genomic purification kit (Promega) from a chicken family, which contained 40 individuals from F_0 generation, 22 individuals from F_1 generation and 370 individuals from F_2 generation.

1.2 Microsatellite markers

The 5'-end labeled primers with HEX, TET or 6-FAM amidite fluorescence were provided by Dr. Hans Chang, Michigan University, United States.

1.3 Determination of different primer (sets)

According to the fluorescent difference, fragment length and avoiding primers pairing, we performed the PCR both with a single marker or M-PCR amplification for all F_0 individuals. Only the markers revealing the identical amplified fragments in both PCR reactions were selected for genotypic linkage and QTL locating analysis within F_1 and F_2 generations. Based on the amplification results of F_0 generation, the sizes of fragments and genotypes within the population could be determined.

1.4 Multiplex PCR amplification

PCR amplifications were performed in a Perkin Elmer 9700 thermocycler, in a 15 μL volume containing 20ng genome DNA, 0.2 pmol fluorescence labeled primer pair, 5 units of Taq DNA polymerase, 200 mmol/L dNTP, 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 10 mmol/L Triton X-100 and 0.01% gelatin. The program started with an initial 5 min denaturation at 94℃, followed by two or three consecutive PCR (40 s at 94℃, 1 min at annealing temperature, 1 min at 72℃, 10～20 cycles), and a final 40 min extension at 72℃. Different annealing temperatures were set for different primer pairs.

1.5 Electrophoresis

For gel analysis, 1 μL of loading buffer, containing 5∶1∶1 mixture of deionized formamide, blue dextran and GeneScan-350 (TARMA) internal size standard (Perkin Elmer Applied Biosystems) was mixed with 1 μL of PCR product. The samples were heated at 94℃ for 3 min and placed on ice immediately for another 5 min prior to gel loading. Electrophoresis was carried out in an ABI PRISM 377 DNA sequencer, using a 4.5% acrylamide gel containing 6 mol/L urea, and run for 2 hours.

2 Results

2.1 Optimization of multiplex PCR conditions

We attempted to optimize our multiplex PCR conditions by varying the following parameters individually and in combination: the annealing temperature (45～55℃), polymerization time (72℃), the number of cycles (10～20) and the concentration of dNTP (200 mmol/L each). On the basis of these optimization experiments, 178 out of 328 polymorphic microsatellite markers were selected into 30 different sets (Table 1). They could be applied to genotyping via the multiplex PCR technique.

Table 1. Selected polymorphic microsatellite markers

Set	Site							
1	MCW61	MCW78	MCW68	MCW35	MCW18	MCW83		
2	MCW95	MCW123	MCW112	MCW97				
3	ADL268	MCW166	MCW160	MCW32	MCW151			
4	MCW81	MCW23	MCW5	ADL158	MCW98	MCW119	MCW64	MCW176
5	MCW178	MCW94	ADL38	MCW58	MCW193			
6	MCW31	MCW29	ADL299	ADL280	MCW104	MCW34	MCW134	
7	ADL123	ADL304	MCW69	MCW145	MCW137	MCW198		
8	ADL146	ADL147	MCW115	MCW88	MCW200	MCW205	MCW116	
9	MCW10	MCW135	ADL284	MCW222	MCW207	MCW100	MCW36	
10	MCW180	ADL270	ADL289	MCW237	MCW184	MCW218	LEI66	
11	MCW82	MCW37	MCW146	MCW185	MCW80	MCW224		
12	MCW62	ADL290	ADL142	LEI85	MCW214			
13	MCW55	LEI146	MCW231	MCW230	LEI97	LEI145		

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Set	Site								
14	MCW148	MCW211	MCW173	LEI144	MCW84	ADL136	LEI107	MCW217	
15	MCW27	LEI73	MCW238	LEI82	LEI141				
16	ADL279	MCW16	MCW240	LEI80	LEI91	ADL266			
17	ADL260	ADL231	LEI70	MCW247	MCW257	MCW256	MCW245		
18	ADL187	MCW66	ADL102	MCW255	MCW251	MCW252			
19	MCW210	LEI79	MCW194	MCW248	MCW165				
20	ADL331	LEI68	LEI81	LEI44					
21	ADL179	ADL324	LEI88	ADL323	MCW264	LEI161	ADL293		
22	ADL328	ADL345	ADL330	ADL322	LEI174	ADL341			
23	ADL336	ADL169	ADL348	ADL257	ADL326				
24	LEI118	ADL250	MCW285	LEI108	LEI115	LEI106	MCW283		
25	MCW295	LEI98	ADL154	LEI84	MCW296	ADL292			
26	MCW323	ADL319	ADL201	ADL273	MCW313	MCW328			
27	ADL320	ADL278	ADL309	ADL310	ADL288	MCW316	ADL314	MCW322	MCW314
28	MCW305	MCW330	MCW300	MCW308	ADL312				
29	MCW128	MCW331	LEI121	MCW294					
30	MCW154	LEI75	MCW241						

Primer sequences can be obtained from: http://www.zod.wau.nl/vf/base_ie.html or http://poultry.mph.msu.edu

Genotype analysis was performed on 370 individuals in the population of F_2 generation via M-PCR technique; the data obtained were analyzed using Genescan™ 3.0 software (Applied Biosystems). After primary evaluation, those 178 microsatellite markers were all segregated from one generation to the next, following Mendelian heritage law; therefore, they are informative markers for linkage analysis and QTL mapping. These polymorphic sites were distributed on 23 autosomes and 1 sex chromosome (Chromosome Z) (http://www.zod.wau.nl/vf/base_ie.html). The number of markers and the distribution of the markers are listed in Table 2.

Table 2. Microsatellite markers of chicken chromosomes

Chromosome No.	Chromosome length(cM)	Marker No.	Marker density(cM)	Chromosome No.	Chromosome length(cM)	Marker No.	Marker density(cM)
1	544	26	20.92	13	52	4	13.00
2	418	23	18.17	14	74	4	18.50
3	322	14	23.00	15	51	5	10.20
4	248	14	17.71	16	60	3	20.00
5	170	12	14.67	17	77	2	38.50
6	104	5	20.80	19	46	3	15.33
7	175	5	35.00	20	49	3	16.33
8	77	4	19.25	22	29	1	29.00
9	79	6	13.17	23	54	2	27.00
10	85	4	21.56	24	19	3	06.33
11	105	11	09.55	26	25	2	12.50
12	57	3	15.67	Z	170	10	17.00

3 Discussion

3.1 Optimization of multiplex PCR amplification condition

Compared with the routine PCR amplification, the procedures were modified to optimize the multiplex PCR conditions. (i) Using different annealing temperatures according to the different primers' T_m; (ii) with the cycle number from 10～20 and extending the annealing time and polymerization time to meet the requirement of specific amplification conditions; (iii) extending the final extension time to 40 min at 72℃ to avoid adding the poly A tail to the amplified products; (iv) adding some supplement reagents into the PCR buffer, for example Triton X-100, and increasing the dNTP concentration; (v) de-

creasing the reaction volume to 15 μL (the routine volume is 25～50 μL), which can lower the experimental costs and made it feasible to large numbers of genomic scanning. Although many parameters were optimized, there were still a lot of microsatellite markers could not be integrated into the current sets, this could be resulted from: (i) some fluorescent labels at the 5'-end were attenuated, leading to some fragments could not be detected by the sequencer, it occupied 15% of the primers tested; (ii) paring of the primers is another factor to influence some amplification reactions; (iii) the current amplification conditions are still not suitable for some primers, for example, annealing temperature and Mg^{2+} concentration, etc.

3.2 Density of marker and QTL localization

All of the 178 polymorphic microsatellite markers were mapped to 23 autosomes and 1 sex chromosome, covering 3080 cM with the average marker density of 18 cM, which meets the requirements of the QTL localization. However, the marker density varies significantly among individual chromosomes, from 6.33 cM to 38.6 cM. It has been indicated in the granddaughter design that some factors such as marker density and the trait heritance, etc. will affect the accuracy of QTL localization[4]. For the better accuracy of QTL mapping, therefore, the markers of high density need to be identified.

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