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ChickRH6: a chicken whole-genome radiation hybrid panel

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Abstract – As a first step towards the development of radiation hybrid maps, we have produced a radiation hybrid panel in the chicken by fusing female embryonic diploid fibroblasts irradiated at 6 000 rads with HPRT-deficient hamster Wg3hCl$_2$ cells. Due to the low retention frequency of the chicken fragments, a high number of clones was produced from which the best ones were selected. Thus, 452 fusion clones were tested for retention frequencies with a panel of 46 markers. Based on these results, 103 clones with a mean marker retention of 23.8% were selected for large scale culture to produce DNA in sufficient quantities for the genotyping of numerous markers. Retention frequency was tested again with the same 46 markers and the 90 best clones, with a final mean retention frequency of 21.9%, were selected for the final panel. This panel will be a valuable resource for fine mapping of markers and genes in the chicken, and will also help in building BAC contigs.

chicken / radiation hybrid / mapping

1. INTRODUCTION

The interest in studying the chicken genome has greatly increased in the past years, driving international efforts towards detailed physical and genetic maps (for a review, see [29]). One of the reasons for this interest is the importance of this species in agriculture. The various current efforts aiming at mapping QTLs (quantitative trait loci), involved in production traits and the resistance to pathogens, will benefit greatly from detailed knowledge of the genome. Other reasons include the importance of the chicken in evolutionary studies as a model organism for birds, and in developmental biology. Also, the chicken

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has the typical genome organisation of birds, with two chromosome subtypes: nine pairs of cytogenetically distinguishable macrochromosomes including the two sex chromosomes Z and W [19] and 30 pairs of small, cytogenetically indistinguishable microchromosomes. The female is the heterogametic sex (ZW) and the male is the homogametic sex (ZZ). The estimated haploid genome size of the chicken is close to 1.2×10^9 bp [2].

Apart from having a small size, estimated to being between 7 and 23 Mb [2], the microchromosomes appear to be more gene dense than the macrochromosomes [3,24,30] and to show a higher rate of recombination [9,25,28]. Twenty-two pairs of them have now been identified by using large insert clones in FISH (fluorescence *in situ* hybridisation) experiments ([8,29], Fillon *et al.*, in preparation).

A consensus genetic map containing close to 1 900 loci was published [12], consisting of 50 linkage groups, with some of them containing as little as two markers. Despite the efforts made to integrate the genetic and cytogenetic maps of the chicken chromosomes [25,31], 16 of the small linkage groups still have to be assigned to a chromosome. The fact that the number of linkage groups is still higher than that of the chromosomes is probably a consequence of the very high recombination rates associated to the microchromosomes, impeding linkage association.

Two BAC libraries were constructed in the chicken [5,20] and a large amount of chicken ESTs were produced [1,34], (see also: http://chick.umist.ac.uk, http://www.chickest.udel.edu).

In this context, whole-genome radiation hybrid (WGRH) panels provide a complementary approach to the different genome mapping techniques currently used in chicken. The resolution that can be achieved is higher than that obtained with recombinant mapping, enabling the ordering of markers otherwise clustered on the genetic map. Another interesting point is the possibility to map markers by a simple PCR, avoiding the necessary development of polymorphism as required for genetic maps. Therefore, the mapping of the now available high numbers of ESTs can be streamlined. The potential resolution of a radiation hybrid panel is tailored by the radiation dose, and panels of different resolutions can be created depending on the needs: aid to BAC contig construction, high resolution transcript maps of a whole genome, or regional fine mapping of candidate regions for QTLs.

WGRH panels are now available in many species including human [13,27, 33], mouse [23], rat [22], dog [35], cat [26], cow [37], pig [38] and horse [17]. Zebrafish WGRH panels and RH maps have been published [11,14], demonstrating that RH technology can also be used for non-mammalian vertebrates. Recently, a first collection of 48 chicken radiation hybrids was published [18]. However, the number of clones produced, the retention frequencies per clone and the DNA quantities available were too low and also varying parameters

such as different radiation doses and recipient cell lines were used. The heterogeneity of the clones thus obtained, with variations in breakage and retention frequencies, may cause problems for the mapping process.

We report here the production of a chicken whole-genome radiation hybrid panel, obtained by fusing irradiated female embryonic diploid fibroblasts to a HPRT-deficient hamster cell line (Wg3hCl$_2$). A radiation dose of 6 000 rads was chosen as a compromise between resolution power and linkage power to build a first chicken RH map. Due to the low retention rate of the chicken genome in the hybrids, a large number of clones (452) was produced, from which the selection of the best ones was performed by using a set of 46 markers chosen across the genome from the genetic map. Ninety hybrids were selected to build the final panel.

2. MATERIALS AND METHODS

2.1. Generation of radiation hybrids

The method was adapted from the one first described by Walter *et al.* [36] and widely used [22,38]. The chicken donor cells used for constructing this panel were normal diploid fibroblasts. They were obtained from six different 9-day-old female chick embryos and propagated in complete RPMI media [RPMI1640 (Sigma Chemical Co.) supplemented with 15% foetal calf serum (Life Technologies), streptomycin and penicillin]. During each experiment, 2×10^7 cells were suspended in 20 mL of RPMI1640 and irradiated at 6 000 rads by gamma rays from a Cesium-137 source. They were mixed to the same number of recipient cells of the hypoxanthine guanine phosphoribosyl transferase (HPRT)-deficient hamster cell line, Wg3hCl$_2$ [7]. Fusion partners were first pelleted and then suspended in 1 mL of polyethyleneglycol (Roche Diagnostics GmbH). After 1 min, 40 mL of RPMI without serum were gradually added and 1 mL of this fusion mixture was added to forty 75-cm^2 flasks containing 30 mL of the complete RPMI media. Twenty-four hours after fusion, HAT (hypoxanthine-aminopterin-thymidine) was added to the media. Four days later, the whole media was changed to discard the non-fused cells. Eight to ten days after the fusion the first hybrid clones were observed. When fully grown after 8 to 20 days of culture, they were picked and transferred to 25-cm^2 flasks. The cells were subsequently transferred to two 75-cm^2 flasks. In order to limit the number of doubling events and the possible loss of chicken genome fragments, all the cells in a flask were passaged. Finally, the cells were harvested from the two 75-cm^2 flasks. Five million were used to extract DNA and the rest were cryopreserved.

2.2. Estimation of chromosome retention frequency

Hybrids were screened for the presence of chicken DNA by using a set of 46 markers distributed across the genome. All of them were microsatellite markers except the *Xho*I-family marker from chromosome W [4]: the primer sequences are available at
http://www.ri.bbsrc.ac.uk/chickmap/ChickMapHomePage.html.

The PCR reaction contained 25 ng hybrid DNA, 2 mM MgCl$_2$, 0.3 U *Taq* DNA polymerase (Life technologies), 1 X buffer (Life technologies), 0.05 X 1% W-1 (Life technologies), 200 μM of each dNTPs, 0.2 μM of each primer and 1 X loading buffer (350 mM sucrose and 0.2 mM cresol red) in a total volume of 15 μL.

The PCR protocol used for the marker *Xho*I was the one described by Clinton (1994) [4], while for the microsatellite markers, a touch-down PCR protocol [6] was performed: denaturation at 94 °C for 3 min was followed by two cycles at each annealing temperature of 57 °C and 55 °C and 33 cycles with an annealing temperature of 53 °C (30 s at the annealing temperature, 20 s at 72 °C and 30 s at 94 °C).

PCR products were analysed using 2% agarose gels and were visualised using ethidium bromide staining. All markers were genotyped in duplicate and scored for the presence or absence of PCR products.

2.3. Large scale culture (LSC)

One hundred and three hybrids were selected based on their high retention rate and cultured in a large scale at the *Centre d'étude du polymorphisme humain* (Dr Claudia de Toma, Fondation Jean Dausset – CEPH – 27, rue Juliette Dodu, 75010 Paris, France). Cryopreserved seed stocks were used to inoculate one 75-cm^2 flask. The cells were subsequently transferred to eight roller bottles to produce the final harvest for DNA extraction. The retention frequencies of these 103 hybrids were then re-evaluated using the same 46 markers as before, to select the 90 best ones in the final panel.

3. RESULTS

3.1. Generation and characterisation of 452 whole genome radiation hybrids

Fifteen fusion experiments were carried out to produce 452 chicken whole-genome radiation hybrids. One clone was recovered per 720 000 chicken fibroblasts, corresponding to a fusion efficiency of approximately 1.4×10^{-6} clones per chicken fibroblast.
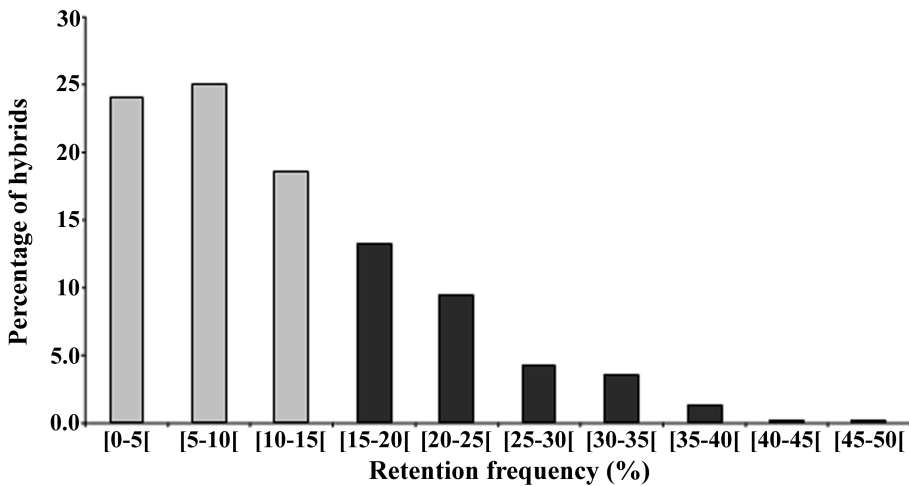
Figure 1. Distribution of the 452 hybrids depending on their retention frequency. Most of the hybrids (67.7%) showed an average retention frequency below 15%. Only 32.3% of the hybrids were satisfactory candidates for the final panel.
[0–5[: retention frequencies below 5%.
[5–10[: comprised between 5% and 9.99%.

To determine the extent to which donor fragments were retained in our hybrids, 46 markers were amplified by PCR from DNA obtained from each of them. Due to the particularities of the chicken genome structure, care was taken in the choice of markers to represent all chromosome types. Half of the markers were from macrochromosomes 1 to 8 and the gonosomes. When several markers from the same chromosomes were used, they were chosen at a minimum distance of 8 cM from one another. Sixteen other markers were from linkage groups assigned to identified microchromosomes. The seven last markers were from undefined regions of the genome: two were from small linkage groups, supposedly corresponding to two different microchromosomes, and five were not linked to any other marker or linkage group in the actual consensus linkage map of the chicken [12]. We supposed that these last markers belong to the smallest microchromosomes and we therefore analysed the results considering two classes of markers: 23 localised on macrochromosomes and 23 localised on microchromosomes.

The average retention rate of the 452 hybrids was 11.3% for the whole genome, but the overall retention rate for markers located on microchromosomes was higher (14.8%) as compared to that of macrochromosomes (9.5%).

Thus, only 32.3% of the hybrids showed a whole genome retention frequency higher than 15% and could be considered as potential candidates for the final mapping panel (Fig. 1). No higher retention frequency could be observed on

chromosome 4 bearing *HPRT* [10], since the chromosome 4 markers used in this study were not close to this gene. The two haploid markers located on chromosome Z showed retention frequencies lower than the others, due to the use of female chicken donor cells while the *Xho*I marker was better retained among the hybrids reflecting its location close to a centromere (Fig. 2). Indeed, the cytogenetic assignment for this marker was proposed to be in Wp1.2-q1.1 by Solari *et al.* [32].

3.2. Pre-selection of 103 candidate hybrids

Considering the low average retention frequency of the chicken-hamster hybrids, as many as 452 clones were produced, with the aim of a final panel of 90. Our first estimations of the chicken genome loss in the hybrids after large scale culture, was around 10%. Therefore, we decided to grow the 103 best candidate hybrids in large scale culture (LSC), in order to be able to choose 90 of them for the definitive panel. As compared to the 452 hybrids, this pre-selection improved the overall whole genome retention frequency from 11.3% to 23.8%, the overall macrochromosome retention frequency from 9.5% to 21.5% and the microchromosome one from 14.8% to 28.5% (Fig. 3).

3.3. Selection of the 90 hybrids for the final panel

After LSC, the retention frequencies of the 103 pre-selected hybrids were estimated using the same 46 markers. The average retention rate was 20.9% for the whole genome, but the overall retention rate for the markers located on the macrochromosomes was 18.6% and for the markers located on the microchromosomes, 25.7% (Fig. 3). The average fragment loss reached 12.5% for the whole genome: 13.5% for the macrochromosomes and 10% for the microchromosomes.

We finally selected the 90 hybrids that constitute the definitive panel. The average retention rates are 21.9% for the whole genome, 20.1% for the macrochromosomes and 25.7% for the microchromosomes (Fig. 3). However, the retention was not always so uniform, with lower values for the largest chromosomes: 15.3% for GGA1 and 17.1% for GGA2.

Two control DNAs were included in the panel: the Wg3hCl$_2$ hamster cell line DNA and a chicken DNA that is a mix of two female embryonic chicken DNAs.

4. CONCLUSIONS

A fusion efficiency of approximately 1.4×10^{-6} clones per chicken fibro-blast was observed. This fusion efficiency was low in comparison to that of
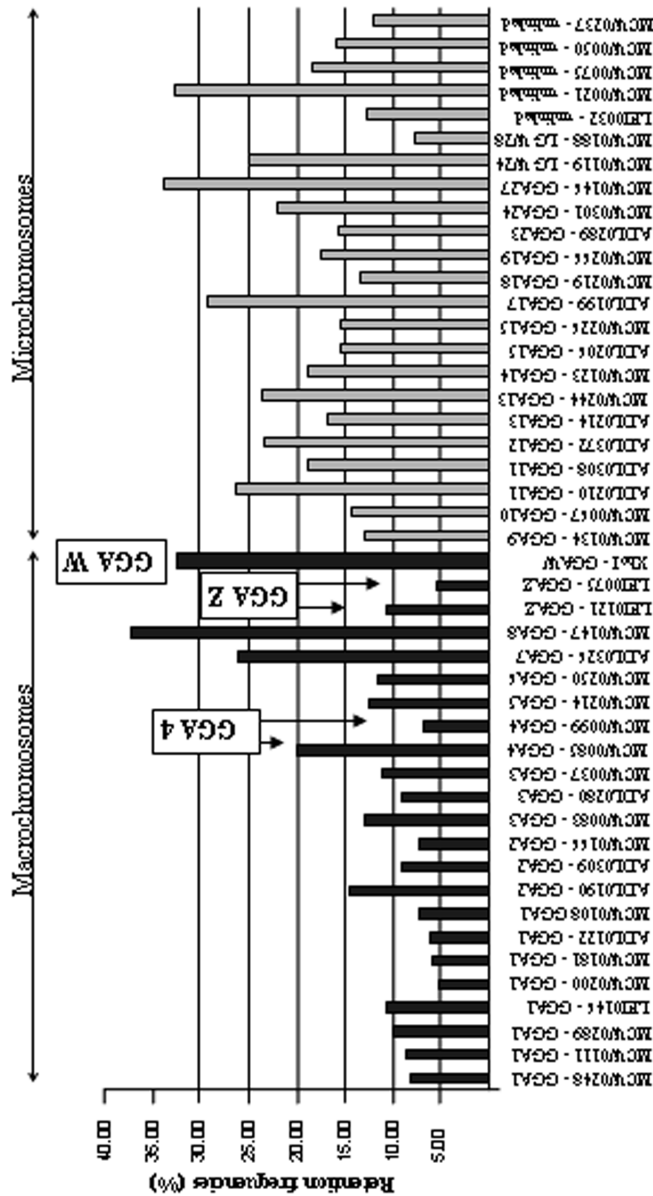
Figure 2. Retention frequencies of 46 genome-wide markers typed on the 452 hybrids. The 46 markers were chosen from linkage groups of the published genetic map [12], 23 are located on macrochromosomes, 2 are on small linkage groups not yet assigned to a chromosome and 5 are genetically unlinked. We thus analysed the results considering two classes of markers: 23 localised on macrochromosomes and 23 localised on microchromosomes. No higher retention frequency could be observed on chromosome 4 bearing *HPRT*. The two haploid markers located on chromosome Z showed retention frequencies lower than the others, due to the use of female chicken donor cells while the *XhoI* marker was better retained among the hybrids reflecting its location close to a centromere. LG W24: linkage group E47/W24; LG W28: linkage group E22/C19/W28; Unlinked: genetically unlinked markers on the chicken consensus map.
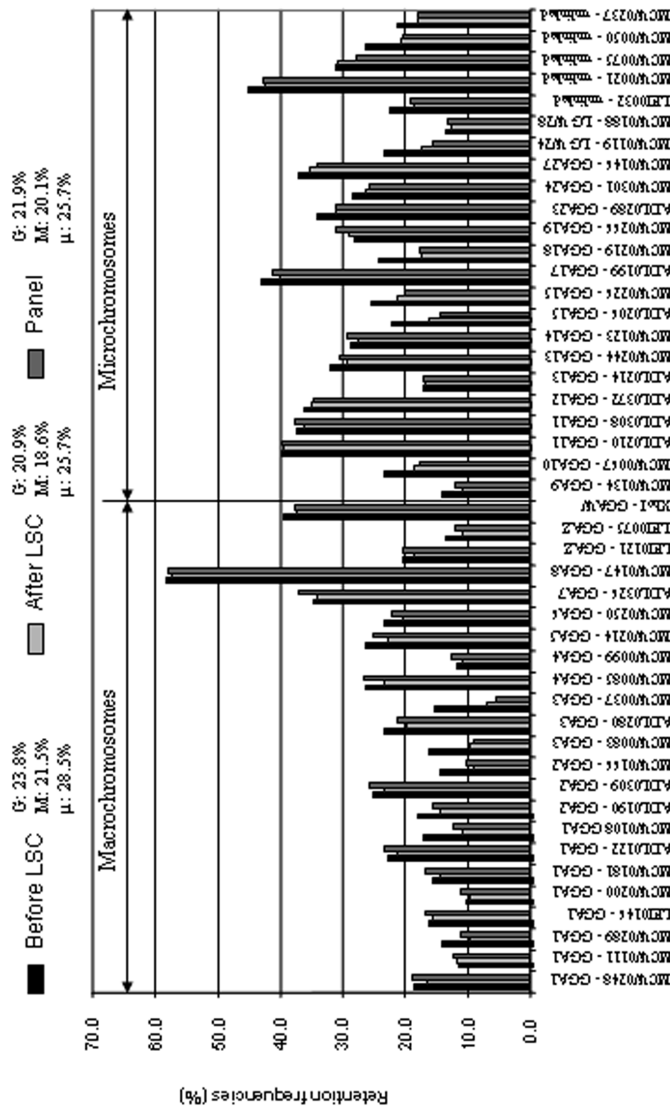
Figure 3. Retention frequencies of the 46 genome-wide markers typed in the 103 candidate hybrids before and after large scale culture (LSC) and in the final panel of 90 hybrids. The 103 candidate hybrids were cultured on large scale and the retention frequency of the 46 markers was re-evaluated for each of them. The average loss reached 12.5% for the whole genome, but the average loss for the macrochromosomes was higher (13.5%) than that of the microchromosomes (10.0%). The average retention rates for the final panel were 21.9% for the whole genome, 20.1% for the macrochromosomes and 25.7% for the microchromosomes. LG W24: linkage group E47/W24; LG W28: linkage group E22/C19/W28; Unlinked: genetically unlinked markers on the chicken consensus map; LSC: large scale culture; G: whole genome retention rate; M: macrochromosome retention rate; μ: microchromosome retention rate.

mammals, *e.g.* 10^{-5} in dogs [35] and 10^{-4} in human [36]. This was expected given the evolutionary distance of the two species, and the results obtained by others ([18], Bumstead, personal communication).

The average retention rate of the 452 hybrids was 11.3% for the whole genome, but the overall retention rate for the markers located on the microchromosomes was higher (14.8%) as compared to that of the macrochromosomes (9.5%). These results were in accordance with those of Kwock *et al.* [18], where retention frequencies were 17.8% for microchromosomes and 10.6% for macrochromosomes. It has been observed that smaller chromosomes are generally retained at a higher rate, as *e.g.* in a human WGRH panel [13]. In the chicken, where the difference in size between the macrochromosomes and the microchromosomes is more pronounced, this trend is more evident. One possible explanation is that in a microchromosome, markers are usually at a closer distance to a centromere, a region where retention frequencies have been shown to be high [15].

For both practical and economical reasons, reducing the number of hybrid DNA samples in a panel to the number that can be easily handled in a microplate is highly desirable. Theoretical data have been reported on the consequences of hybrid selection on the mapping power of RH panels and it is generally admitted that a retention frequency over 20% is a minimum prerequisite for a good efficiency of a panel [16, 21, 35]. Despite a drastic selection (90 hybrids selected out of 452 developed), we could not manage to improve the retention frequency of the two largest macrochromosomes to reach 20%. However, the overall retention frequency of this panel remains suitable for RH mapping. DNA extraction produced more than 3 mg of DNA for each hybrid, an amount sufficient for an estimated 150 000 PCR assuming 20 ng per reaction.

This RH panel is a powerful tool for integrating genetic and physical maps. Mapping STSs from BAC contig ends will help find the location and orientation of linkage groups. An obvious exploitation is the mapping of cDNAs and ESTs onto a framework of microsatellite markers, providing the basis for the biological characterisation of specific chromosomal regions of interest. Such RH-mapping data will also be a powerful method in comparative gene mapping since chromosomal order can be established for expressed genes that are usually conserved between species but are often recalcitrant to linkage mapping for a lack of readily detectable allelic variations (for chicken-human comparative mapping see Schmid *et al.* [29]).

Approximately 1 000 markers are currently being screened across the RH panel; a part of them are microsatellite markers anchored on the genetic map and the others are ESTs. This WGRH panel, named ChickRH6, is available to the academic community, upon request to the authors (mmorisso@toulouse.inra.fr; vignal@toulouse.inra.fr).

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