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## Construction and characterization of a high-resolution, 9000-rad canine radiation hybrid panel

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*Description:* To advance the development of the canine genome map, a whole-genome radiation hybrid panel, RHDF5000, was previously constructed using a radiation dose of 5000 rad.[1] This panel was used for several years to produce maps of increasing density, which were then integrated with genetic linkage and physical fluorescent *in situ* hybridization maps.[2–4] The culmination of this work was a map containing 4249 markers, which is close to the resolution limit of this panel. In response to the need to map more markers, we report here the construction and characterization of a higher-resolution panel (RHDF9000).

*Experimental approach:* Primary dog fibroblasts were irradiated at 9000 rad and fused with HTK3- (thymidine kinase-deficient) hamster cells (Supplemental Methods). Five identical fusions produced 429 clones harbouring dog genomic DNA, as shown by detection of the canine SINE marker. DNA was extracted from 429 clones, and marker retention frequency was assessed using a set of 96 markers randomly distributed throughout the dog genome (Supplemental Methods). Based on their retention frequencies, we selected 121 hybrid cell lines with a mean retention frequency of 25.8% (range 15–60%). These 121 cell lines were then expanded, and large-scale DNA preparations were produced (Supplemental Methods). The same set of 96 markers was typed again, and radiation hybrid (RH) data analysed with the tsp_rh_map package.[5] Cell lines with very similar patterns were discarded. The final 88 hybrid cell lines had a mean retention frequency of 28% (Fig. S1).

*RH map development:* With this panel, we mapped 10 348 gene-based markers, derived from the dog 1.5 × survey sequence,[6] to 9081 positions (which is impossible with a lower-resolution panel such as RHDF5000). This panel was also used to identify 264 conserved blocks of human-dog synteny larger than 500 kb,[7] consistent with the whole-genome assembly CanFam2.[8]

*Comments:* From these results, it is clear that the construction of a dense, high-resolution RH map of each of the mammalian genomes selected by the NIH-NHGRI for survey sequencing (2 × redundancy) is desirable in the context of a mammalian genome project. These panels could be used to identify blocks of synteny after survey sequencing.

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### Supplementary Material

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**Figure S1** RH clone retention frequencies.

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## Linkage analysis excludes the involvement of the canine *PKD2* homologue in bull terrier polycystic kidney disease

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*Source/description: PKD2* is mutated in 10–15% of human patients with autosomal dominant polycystic kidney disease, which is clinically and pathologically similar to bull terrier polycystic kidney disease (BTPKD), making it a candidate gene for BTPKD. Fifteen microsatellites (Table S1) were amplified from genomic DNA of bull terriers with and without BTPKD[1] from a pedigree (Fig. S1). Thirteen microsatellites were near *PKD2* on CFA32 (http://research.nhgri.nih.gov/dog_genome/). Two microsatellites were identified from dog genome sequence using RepeatMasker (http://www.repeatmasker.org/): *PKD2MIC1* was a $(TA)_{20}$ repeat in predicted intron 2 of *PKD2*, and *PKD2MIC2*, which was 23.7 kb from the end of the *PKD2* 3′-UTR, included a $(TC)_{11}$ repeat, an imperfect $(GAAA)_{29}$ repeat and a $(CA)_{14}$ repeat.

*Genotyping:* Primers for *PKD2MIC1* (TTTTCAGCAAAATGACTTTCCA, GCCGGTGATCACTGAAGATT) and *PKD2MIC2* (GAGATTGAGCCCTGTGTCAAG, TGGCATTAAATACATTCC-

CATC) were designed using Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) and analysed using OLIGOANALYZER 3.0 (http://www.idtdna.com/analyzer/Applications/OligoAnalyzer/Default/aspx). Primer sequences for all other markers are available at http://research.nhgri.nih.gov/dog_genome/. Amplifications were optimized in singleplex and multiplex reactions to ensure consistent results. M13 tailing was used to fluorescently label amplicons for genotyping.[2] Genotyping was on an ABI Prism 3700 DNA Analyzer, and Genotyper 3.7 software (ABI) was used for analysis. Parentage verification panels 1 and 2 (http://www.vgl.ucdavis.edu/research/canine/ISAG/index.html) from ISAG (http://www.isag.org.uk/) confirmed pedigree relationships in 10 related animals.

*Statistical analyses:* The pedigree contained 51 bull terriers with polycystic disease and 202 unaffected animals, of which 20 affected dogs and 16 unaffected dogs were genotyped (Fig. S1). Pedigree analyses, including testing for Mendelian errors, were carried out using the Sib-pair program (http//http://www.qimr.edu.au/davidD). A segregation analysis confirming dominant inheritance was carried out using PAP version 5.0 (http://hasstedt.genetics.utah.edu/pap5). The relative program[3] confirmed pedigree information for first-degree relationships. The markers were ordered using positions in the canine sequence and genetic map positions,[4] and confirmed using SIMWALK2.

The observed recombination between markers was consistent with previously published maps (http://research.nhgri.nih.gov/dog_genome/; http://www.ncbi.nlm.nih.gov/genome//guide/dog/) (Table 1). Marker *FH3744* was not included in the linkage analysis because it exhibited Mendelian inconsistencies. Multipoint parametric linkage analyses were performed using the SIMWALK2 package using conventional thresholds for assessing significance,[5] assuming a dominant model (1% disease allele frequency in founders, 50% penetrance). Linkage of BTKPD to the tested region of CFA32 was excluded with a LOD score of −9.0 at the position of *PKD2*, which is located in the region containing markers *CPH2*, *PKD2MIC1*, *PKD2MIC2* and *FH2875* (Fig. 1). Nonparametric linkage analysis also failed to support linkage. Affected dogs were either heterozygous or homozygous, but only heterozygosity would be expected because homozygosity in other species is lethal.[6]

**Table 1** Observed and expected recombination events between microsatellite markers used in this study.

| Marker | Observed recombination | Expected events | Significance (*P*-value) | Intermarker distance (cM) |
|---|---|---|---|---|
| *CPH26–PKD2MIC1* | 13.0 | 10.9 | 0.30 | 2.3 |
| *PKD2MIC1–PKD2MIC2* | 0.5 | 0.6 | 0.71 | 0 |
| *PKD2MIC2–FH2875* | 4.4 | 5.0 | 0.66 | 0.8 |
| *FH2875–D03908* | 21.5 | 21.4 | 0.52 | 3.9 |
| *D03908–FH3236* | 5.4 | 5.5 | 0.57 | 1.0 |
| *FH3236–AHT127* | 39.9 | 36.3 | 0.29 | 7.3 |
| *AHT127–FH4036* | 5.9 | 5.5 | 0.49 | 1.1 |
| *FH4036–FH3294* | 11.9 | 10.9 | 0.42 | 2.1 |

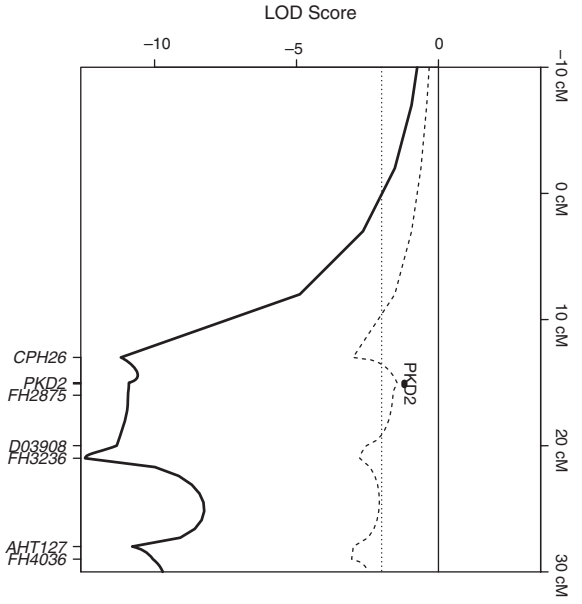


**Figure 1** Multipoint LOD scores for polycystic kidney disease versus eight CFA32 microsatellite markers in a bull terrier pedigree. The solid curve was obtained by analysing the entire pedigree, whereas the broken curve was obtained by analysing the subset of the pedigree shown in Figure S1.

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## Supplementary Material

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**Table S1** Microsatellite markers from the region of the canine *PKD2* homologue used for linkage analysis.

**Figure S1** Pedigree of bull terriers segregating for polycystic kidney disease.

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