

used for the *Bam*HI library and pECBAC1⁹ for the *Eco*RI and *Hind*III libraries. Each library contains 38 400 clones for a total of over 115 000 BACs. The BAC insert sizes were determined as described in Fig. 1. For the *Bam*HI library, 15 clones with no insert (4.8%) were observed among 314 random BACs. Maximum and minimum insert sizes were 195 and 45 kb, with an average of 150 kb. For the *Eco*RI library, 321 BACs were sized, with 11 clones (3.4%) lacking inserts and maximum and minimum inserts of 230 and 48 kb and an average of 152 kb. For the *Hind*III library, 309 BACs were sized, giving eight clones (2.6%) lacking inserts, maximum and minimum inserts of 290 and 45 kb and an average insert of 172 kb. Other than the fact that the *Hind*III library contained more inserts greater than 181 kb, the three libraries were very similar in their overall properties (Fig. 1). Together they provide over 15-fold coverage of the haploid chicken genome.

Gene/marker content: Hybridization to filter arrays of the *Bam*HI BAC library was performed using ³²P-labelled, PCR-amplified DNA fragments (typically, pools of five probes at once) from 30 genes or markers using standard techniques¹⁰. BAC identities (Table 1) were determined by Southern hybridization of digested BAC DNA with individual probes, and independently confirmed by either PCR amplification or overgo¹¹ hybridization. On average, two positive BACs were obtained per probe, less than would be predicted by the estimated 4.8-fold coverage of the *Bam*HI library. This discrepancy is typical of many, if not all, large-insert DNA libraries. Possible explanations include false-negatives that arise in screening because of inadequate membrane spotting, low specific activity of an individual probe relative to others in its pool, or failure to detect a true-positive BAC in a confirming PCR reaction. In all but three cases (*BRD3*, *ROS0200* and *TPM2*), at least one *Bam*HI BAC clone containing the gene of interest was obtained. Recent screening with several hundred overgo¹¹ probes (data not shown) confirms that each library is approximately equivalent in overall gene/marker content.

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Correspondence: J Dodgson (dodgson@msu.edu)

Linkage mapping of the porcine testis enhanced gene transcript (*TEGT*) gene to chromosome 5^{1,2}

J. G. Kim, D. Nonneman, J. L. Vallet and R. K. Christenson

US Department of Agriculture, Agricultural Research Service, US Meat Animal Research Center, Clay Center, NE, USA

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Source/Description of Primers: A cDNA clone containing the partial coding region of the porcine testis enhanced gene transcript (*TEGT*) was isolated (GenBank accession no. AY166682) from the 'Meat Animal Research Center (MARC) 2PIG' expressed sequence tag (EST) primary library¹ by iterative screening and sequenced. The carboxy-terminal 15 amino acids derived from the partial porcine *TEGT* cDNA are identical with the 15 carboxy-terminal amino acids of the human *TEGT* (GenBank accession no. NM_003217) and the 3' untranslated region of the porcine *TEGT* shares 75% identity with the human *TEGT*. The forward (*TEGT* F1) and reverse (*TEGT* R1) primers that were used for PCR amplification to screen the MARC 2PIG EST library correspond to bases 6–24 and 374–356 of the porcine cDNA sequence (GenBank accession no. AY166682), respectively. The forward (*TEGT* F2) and reverse (*TEGT* R3) primers that were designed to amplify a 927-bp product in the 3' untranslated region of the cDNA correspond to bases 501–520 and 1445–1426 of the porcine *TEGT* cDNA, respectively.

PCR Primer sequences and a flanking sequence for a single nucleotide

polymorphism: *TEGT* F1: GCGTGGGTGATGATTCTGG

TEGT R1: CTGGCGTTTGCTTCCTCTG

TEGT F2: CCTGGGAAAGAAGTTTGCTG

TEGT R3: CAAGAAGCCAGACCAACAG

Flanking sequence: TGTCTACCC(T/G)CAGGGAACCT

TEGT probe primer: GTCCCTTGCTCACCC

PCR conditions: PCR reactions were carried out in a 25- μ l volume containing 100-ng genomic DNA, 1.5 mM MgCl₂, 20 pmol of each primer, 100 μ M dNTP, and 0.35 U HotstarTM.

¹Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

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Taq polymerase (Qiagen Inc., Valencia, CA, USA). Amplification was performed under the following PCR conditions; 15 min at 94 °C; 45 cycles of 30 s at 94 °C, annealing for 45 s at 61 °C, 1.5 min at 72 °C; and a final extension of 5 min at 72 °C. The amplified genomic DNA of eight parents (seven F1 sows and one white composite boar) from the MARC Swine Reference Population² was bidirectionally sequenced and evaluated for polymorphisms³.

Polymorphism and chromosomal location: A G/T single nucleotide polymorphism was detected at position 715 of the porcine *TEGT* cDNA (GenBank accession no. AY166682). This polymorphism was heterozygous in two of the seven F1 sows. An assay was designed to genotype this polymorphism using primers *TEGT* F2 and R3, primer extension with the *TEGT* probe primer, and analyte detection on a MALDI-TOF mass spectrometer⁴ with 50- μ l PCR reactions. PCR conditions were same as above. This marker generated 29 informative meioses in the MARC swine reference population. The *TEGT* gene was mapped to chromosome 5 position 52 cM, which is the same position as marker SWR453 on the current MARC swine chromosome 5 linkage map (<http://www.marc.usda.gov/>) using CRI-MAP. The most significant two-point linkage detected was with SWR453 (LOD = 6.62) at 0 recombination. The *TEGT* gene in human is located on chromosome 12q12–q13, which shares homology with swine chromosome 5. *TEGT* was identified as Bax inhibitor-1 in mammalian cells, which functions as a suppressor of apoptosis⁵. The transcript of *TEGT* is abundant in adult rat testis⁶ and may support the survival of certain cell types in the testis.

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Correspondence: R. K. Christenson
(christenson@email.marc.usda.gov)

Characterization and mapping of 19 polymorphic microsatellite markers for rainbow trout (*Oncorhynchus mykiss*)

Y. Palti*, R. G. Danzmann[†] and C. E. Rexroad III*

*National Center for Cool and Cold Water Aquaculture, USDA-ARS, 11876 Leetown Road, Kearneysville, WV 25430, USA.

[†]Department of Zoology, University of Guelph, Guelph, ON N1G2W1, Canada

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Source/description: A rainbow trout microsatellite-enriched library (GA) was constructed by Genetic Identification Services

(Chatsworth, CA, USA) as previously described¹. Clones were sequenced with M13 forward and reverse primers using Big Dye Terminator Sequencing kit (ABI, Foster City, CA, USA). Electrophoresis and fragment detection were performed with an ABI 3700 automated DNA sequencer (ABI). Sequences were viewed with Chromas 2.13 (Technelysium Pty Ltd, Helensvale, Queensland, Australia). Base-calling to assign quality scores and to trim low-quality sequence was conducted with the Phred for Windows software (CodonCode, Dedham, MA, USA). The Sequencher software (Gene Codes Corporation, Ann Arbor, MI, USA) was used to remove vector contamination, group sequences containing the same core repeat, and analyse sequences within each group to eliminate redundant clones. The PCR primers were designed to amplify unique microsatellites using Oligo 6.0 software (Molecular Biology Insights, Cascade, CO, USA).

PCR conditions: Primers were obtained from commercial sources (forward primers labelled with FAM or HEX; Alpha DNA, Montreal, Quebec, Canada) and PCR conditions were optimized using DNA from two Kamloop strain rainbow trout, the clone of origin and a negative control. Primers were optimized for amplification by varying annealing temperatures and MgCl₂ concentrations. The PCR reactions (12 μ l total volume) included 50 ng DNA, 1.5–2.5 mM MgCl₂, 1.0 μ M of each primer, 200 μ M dNTPs, 1X manufacturer's reaction buffer (GeneAmp[®] 10X PCR Gold Buffer II), and 0.5 unit AmpliTaq Gold Polymerase (ABI). Amplifications were conducted in an MJ Research DNA Engine thermal cycler model PTC 200 (MJ Research, Waltham, MA, USA) as follows: an initial denaturation at 94 °C for 10 min, 36 cycles consisting of 94 °C for 30 s, annealing temperature for 30 s, 72 °C extension for 30 s; followed by a final extension of 72 °C for 10 min. Specific repeat information, primer sequences and GenBank accession numbers are given for each primer in Table 1. The optimal annealing temperature was 58 °C for all markers, except *OMM1371*, for which it was 54 °C. The optimal MgCl₂ concentration was 2.5 mM for all markers, except *OMM1369*, for which it was 1.5 mM.

Polymorphism and allele size: The PCR was performed to amplify each marker from a genomic DNA panel consisting of 47 animals. We used homozygous animals from near isogenic lines² to aid in determining the copy number of the microsatellite loci. In rainbow trout androgenesis is used for generating homozygous individuals³. Androgenetic animals from several strains were previously used as founders of clonal lines, which are being propagated by androgenesis (male line) or gynogenesis (female line). The panel was composed of nine homozygotes [OSU (2), Arlee (1), Swanson (2), Hot Creek (2) and Clearwater (2)], 18 unrelated rainbow trout [Kamloop (4), Klamath (2), Redband (5), Tucanon steelhead (5) and Skokumchuck steelhead (2)] and 20 other salmonids [Yellowstone Cutthroat (4), Westslope Cutthroat (1), Brook trout (4), Arctic char (2), Atlantic salmon (2), Sockeye salmon (1), Coho salmon (4) and Chinook salmon (2)]. The PCR amplification was verified on 3% agarose gels. The PCR reactions were then combined according to label and expected size for fragment size analysis with an ABI 3100 genetic analyzer and the Genescan software (ABI, Foster City, CA, USA). Fragment size data were analyzed with the Genotyper software (ABI). The number of alleles per marker, size range, per cent heterozygosity