Supporting Information

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SI Text

Linkage Mapping Population. Fig. S1 is a schematic showing relationship structure between the individuals used for linkage mapping of the dwarfism disease locus.

General Lab Techniques. DNA was extracted from several tissues. DNA was collected from whole blood, semen (Wizard DNA Kit; Promega), and blood on Whatman FTA cards (Whatman). PCR was performed as described (Table S1). RNA was isolated by TRIzol method (Invitrogen).

Preliminary Linkage Analysis. Chromosomes were prioritized for linkage analysis based on number of candidate genes known to cause dwarfism in humans or cattle on each chromosome. These chromosomes included BTA 1, 6, 14, and 19. Ten microsatellite markers were genotyped on each chromosome and used for linkage analysis to provide a coarse position for the mutation. An adaptation of the Elston-Steward methodology was used to perform linkage mapping, described in detail below. This method allowed us to accommodate missing genotypes by summing over all genotype combinations during the likelihood calculation. Based on these preliminary results, an additional 19 microsatellite markers were genotyped within and surrounding the critical region containing the highest LOD score. Marker positions were obtained from USDA-MARC at http://www.marc.usda.gov/genome/genome.html (accessed August 16, 2004). Missing genotypes were accommodated in a pedigree with loops, common ancestors, using the method of Fernandez et al. (1).

Template Sequence Retrieval. Linkage and human/bovine radiation hybrid map comparisons allowed us to determine the gene content within the critical region. We identified the segment between 79.18 and 84.73 Mbps in human as the most likely region to contain the Angus dwarf mutation. Radiation hybrid mapping data as well as sequence maps in human, rat, and mouse indicated a highly conserved syntenic block of ≈20 genes. We hypothesized that cattle would also retain this syntenic gene block. All template sequences were retrieved at http://www.ensembl.org/index.html (accessed December 2, 2004) and http://www.genome.ucsc.edu/(accessed December 2, 2004).

Sequence Analysis of Positional Candidate Genes and Genotyping. We performed PCR with primers that flanked each exon of the 4 candidate genes that were investigated. Primer sequences are given in Table S1. Primers were designed from these sequences using Primer3 at http://frodo.wi.mit.edu//primer3/ (accessed December 10, 2004). Genotypes were obtained by RFLP and single-base extension using SNaPshot chemistry (Applied Biosystems). Products were analyzed on an ABI-3100 and results were analyzed using Genescan software (Applied Biosystems). The SNaPshot reaction was performed as described by the manufacturer. Primers used for Snapshot are provided in Table S2. RFLP assays are described in Table S3. Genotypes used for linkage analysis are provided in Fig. S2 for all dwarfs and 2 unaffected full sibs. Allele frequencies for these SNP and microsatellite markers are provided in Fig. S4.

Comparative Modeling of the Wild-Type and R678X PRKG2 Proteins. Because no crystallized structure of PRKG2 has been imaged, we used PRKG1 as a template for modeling because of the high protein sequence similarity between these 2 paralogues. Fig. S3 displays the model of each protein and the changes that occur in the dwarf PRKG2 structure. These changes would appear to drastically change the protein structure of PRKG2. A multiple-species comparison of PRKG2 peptide sequence is provided in Fig. S4.

Histological Analysis of Bovine *PRKG2*$^{R678X/R678X}$ (Dwarf) Individuals. To determine the specific defects in growth plate development of Angus dwarfs, we examined histology of bovine dwarf growth plates. Fig. S5 shows a dwarf Angus growth plate stained by alkaline phosphatase (ALP). The growth plate is very disorganized, and there appears to be excessive calcification encasing the chondrocytes. The histology shows a defect in endochondral ossification, likely responsible for the reduced long bone growth in Angus dwarfs.

1. Fernandez SA, Fernando RL, Guldbrandtsen B, Totir LR, Carriquiry AL (2001) Sampling genotypes in large pedigrees with loops. *Genet Sel Evol* 33:337–367.
2. Delano WL (2002) PyMOL Molecular Graphics System. Available at http://pymol.org/.

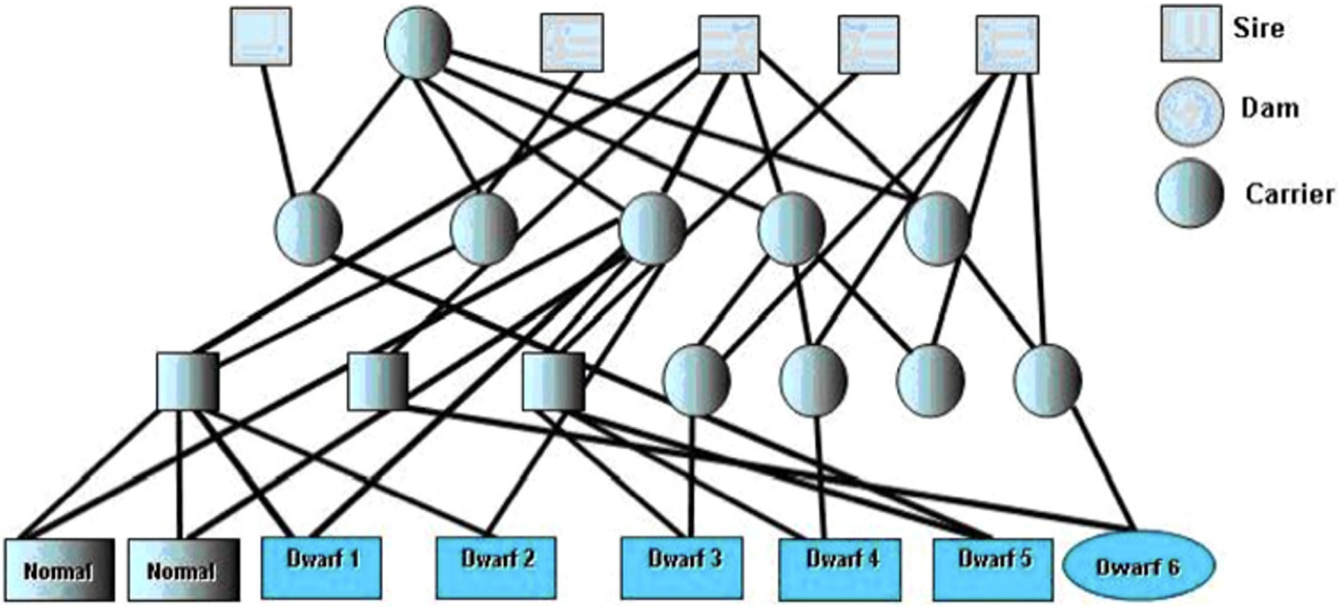
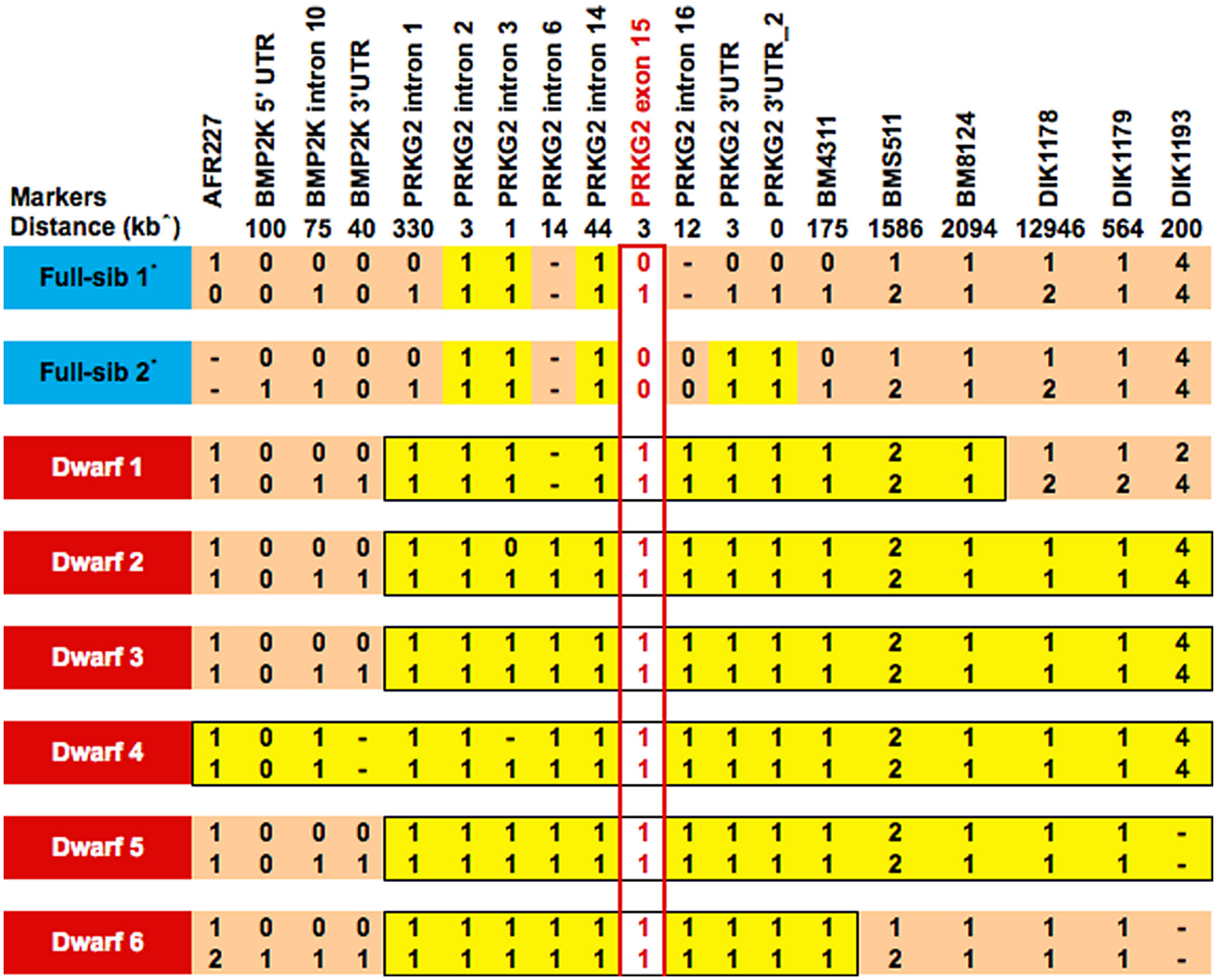


Fig. S1. A 4-generation pedigree consisting of 26 individuals of individuals was used for linkage mapping. These 26 individuals included 8 males (sires), 10 females (dams), and 8 calves (dwarf and normal phenotypes).



Markers	AFR227	BMP2K 5' UTR	BMP2K intron 10	BMP2K 3'UTR	PRKG2 intron 1	PRKG2 intron 2	PRKG2 intron 3	PRKG2 intron 6	PRKG2 intron 14	PRKG2 exon 15	PRKG2 intron 16	PRKG2 3'UTR	PRKG2 3'UTR_2	BM4311	BMS511	BM8124	DIK1178	DIK1179	DIK1193
Distance (kb^)		100	75	40	330	3	1	14	44	3	12	3	0	175	1586	2094	12946	564	200
Full-sib 1*	1	0	0	0	0	1	1	-	1	0	-	0	0	0	1	1	1	1	4
	0	0	1	0	1	1	1	-	1	1	-	1	1	1	2	1	2	1	4
Full-sib 2*	-	0	0	0	0	1	1	-	1	0	0	1	1	0	1	1	1	1	4
	-	1	1	0	1	1	1	-	1	0	0	1	1	1	2	1	2	1	4
Dwarf 1	1	0	0	0	1	1	1	-	1	1	1	1	1	1	2	1	1	1	2
	1	0	1	1	1	1	1	-	1	1	1	1	1	1	2	1	2	2	4
Dwarf 2	1	0	0	0	1	1	0	1	1	1	1	1	1	1	2	1	1	1	4
	1	0	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	4
Dwarf 3	1	0	0	0	1	1	1	1	1	1	1	1	1	1	2	1	1	1	4
	1	0	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	4
Dwarf 4	1	0	1	-	1	1	-	1	1	1	1	1	1	1	2	1	1	1	4
	1	0	1	-	1	1	1	1	1	1	1	1	1	1	2	1	1	1	4
Dwarf 5	1	0	0	0	1	1	1	1	1	1	1	1	1	1	2	1	1	1	-
	1	0	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	-
Dwarf 6	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	-
	2	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	-

Fig. S2. Genotypes for 6 affected individuals and 2 unaffected full sibs for 9 markers flanking the *PRKG2* exon 15 *R678X* nonsense mutation. Homozygous marker genotypes in dwarfs are in yellow as are homozygous markers in the unaffected full sibs. For genes, distances are based on sequence data. For microsatellite markers, distances are based on genetic maps at the USDA website (http://www.marc.usda.gov/genome/genome.html), where each cM is considered as one megabase. A dash (-) indicates a missing genotype; an asterisk (*) indicates that both individuals are full sibs with dwarfs 4 and 5.

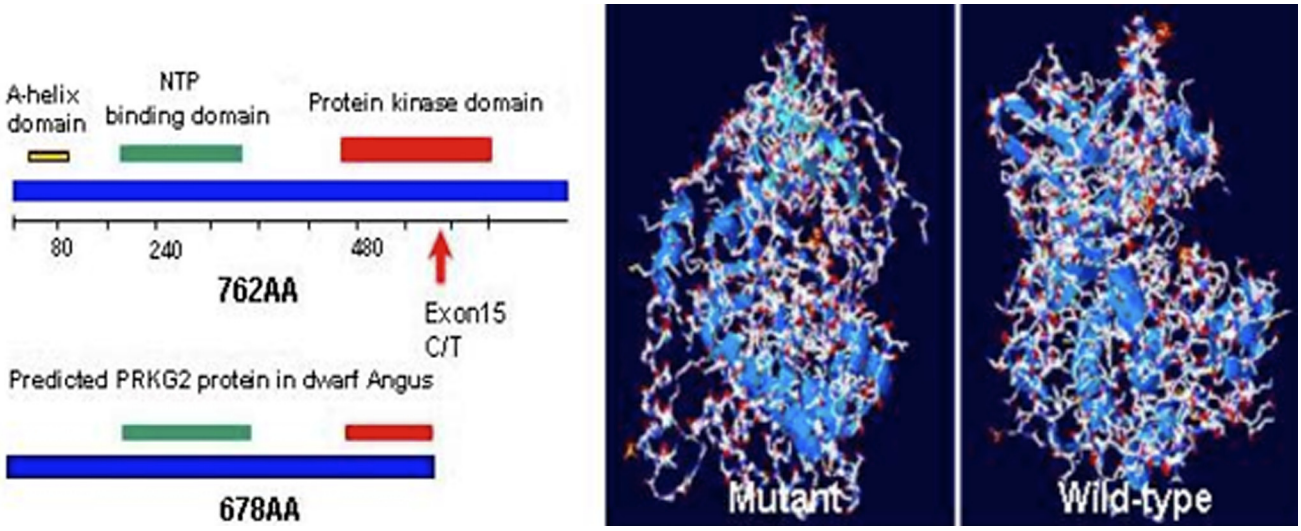


Fig. S3. Comparison between normal and dwarf cattle PRKG2 proteins.

Fig. S4. Comparison of the primary amino acid sequence across 7 species at the exon 15 *PRKG2* mutation. The position of the R678X mutation in Angus dwarfs is marked by a red arrow.

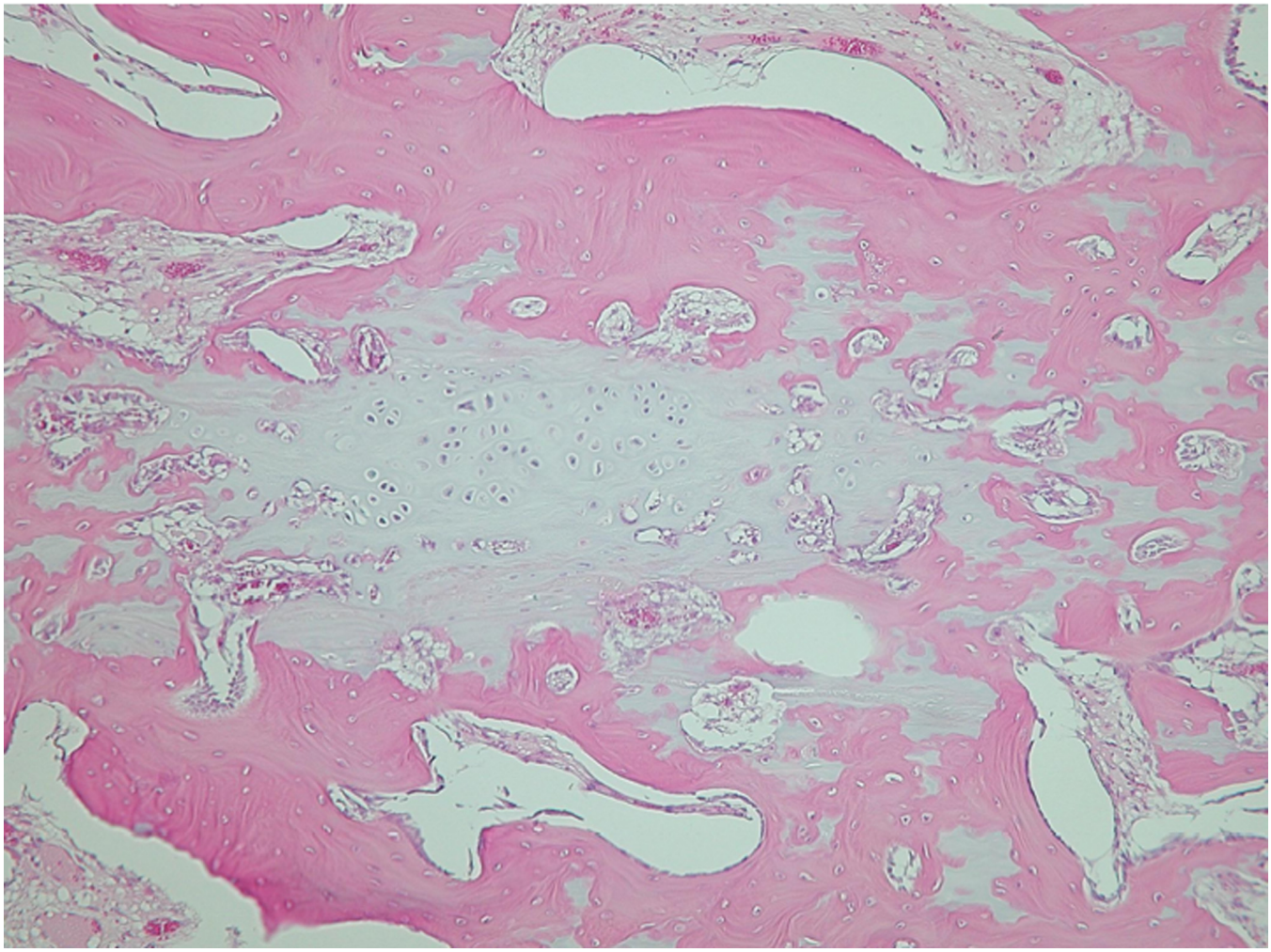


Fig. S5. Histological examination of a Angus dwarf bovine growth plate by alkaline phosphatase staining. The growth plate shows a general loss of organization and mixing of hypertrophic and proliferative chondrocytes.

Table S1. Primer sequences and PCR conditions for markers used in bovine dwarfism linkage mapping

Gene	Position	SNP	Primers	PCR conditions[a]
BMP2K	5′ UTR	A/G	GTGCCAACCCTGTGCTACTT TATATCGGGTCTCCGCTCTG	(1) 95 °C, 5 min; (2) 95 °C, 30 s; (3) 60 °C, 75 s; (4) 72 °C, 75 s; (5) go to step 2, 35×; (6) 72 °C, 10 min
	Intron 10	C/T	CATTGCTGAGAACATTAGGAAAA CCAGCTTCAGTGGCAGACTA	(1) 95 °C, 5 min; (2) 95 °C, 30 s; (3) 60 °C, 75 s; (4) 72 °C, 75 s; (5) go to step 2, 35×; (6) 72 °C, 10 min
	3′ UTR	C/T	GCAAAATTCAGGCCATCTTC GCACACACACACCCAAGACT	(1) 95 °C, 5 min; (2) 95 °C, 30 s; (3) 60 °C, 75 s; (4) 72 °C, 75 s; (5) go to step 2, 35×; (6) 72 °C, 10 min
PRKG2	Intron 1	A/G	AGCCGGAAACTCCACTAGGT GCAAAGCAAACCACTTCCTC	(1) 95 °C, 5 min; (2) 95 °C, 30 s; (3) 65 °C, 75 s; (4) 72 °C, 75 s; (5) go to step 2, 35×; (6) 72 °C, 10 min
	Intron 2	C/T	GACAAGGAACCCATGCAGAG GGCACAGCAGGAGACAAAA	(1) 95 °C, 5 min; (2) 95 °C, 30 s; (3) 65 °C, 75 s; (4) 72 °C, 75 s; (5) go to step 2, 35×; (6) 72 °C, 10 min
	Intron 3	A/G	TGTGAAATGACTCCATCAACA GGAAATCTTTTGCTGCTCACTT	(1) 95 °C, 5 min; (2) 95 °C, 30 s; (3) 60 °C, 75 s; (4) 72 °C, 75 s; (5) go to step 2, 35×; (6) 72 °C, 10 min
	Intron 6	A/C	ATCCCCTTCCCCCTCCTC CCTTGCTCTCATTTCTCTTCA	(1) 95 °C, 5 min; (2) 95 °C, 30 s; (3) 65 °C, 75 s; (4) 72 °C, 75 s; (5) go to step 2, 35×; (6) 72 °C, 10 min
	Intron 14	INDEL G	GGGAAACCCTTACGAAGTTAAA TGTGAAGAGGAAAAGGCATCA	(1) 95 °C, 5 min; (2) 95 °C, 30 s; (3) 60 °C, 75 s; (4) 72 °C, 75 s; (5) go to step 2, 35×; (6) 72 °C, 10 min
	Exon 15	C/T	AGGAGGGAAAGAGGGAGGAT GGGAAGCCCAAAGTAGAAATG	(1) 95 °C, 5 min; (2) 95 °C, 30 s; (3) 60 °C, 75 s; (4) 72 °C, 75 s; (5) go to step 2, 35×; (6) 72 °C, 10 min
	Intron 16	C/G	TGCATAGAATCACAACTGCAA GAGTGGAAAAACAGGGAGACC	(1) 95 °C, 5 min; (2) 95 °C, 30 s; (3) 60 °C, 75 s; (4) 72 °C, 75 s; (5) go to step 2, 35×; (6) 72 °C, 10 min
	3′ UTR	(1) G/T; (2) A/C	TCCTCACCTTTGGTTTCATCTT TTTGCCTTTCCCCAGACTAC	(1) 95 °C, 5 min; (2) 95 °C, 30 s; (3) 60 °C, 75 s; (4) 72 °C, 75 s; (5) go to step 2, 35×; (6) 72 °C, 10 min

[a]PCR reaction conditions were as described by manufacturer (Promega).

Table S2. SNaPshot primers used for genotyping in bovine dwarfism linkage mapping

Gene	Position	SNP	Primers[a]
BMP2K	5′ UTR	A/G	TCATTTATCTTTAGCTTGCTGCTGCTGCTAAGTCACTTCAGTTGTGTCCGACTCTGTGCA
	Intron 10	C/T	AATATACCTGCTTCACAATAAGTCTGCCAATAATTTACACTGCTAAGTAACCAAGCCATAAAAGGTTT ATGTAAA
	3′ UTR	C/T	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTATTTCTAAAATATGTACGGTGA
PRKG2	Intron 6	A/C	GACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGCAGCAGA GATATACAGATGAAT
	Exon 15	C/T	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCCCAGAAAGATAACAAGA
	Intron 16	C/G	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCTGTTGCCTGCTTCCTGCCT

[a]All primers are given in 5′ to 3′ orientation. Conditions of all single-base extension was the same: (1) 96 °C for 10 s; (2) 50 °C for 5 s; (3) 60 °C for 30 s; (4) repeat steps 1–3 for 24 more cycles; (5) store product at 4 °C.

Table S3. RFLP assays in the *PRKG2* gene

Position	SNP	Enzyme[a]
Intron 1	A/G	Mse I
Intron 2	C/T	BstZ17 I
Intron 3	A/G	Bsr I
Intron 14	INDEL G	Hyp166 II
3′ UTR	G/T	BsaH I
3′ UTR_2	A/C	Taq I

[a]Digest conditions were as described at http://www.neb.com/nebecomm/products/category1.asp?#2.
All digests were done overnight.

Table S4. Single-nucleotide polymorphism frequencies for 12 SNPs in positional candidate genes BMP2K and *PRKG2* used for fine mapping the Angus dwarfism mutation

Gene	Position	Alleles	Frequency, %
BMP2K			
	5′ UTR	A/G	A = 82 G = 18
	Intron 10	C/T	C = 44 T = 56
	3′ UTR	C/T	C = 62 T = 38
PRKG2			
	Intron 1	A/G	A = 40 G = 60
	Intron 2	C/T	C = 14 T = 86
	Intron 3	A/G	A = 22 G = 78
	Intron 6	A/C	A = 38 C = 62
	Intron 14	Ins/del G	delG = 78 insG = 22
	Exon 15	C/T	C = 46 T = 54
	Intron 16	C/G	C = 42 G = 58
	3′ UTR	G/T	G = 85 T = 15
	3′ UTR2	A/C	A = 85 C = 15