ANIMAL GENETICS Immunogenetics, Molecular Genetics and Functional Genomics

BRIEF NOTES

doi:10.1111/j.1365-2052.2009.02013.x

Development of fibroblast cell lines from the cow used to sequence the bovine genome

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Accepted for publication 12 November 2009

Source/methods: Two cell lines were initiated from 6-mm skin biopsies obtained from the Hereford cow whose DNA was used in sequencing the bovine genome.[1] Biopsies were mechanically disrupted and digested in 0.25% trypsin to release individual cells.[2] Immunohistochemistry was performed using a HistoMark® Biotin Streptavidin Kit – AP System (KPL), as per the manufacturer's instructions. The primary antibodies used were mouse monoclonals against cytokeratin (1/5/10/14; Vector Laboratories, Inc.) and vimentin (Invitrogen), and the secondary antibody used was goat-anti-mouse IgG (KPL). The colorimetric portion of the analysis was performed using the HistoMark® RED Phosphatase System (KPL). Metaphase chromosome spreads were prepared for cytogenetic analysis by the Cytogenetics Laboratory at the Corriel Institute for Medical Research, Camden, NJ, USA, as previously described.[3] See Appendix S1 for more information.

Results/description: Immunohistochemical staining showed that the cell lines were negative for the expression of pan-cytokeratin and positive for the expression of vimentin, indicating that the cell lines were mesodermal in origin. The cell lines were designated MARC.BGCF.2 and MARC.BGCF.1–3 (MARC Bovine Genome Cow Fibroblasts). No normal cells were observed during cytogenetic analysis of MARC.BGCF.2 (Fig. S1A–F). Thirteen different types of abnormal cells were seen. The cell line was female; therefore, two X chromosomes were seen in each cell. The predominant abnormalities were trisomy for chromosome 6 and additional but unknown chromosomal material added atop the centromere of chromosome 18. Most cells also suffered gains and losses of other autosomes, and structural abnormalities affecting chromosome 2 were seen in two cells. Cytogenic analysis of cells from cell line MARC.BGCF.1–3 indicated that more than half of this culture is tetraploid, as seen by a scan of 100 metaphases for size (Fig. S1G–L). However, we were rarely able to obtain tetraploid metaphase spreads without overlapping chromosomes. It is most likely that the chromosomal abnormalities in both cell lines occurred *in vitro*, which is not uncommon in cell lines. Because genomic sequence has not been obtained for these cells, prior to using them as a source of DNA, it would be prudent to compare the alignment between the published bovine genome sequence and the cell lines to ensure that mutations do not occur at areas of interest. Both cell lines have been submitted to the ATCC (http://www.atcc.org). Following expansion, they will be available as CRL-2874 (MARC.BGCF.2) and CRL-2875 (MARC.BGCF.1-3). At this time, MARC.BGCF.2 has been subcultured 103 times and MARC.BGCF.1-3 20 times.

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Acknowledgements: The authors would like to thank Stacy Bierman for excellent technical assistance; Joan Rosch and Sherry Kluver for secretarial assistance; and Drs. Tim P. L. Smith, Tad S. Sonstegard, Melissa J. Landrum and Tara G. McDaneld for critical review.

References

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2 Freshney R.I. (1987) Culture of Animal Cells: A Manual of Basic Technique, 2nd edn, p. 117. Alan R. Liss, Inc., New York.
3 The Bovine Genome Sequencing and Analysis Consortium *et al.* (2009) *Science* **24**, 522–8.

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Supporting information

Additional supporting information may be found in the online version of this article.

Figure S1 The composite karyotype.

Appendix S1 Supplemental methods.

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doi:10.1111/j.1365-2052.2010.02021.x

East Friesian sheep carry a *Myostatin* allele known to cause muscle hypertrophy in other breeds

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Accepted for publication 14 December 2009

Background: The East Friesian breed of sheep was developed in northern Germany and the Netherlands, and has become one of the world's most productive dairy sheep. It is likely to have contributed to the foundation of other breeds, such as the Texel, which originated in the Netherland's chain of West Friesian islands. The Texel is a meat breed that displays a muscle