Production and use of bovine DNA libraries: DNA-sequencing

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Summary: An important part in the use of genomic DNA libraries is the sequencing of identified clones for detailed information. In this study, methods for DNA sequence analysis were elaborated and employed for the k-casein gene, a bovine milk protein. The results encourage further research.

KEYWORDS: Cattle - DNA-sequencing - Genomic DNA.

A study of the use of DNA sequencing methods was carried out by the Institute of Physiological Chemistry in collaboration with the Institute of Animal Breeding and Genetic Research as part of the research project "Production and use of bovine DNA libraries".

This study aimed to show whether DNA sequencing would be suitable for veterinary purposes. For example, could the method be applied and adapted to a DNA fragment from a Cosmid library of bovine genomic DNA (1)?

A cDNA fragment of known sequence, a bovine k-Casein-DNA fragment (3), was isolated, cloned and sequenced. The nucleotide sequence determined corresponded to that expected for a sub-fragment of k-Casein-DNA. This showed that the newly established sequencing method was reliable.

The sequencing method was then used to analyse a DNA fragment of unknown sequence. A cosmid library of bovine genomic DNA (2) was first screened with a bovine α_{s1}-Casein-cDNA probe (3) and a clone was chosen which showed clear and reproducible hybridisation to a Pst I fragment of about 2.0 kb in size.

After digestion with Pst I and agar gel electrophoresis, the relevant size fraction of fragments was electro-eluted and digested into about ten sub-fragments using the restriction enzyme Alu I. This mixture of fragments was cloned into the Sma I site of the M13 vector. After ligation and transformation, 36 colourless plaques were identified and single-stranded recombinant DNA phages isolated. Ten clones were chosen at random for further analysis. First, identical clones were identified on the basis of A-tracks (application of only one sequencing reaction per clone), then a representative of each group was chosen for further sequence analysis. In all, six different clones were sequenced.

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The results were as follows:

- The nucleotide sequences determined for clones began with a sequence which was derived from the vector. Following the sequence of the Sma I cloning site, the insert sequence began as expected.

- Two of the clones contained the same DNA fragment insertion, but inserted in opposite directions. A comparison of the two sequences shows that the sequencing method works very reliably.

- No homology was detected between the sequences of any of the clones and previously determined sequences of the αs1-Casein-cDNA (3) or the known sequence at the 5' ends of the gene (4).

From the results so far, it cannot be determined whether the sequenced fragments are derived from Intron DNA or DNA which non-specifically cross-reacts with the probe. Further experiments are necessary to answer this question.

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Résumé: Les banques d'ADN génomique ont une utilisation importante dans le séquençage de clones identifiés, pour obtenir des informations détaillées. Les auteurs ont mis au point des méthodes d'analyse des séquences d'ADN et les ont appliquées au gène k de la caséine, qui est une protéine du lait de vache. Les résultats obtenus invitent à poursuivre les recherches.

MOTS-CLÉS : ADN génomique - Bovins - Séquençage de l'ADN.

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Resumen: El uso de las bibliotecas de ADN genómico desempeña un papel importante en la determinación de secuencias de clones identificados para obtener informaciones detalladas. Los autores han establecido métodos de análisis de las secuencias de ADN y los han aplicado al gene k de la caseína, proteína de la leche vacuna. Los resultados alientan a proseguir con las investigaciones.

PALABRAS CLAVE: ADN genómico - Bovinos - Determinación de secuencias de ADN.

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REFERENCES

