

RAPID SOUTHERN BLOT PROTOCOL FOR THE ANALYSIS OF TARGETED MUTAGENESIS IN THE MOUSE IGHG1 LOCUS

PROTOCOL RAPID DE ANALIZĂ SOUTHERN BLOT A MUTAȚIILOR ȚINTITE ÎN LOCUSUL MURIN IGHG1

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The efficiency of homologous recombination relies on the length of the homology arms and the chromatin compactation of the locus. In ES cells the immunoglobulin locus is compacted. Thus, the homologous recombination events occur at a very low frequency. A high number of ES cell colonies have to be analysed to detect a homologous recombination event. Here we describe a reliable and rapid protocol for the analysis of targeted mutagenesis in the immunoglobulin locus using Southern Blot analysis.

Key words: Ighg1 gene, immunoglobulin, southern blot, embryonic stem cells, gene targeting.

Introduction

Gene targeting provides a powerful technology to introduce specific mutations into any desired locus in the mouse genome. Several studies demonstrate that mouse B cells express full-length chimeric or fully humanized antibodies showing that the mouse system upon antigenic stimulation can elicit an immune response at the same level as B cells in wildtype mice (1). Thus knock in technology provides the mean to reconstitute the mouse genome with human homologous immunoglobulin genes of interest and produce high affinity chimeric monoclonal antibodies to a wide spectrum of antigens via somatic hypermutation and selection mechanism. The immunoglobulin IgG1 is expressed at the highest level among the immunoglobulins and induces complement activation. The replacement of this gene with human counterparts is therefore of interest. Here we show the protocol for the Southern blot analysis of targeted mutagenesis of the mouse Ighg1 gene.

Materials and Methods

A 59,641 kb genomic sequence that encompasses the *Ighg1* to the *Ighg2a* was used to develop the Southern blot Strategy (2).

The map of this sequence was created using the software Laser Gene (DNA STAR) and a PC Unit IBM R51. The enzymes *Bam*HI was chosen for restrictive digestion of the genomic DNA. The methods of molecular biology for the restrictive digestion of the genomic DNA and that of the Southern blot analysis were used as described before (3).

Different probes have been designed to monitor the replacement of the secretory form of the *Ighg1* gene. The sequence of the primers used for the PCR amplification of the probes was presented in the table 1.

Table1- Oligonucleotide sequence used for PCR amplification.

Primer	Description	Primer sequence
-	5'H for.	AAAAGCGGCCGCGATGCACAGGCAGGAAGATC
-	5'H rev.	CCTGAATTCTACATGGAGTAGGTGATATCCAAC
P 284	3'S for	TCCTGAAGCTGGAAGTCCCG
-	P 3 Hom	AAATCGATGGCATGCCTTCCACATGGCTCTC
P 295	3'S++ for.	GAGTCACACCCTATCTTGGATG
P 296	3'S++ rev.	GGGGTCAGTGCTTAGCAGTC

The PCR conditions were adapted after the protocol described by supplier for the *Taq* polymerase used (Invitrogen). The PCR fragments were subcloned into pGMTe plasmids (Promega). In the table 2 are listed the probes generated for the Southern blot analysis.

Table 2 - Probes for Southern blot analysis of the *Ighg1* gene.

Probe	Primers	Length (bp)	Vector (pGMTe)	References
5'Hom	P5'Hfor/ P5'Hrev	843	5HomTe	3
3'SL	P284/ 3Hom	618	3SLTe	3
3'S++	P295/ PP296	311	3S++Te	3

Results and Discussions

The bioinformatical analysis of the *Ighg1* locus sequence showed that there are two restriction enzymes *Bam*HI and *Eco*RI suitable for the Southern blot analysis of the targeted mutation into the *Ighg1* locus. In the following experiments *Bam*HI was used. In the figure 1 was presented the partial restriction maps of the mouse. The figure comprises the genomic organization exons coding for the secretory and

bound form of the IgG1 immunoglobulin, the position of the *Bam*HI restriction sites and the length of the expected fragments after labeling with different radioactive probes.

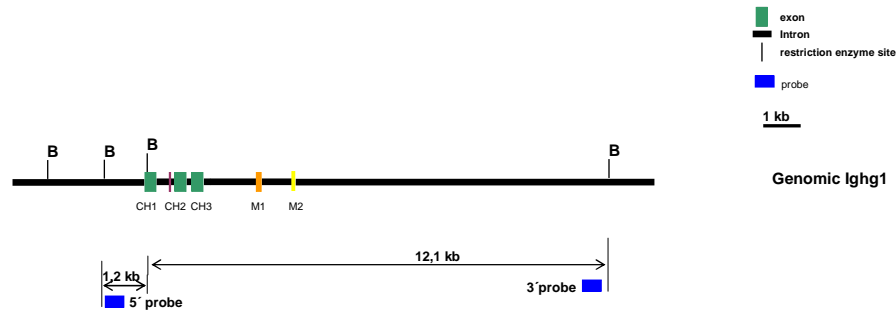


Figure 1: Restriction map of the *Bam*HI enzyme in the wild type IgH1 locus.

To establish a Southern blot protocol with reliable and reproducible analysis results after over night exposure, two different positive charged membranes (Amersham XL and Amersham N+) and three different hybridisation buffers (Denhardt's, FBI, Quick Hybridisation Buffer) were compared.

The criteria used to appreciate the suitability of the positive charged nylon membranes for this application were: the signal and the background intensity. In the figure 2 are presented the hybridisation results using the 3SL and 5Hom probes.

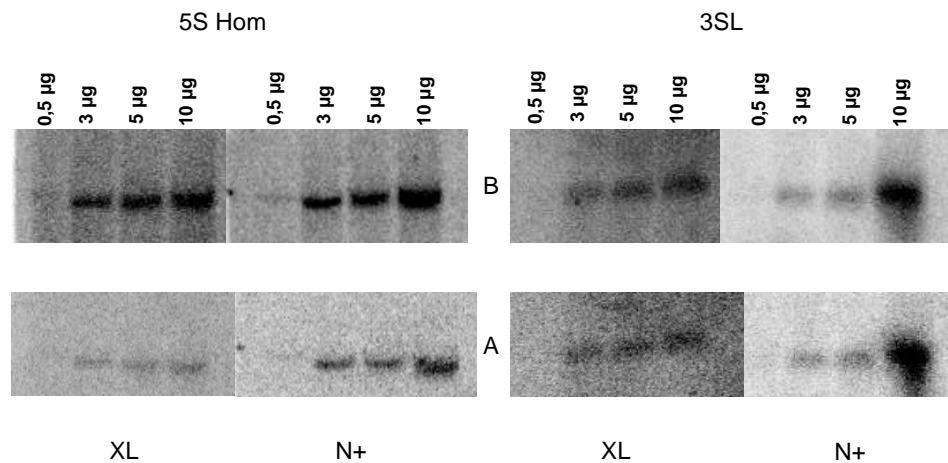


Figure 2: Signal and the background intensity of different nylon positive charged membranes. A- short exposure (o/n), B- long exposure.

The result show that the N+ membrane leads to signals of higher intensity than the XL membrane after over night exposure. The detection occurred in amount of genomic DNA of 3 μg genomic DNA. The background of the N+ membrane using the same hybridisation and washing conditions was of lower intensity compared to that of the XL membrane. This result was reproduced using different probes.

To determine the minimum level of detection of one homozygous gene, different amounts of DNA ranging between 0,5- 3 μg were blotted. The hybridisation was done with the 3S++ probe. In the figure 3 are presented the results obtained after short (over night, o/n) and long exposure (5 days) of the screens.

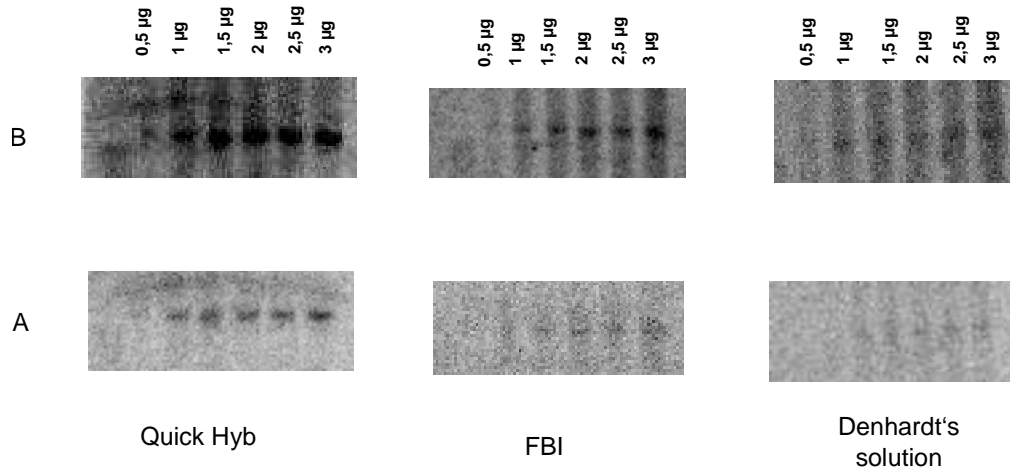


Figure 3: Minimum level of detection of the Ighg1 gene. A- short exposure (o/n), B- long exposure (5 days).

The result of the experiment shows that the Quick Hyb Buffer (Stratagene) enables the detection of one homozygot gene in a DNA quantity as low as 1 μg after over night exposure.

Conclusions

A reliable and reproducible Southern blot analysis protocol was established for gene targeting of the Ighg1 gene. The use of the nylon N+ positive charged membrane (Amersham) and Quick Hyb Buffer (Stratagene) leads to the detection of homozygous events in amount of DNA as low as 1 μg , after over night exposure.

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