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# Search for mouse gene related to GA733 human tumor antigen gene

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**Abstract.** Human antigen GA733-1, defined as 40 kDa cell glycoprotein, is one of the antigens associated with gastrointestinal carcinomas. Its studies may contribute to the tumor etiology and therapy effects in animal model.

**Key words:** GA733 oncoantigen, gene sequences, mouse genomic DNA.

Monoclonal antibody (GA733) derived from mice immunized with a human stomach adenocarcinoma cell line exhibits very strong affinity to numeruos human carcinomas (HERLYN et al. 1986). GA733 antigen has been found in very high densities on the surface of endodermal epithelium derived neoplasms and also in lower degree in normal epithelial tissues. It is very probable that there exist at least two glycoproteins of GA733 antigen family in human cells. Monoclonal antibody CO17-1A binds to overlapping epitopes of GA733 Ags or more likely to cross-reacting epitopes present on related antigens (ROSS et al. 1986). CO17-1A antigen has got narrow specificity in comparison to GA733 Ag and is not expressed for instance in cervix or lung carcinomas (HERLYN et al. 1986).

GA733 Ag–related gene have been isolated and sequenced (SZALA et al. 1990). This GA733-1 Ag human gene is very stronly expressed (on mRNA level) in human pancreatic carcinoma and colorectal carcinoma cell lines (GOTTLINGER et al. 1986). Monoclonal antibodies agaist both GA733 and CO17-1A epitopes inhibit growth of the tumor xenografts in nude mice (HERLYN et al. 1984) and have been successfully applied in colorectal cancer therapy (DOUILLARD et al. 1986). It would be interesting to find GA733 related antigen

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in the mouse cells and GA733 Ag genes in mouse genome, first in order to get more safe animal model of colorectal cancer therapy and additionally, to make some comparisons with human intronless GA733 Ag gene (BERGSAGEL et al. 1992).

In the presented study we looked for GA733 Ag–related sequences in the mouse genomic library.

## Material and methods

### Library plating

Commercial preparation of mouse genomic library (DNA of Balb/c mouse liver digested with ECoRI enzyme and placed in Charon 4A vector) have been used. *E. coli* LE392 host strain growing in NZY medium supplemented with 0.2% maltose was infected with $1\times10^8$ plaque forming units incubated for 20 minutes at 37°C for efficient phage adsorption and transferred into 8 ml of top agarose (temp. 50-55°C) on the bottom agarose plates. Six plates (totally 6×10 pfu) were incubated for 10 hours at 37°C.

### Bacteriophage library screening

After transferring to WHATMAN nitrocellulose filters (two replaces of each plate) the obtained discs were denaturated in 0.5 M NaOH and 1.5 M NaCl, neutralized with 0.5 M TRIS, pH 8 and 1.5 M NaCl, shortly washed with 2×SSPE (0.36 M NaCl, 0.02 Na-phosphate buffer pH 7.4 and 2 mM EDTA).

After baking (2 hours 80°C) the filters were washed in 6×SSC buffer (SSC buffer: 0.15 M NaCl and 0.015 M Na-citrate), soaked with 50 mM TRIS pH 8, 1 M NaCl, 1 mM EDTA and 0.1% SDS, prehybridized and hybridized.

### Hybridization

Prehybridization was carried out for 4 hours at 37°C with prehybridization solution (1×Denhardt solution, 4×SSC, 50% deionized formamide and 100 mg/ml denaturated carrier DNA (*E. coli* DNA) depolimerized by sonication). Hybridization was carried out up to 36 hours at a room temperature in the same prehybridization solution containing additionally 0.2% SDS and about 0.1 mg/ml radioactive DNA probe (PstI – PstI fragment of human GA733-1 gene) nick translated with a $^{32}$P-dCTP (Amersham UK) to a specific activity

$10^8$-$10^9$ dpm/mg DNA. After hybridization, the filters were washed in middle stringency conditions with prehybridization solution, 3×SSC buffer, 2×SSC and 1×SSD buffer supplemented with 0.5% SDS for 10 minutes at 65°C and finally – two times (for 120 and 30 minutes, respectively) in 0.5×SSC and 0.5% SDS at 67°C.

**Extraction of bacteriophage**

Charon 4A particles were eluted from plates twice with 10 ml and 5 ml of SM buffer (for 1 hour and 30 min, respectively) at a room temperature. Composition of SM buffer: 100 mM NaCl, 10 mM $MgSO_4$, 50 mM TRIS pH 7.5, 0.01% gelatine. Combined eluates were centrifugated to pellet bacterial debris, supernatant was incubated with 1 mg/ml of DNase and pancreatic RNase for 3 hours at a room temperature. Phage particles were precipitated with polyethylene glycol (PEG6000) and 1 M NaCl overnight at 4°C. The pellet was dissolved with SM buffer and dialyzed, according to the procedure described by MANIATIS et al. (1989).

**Isolation of bacteriophage DNA**

DNA was isolated from bacteriophage suspension by dialysis for 2 hours at 4°C with TEM buffer (10 mM $MgSO_4$, 10 mM TRIS pH 8.0 and 1 mM EDTA), incubating during 15 minutes at 65°C with 10 mM EDTA and 0.2% SDS followed by digestion with 50 mg/ml proteinase K during 1 hour at 37°C. DNA was extracted with phenol : chloroform mixture (1:1) and twice with chloroform : isoamyl alcohol (1:24). Aqueous phase was dialysed for 4 hours at 4°C with TE buffer (10 mM TRIS pH 8.0 and 1 mM EDTA) and precipitated with two volumes of 96% ethanol and 0.1 vol. of 3.0 M sodium acetate. After centrifugation, the obtained pellet was washed twice with 70% ethanol, dryed under vacuum and dissolved in 50 ml TE buffer.

**Electrophoresis of DNA**

Charon 4A DNA clone with appropriate insert was electrophoresed before and after ECoRI digestion (1 to 2 μg of DNA digested for 2 hours with 5 units of ECoRI), on 1% agarose gel in 0.5×TBE (MANIATIS et al. 1989).

**Southern blotting and hybridization conditions**

Blotting of DNA electrophoresis was essentially the same as described in MANIATIS manual. Prehybridization and hybridization were similar to those used for phage libraries screening.

## Results

After the first screening of mouse genomic library we have got six strong signals after hybridization to probe derived from human GA733-1 Ag gene. Additionally we have found 22 weaker signals. After the secondary screening there remained only 5 strongly hybridizing clones and 9 clones giving weaker signals when hybridization was performed in middle stringency conditions (Figs. 1, 2). Finally after the third and fourth screening we have purified eleven mouse genomic clones (in Charon 4A vector) related to the human gene GA733-1 (shown in Fig. 3). After minipreparations procedure we have purified DNA of all clones. All preparations were digested with EcoRI restriction enzyme and electrophoresed on 1% agarose (Figs. 4, 5).

Agarose gels were blotted to nitrocellulose and hybridized to the same human GA733 Ag gene probe (Figs. 6-8). Clone 90311 can be related to 3.8 kb DNA fragment visible in Fig. 4 (line 7), the observed signal was very strong (Fig. 6). Clones 70441 and 20341 gave two radioactive DNA fragments related to 2,3 kb and about 2,35 kb band, respectively (Fig. 7). Clones 60522 and 50441 gave three specific radioactive restriction fragments of 4.3 kb and 5.4 kb and about 5.5 kb band, respectively (Fig. 8).

## Discussion

The present study indicates the presence of several clones of mouse genomic DNA, related in various degree to the human GA733-1 antigen gene sequences. Generally, restriction maps of these clones appear to be different in comparison to GA733-1 Ag gene. Therefore, all examined clones appear to be unique. It will be very interesting to compare the structure and sequences of other members of human GA733-1 antigen gene family and related genes of other species (ZIELEWICZ 1994). Strong evolutionary conservation of this gene was preliminary noticed on the basis of DNA studies in mouse, chicken, fish, frog and snail species. According to Linnenbach studies the GA733-1 neoantigen gene derived probably from human retrotransposone and is an interesting example of intronless functional gene. Its functional involvement in tumorigenesis can be eventually deduced (LINNENBACH et al. 1993). The similarity of Interleukin 2 receptor sequences (especially in growth factor binding domain) to the GA733-1 antigen gene (LINNENBACH et al. 1989) and multiplicity of sequences homologous to Interleukin 2 gene in the human genome (MITA et al. 1986) is very intriguing.

On the other hand, it will be interesting to establish more precisely restriction maps and detailed sequences of isolated mouse clones. Structural mutational

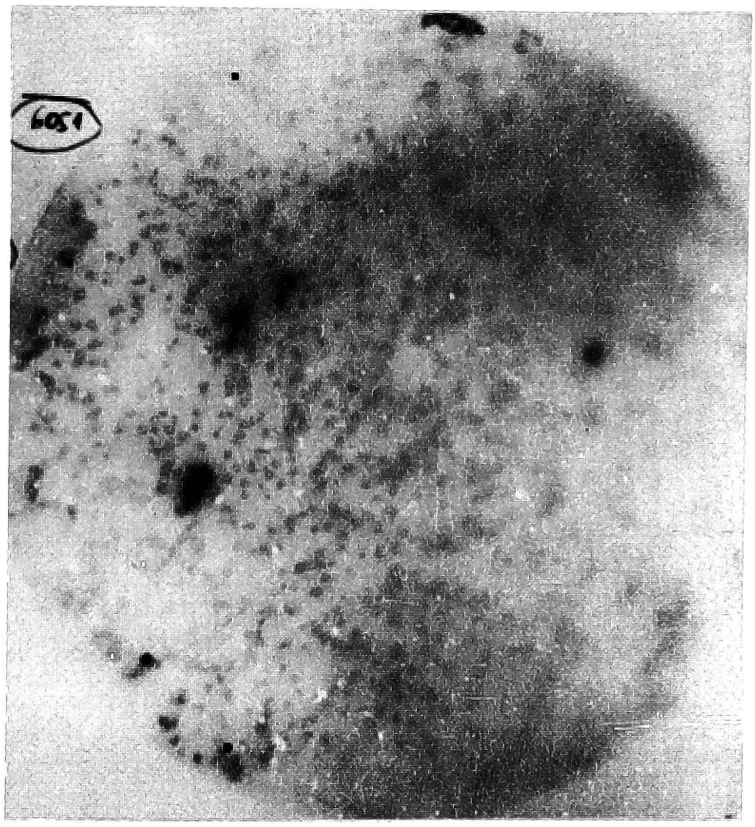


Fig. 1. Secondary screening of mouse genomic library with human GA733-1 antigene gene derived DNA probe (clone 605)

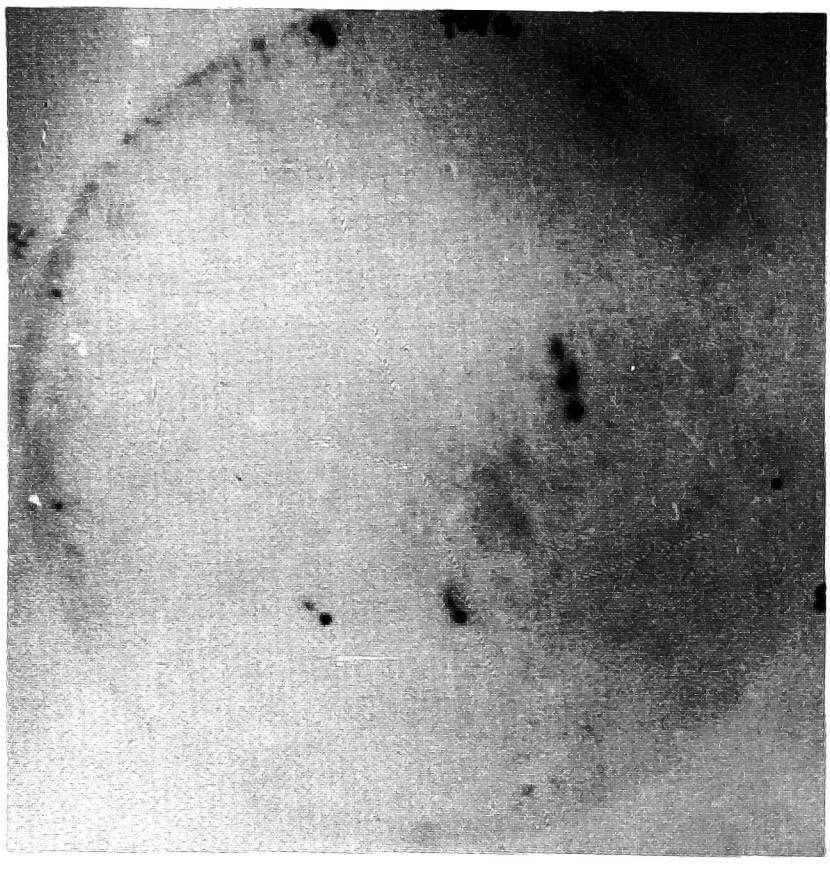

Fig. 2. Secondary screening of mouse genomic library with human GA733-1 antigene gene derived DNA probe (clone 704)

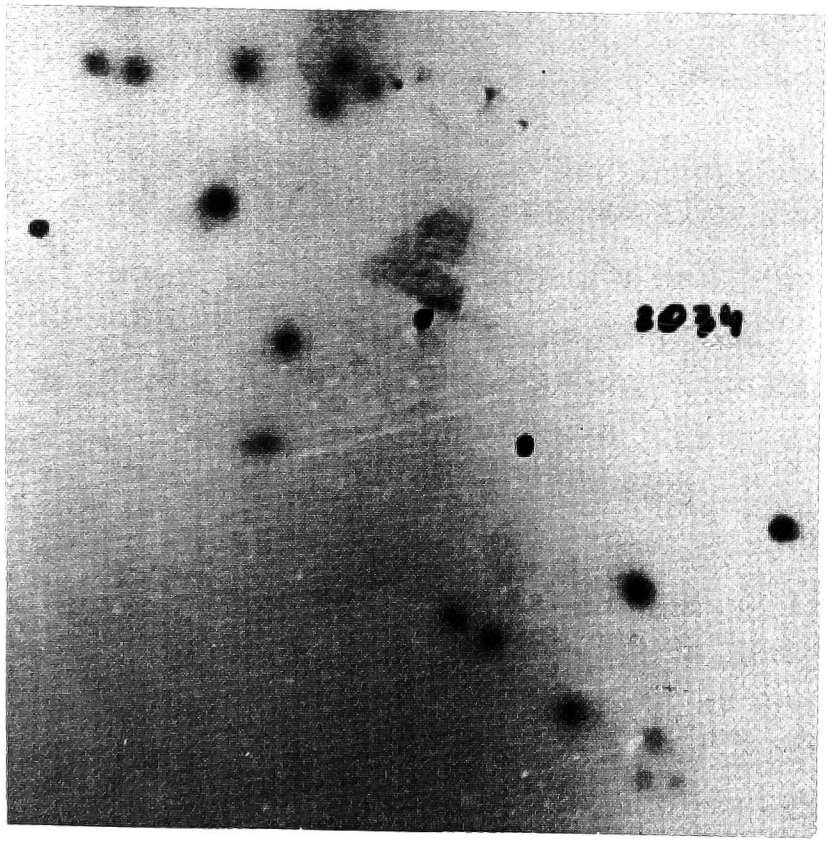


Fig. 3. Tertiary screening of mouse genomic library with human GA733-1 antigene gene derived DNA probe (pure 2034)

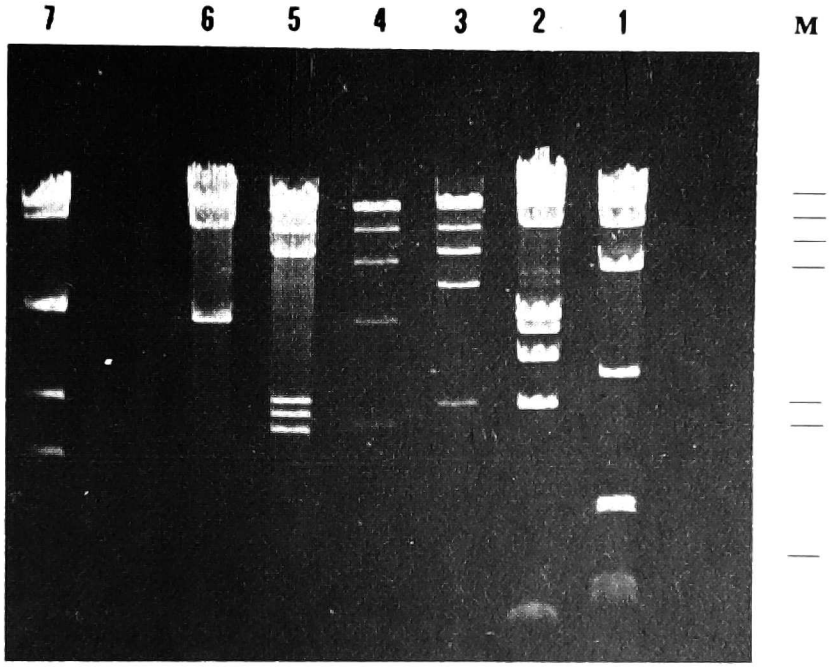


Fig. 4. Agarose gel electrophoresis of ECoRI digested DNA, isolated from different pure clones of mouse genomic library. Lines on right margin – marker DNA (λ phage DNA digested with HindIII). Marked clones 20341 line 2, 70441 line 3, 90311 line 7

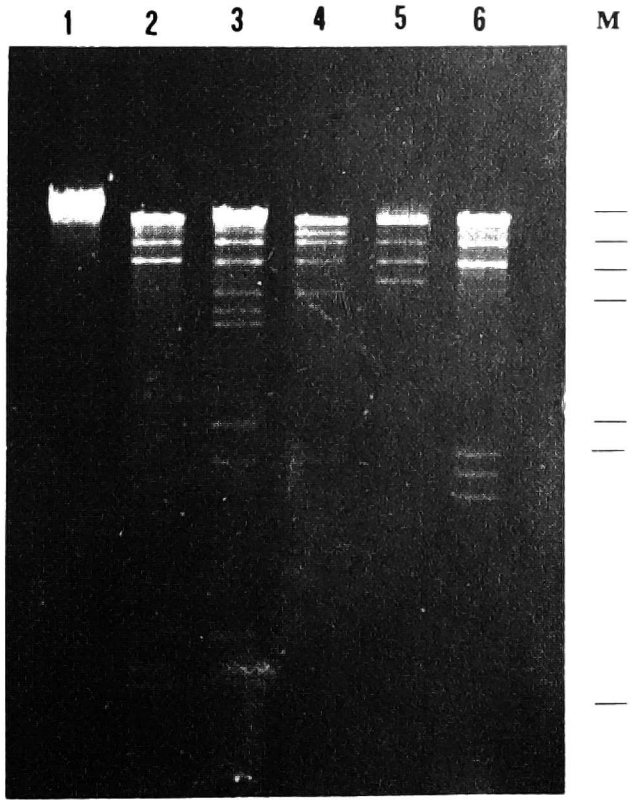


Fig. 5. Agarose gel electrophoresis of ECoRI digested DNA, isolated from different pure clones of mouse genomic library. Lines on right margin – marker DNA (λ phage digested with HindIII). Marked line 3 of 60522 and line 4 of 50441 clones

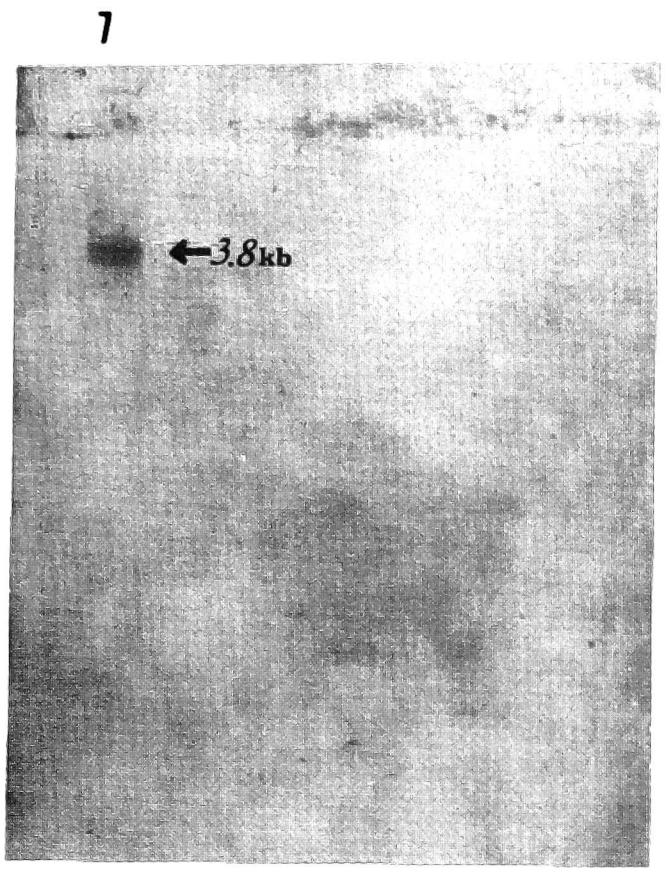


Fig. 6. Autoradiography of hybridization of GA733-1 antigene gene derived DNA probe (labelled with $\alpha$-$^{32}$P dCTP) to the ECoRI digested DNA, prepared from isolated pure clone (after Southern bloting of gel shown on Fig. 4). Position of 90311 clone marked line 7

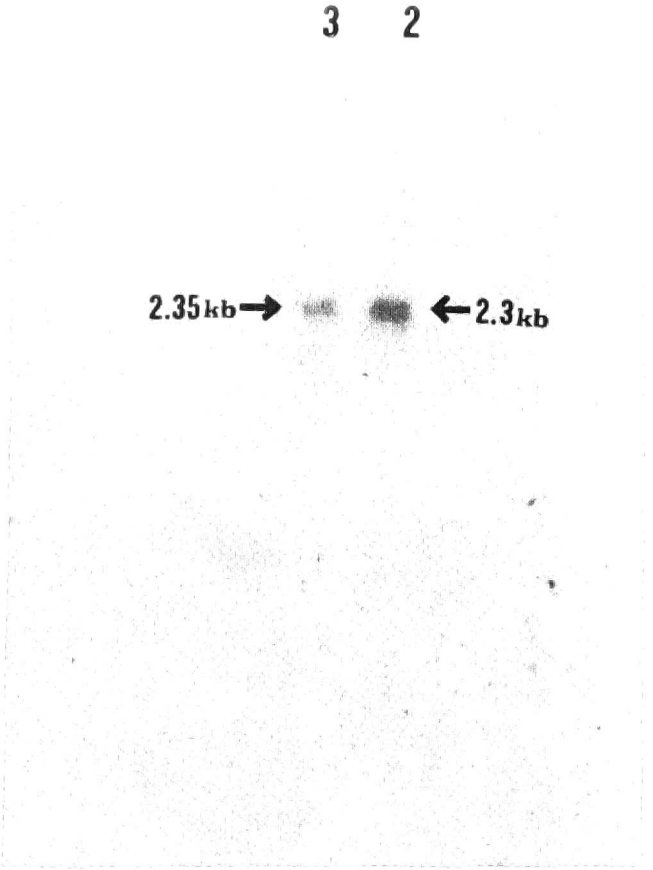


Fig. 7. Autoradiography of hybridization of GA733-1 antigene gene derived DNA (labelled with $\alpha$-$^{32}$P dCTP) to the EcoRI digested DNA, prepared from isolated pure clones (after Southern blotting of gel shown in Fig. 4). Marked positions of clones 70441 (line 3) and 20341 (line 2)

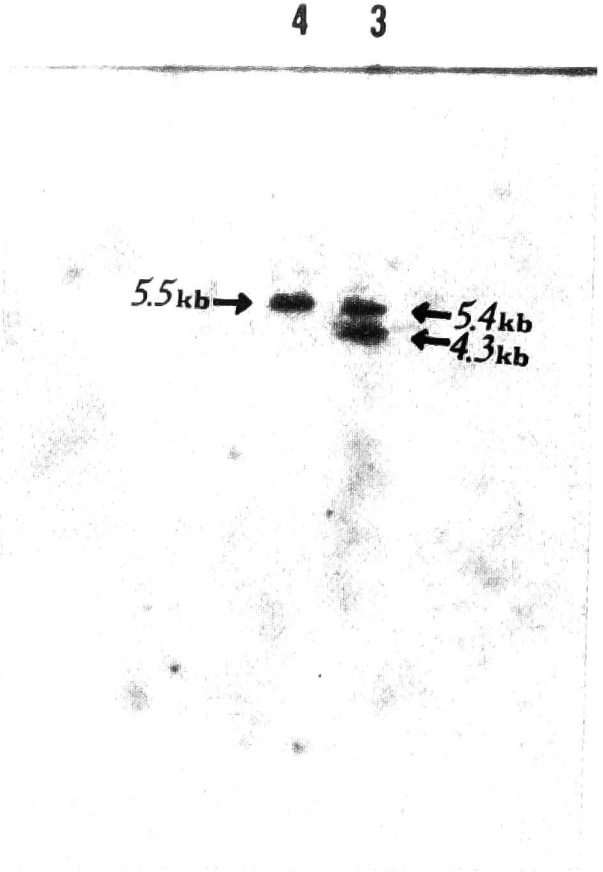


Fig. 8. Autoradiography of hybridization of GA733-1 antigen gene derived DNA (labelled with $\alpha$-$^{32}$P dCTP) to the EcoRI digested DNA, prepared from isolated pure clones (after Southern blotting of gel shown on Fig. 5). Marked positions of clones 60522 line 3 and 50441 line 4

events of GA733-2 gene have not been found yet (LINNENBACH et al. 1989). Additionally GA733 gene promoter function studied by Siemieniako revealed a complex mechanism of this gene repression (SIEMIENIAKO et al. 1994).

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