

B. Wirth
J. Schönling
A. El-Agwany
J. Seehafer
K. Rütter
H. Raschke
A. Leutner
B. Piechaczek-
Wappenschmidt

Institute of Human Genetics,
Bonn, FRG

Efficiency of Various Strategies and Materials to Generate New Markers: Saturating the Region 5q11.2-q13.3 with 30 New Randomly Distributed Clones

Abstract

Several different strategies and materials were used for saturating the region 5q11.2-q13.3 with new, randomly distributed markers: isolation of human clones from three chromosome-5-specific libraries (a *Bss*HII endclone phage library from the somatic cell hybrid H64 and two total genomic phage libraries from radiation hybrids IH12 and IH132), as well as Alu-PCR from chromosome-5-specific radiation hybrids with overlapping fragments in the region around the spinal muscular atrophy locus, followed either by direct isolation of Alu-PCR products or hybridization of Alu-PCR products to chromosome-5-gridded cosmid libraries. 253 human phage and cosmid clones were mapped to various parts of chromosome 5 by deletion mapping to somatic cell hybrid panels. 30 of these clones were mapped into the region 5q11.2-q13.3, 9 of which are flanking rate cutting *Bss*HII-sites, known to be, often, starting points for genes. They represent excellent starting material for the development of new polymorphic markers and sequence-tagged sites, for YAC screening and building of contigs, as well as for direct isolation of genes.

Key Words

Markers from 5q11.2-q13.3
Endclone library
Radiation hybrids
Alu-PCR
Screening strategies
Spinal muscular atrophy

Introduction

The chromosomal region 5q11.2-q13.3 covers about 1% of the human genome. In addition to the important and as yet uncloned gene for autosomal recessive spinal muscular atrophy (SMA), this region contains quite a

few cloned genes such as dihydrofolate reductase (DHFR), hexosaminidase B (HEXB), microtubule-associated protein 1B (MAP1B), RAS p21 protein activator (RASA), 5-hydroxytryptamine receptor 1A (HTR1A), cyclin B1 (CCNB1), phosphatidylinositol-3-kinase-associated p85 protein (GRB1), zinc fin-

ger protein 5 (ZNF5), arylsulfatase B (ARSB) and cartilage linking protein (CRTL1) [1]. Many more genes are expected to be encoded in this chromosomal region. This assumption is also based on the fact that roughly 70% of the chromosomal segment consist of Giemsa light bands which are usually gene-rich portions of the human genome. The above-mentioned genes were isolated by methods other than positional cloning. Thus, relatively little is known about the physical map of this region.

Our main interest is directed towards the isolation of the gene responsible for SMA by methods of positional cloning. The SMA gene has previously been localized on 5q11.2-q13.3 between the loci D5S6 (proximal) and D5S39 (distal) by genetic linkage analysis which corresponds to a genetic interval of about 10 cM [2, 3]. Since then, new flanking markers were identified which narrowed down the SMA region to 6 cM [4-6] and recently to 4 cM [7].

Whenever positional cloning is required, the isolation of many region-specific clones is important for rapid progress in the construction of (yeast artificial chromosome) YAC-contigs, physical mapping, isolation of polymorphic markers and the cloning of gene(s).

In order to enrich the region 5q11.2-q13.3 with further markers, we used various methods to select new region-specific human clones, and various starting materials based on chromosome-5-specific somatic cell hybrids, radiation hybrids (RHs) and flow-sorted gridded libraries.

Four different strategies were used here for the isolation of region-specific clones. The efficiency, advantages and disadvantages of each will be compared and discussed.

(1) Isolation and mapping of CpG flanking phage clones derived from a size-selected *Bss*HII endclone library (kindly provided by Drs. Frischauf and Varesco), made from the

somatic cell hybrid H64 (chromosome 5 and 4 on hamster background).

(2) Construction of two genomic phage libraries from chromosome-5-specific radiation hybrids and isolation of human clones.

(3) Alu- and Line-PCR from 10 partly overlapping RHs and isolation of common bands from ethidium bromide (EtBr)-stained gels.

(4) Alu-PCR from 4 overlapping RHs and the somatic cell hybrid HHW1064 (del5q11.2-q13.3) used as a negative control; hybridization of the PCR products to flow-sorted gridded cosmid filters and isolation of clones giving signals with at least two RH and not with the cell line HHW1064. The physical location of all clones was carried out by deletion mapping using somatic cell hybrids with the whole of chromosome 5 or part of it.

Genomic libraries could either represent a collection of fragments of total genomic DNA or of only some specific regions, flanking or including CpG islands, like endclone libraries [8], linking libraries [9] or jumping libraries [10]. Clones flanking CpG islands often identify conserved or transcribed sequences and are extremely useful for the construction of long-range restriction maps. However, there is the risk of choosing a rarely cutting enzyme that does not cut in or near the gene one is looking for, or that the library contains size-selected fragments and the fragment bearing the gene of interest has not been subcloned. Furthermore there are genes which do not start with a CpG island.

Therefore, the additional use of total genomic libraries might turn out to be very important. In order to minimize the efforts put into the selection of specific from unspecific clones, the use of chromosome- or region-specific somatic cell hybrids or RHs is very advantageous.

Since the construction of libraries and selection of human-specific clones involve mul-

tiple labor-intensive steps, we used another very powerful method, the Alu-sequence-primed polymerase chain reaction (Alu-PCR) from RHs containing overlapping regions. This method allows specific amplification of human fragments from somatic cell hybrids or RHs [12–14] and therefore enables the direct generation of specific sequences without the construction of libraries and all the consequent laborious steps mentioned above.

The disadvantage of most RHs is that they usually contain human fragments additional to those of interest. In order to select Alu-PCR products from the overlapping region, one can either compare the Alu-PCR bands in EtBr-stained agarose gels or hybridize them to each other and isolate common bands. Another more efficient strategy is the hybridization of the Alu-PCR products from different overlapping RHs to chromosome-specific gridded libraries and the selection of common positive clones [15].

Here we report the isolation and mapping of 253 human clones, using four different strategies and various starting materials. 30 of these clones were mapped into the region 5q11.2-q13.3.

Materials and Methods

Somatic Cell Hybrids

The following cell lines on a hamster background were used: PN/TS containing a single human chromosome 5 [11]; HHW1064 containing a single human chromosome 5 with a large cytogenetically detectable deletion of the region 5q11.2-q13.3 [16] and two smaller microdeletions on 5p and 5cen-q11, proximal to D5S76 [17], and HHW213 containing 5pter-q11 and a small fragment from the distal 5q [18].

Radiation Hybrids

27 chromosome-5-specific RHs (kindly provided by Drs. E. Solomon and H. Thomas) were selected out of 173 RHs based on signals obtained with three SMA flanking markers pM4, pJK53 and p105-153 [H. Thomas, pers. commun.].

Probes

All probes used for characterization of the RHs are polymorphic markers flanking the SMA locus: pM4 (D5S6), pJK53 (D5S112) and p105-153 are RFLPs [19], while EF(TG/AG) (D5S125), YN(CT)(D5S127) [7], MIT-I105 (D5S351) [20] and RB110/111 (MAP1B) [6] are polymorphic microsatellites.

The human *Bss*HII endclones derive from a somatic cell hybrid H64 containing only human chromosomes 5 and 4 in a hamster background [11]. The library has been size-selected by pulse field gel electrophoresis (PFGE). *Bss*HII fragments in the range of 0.5–2 Mb were isolated and used to construct the library. 365 clones have previously been isolated and mapped to chromosome 5q, 5p, or 4 by Dr. Varesco. Of these, we chose 111 clones which mapped to 5q or had not yet been mapped at all.

Construction of Libraries

High-molecular-weight genomic DNA from the RHs was partially digested with *Mbo*I and ligated into the *Bam*HI site of the vector arms of EMBL3 (IH12) or Lambda-Dash 12 (IH132), packaged with Gigapack-Gold (Stratagene) and plated onto bacterial strain NM646 as described in Frischauf [21]. About 100,000 pfu of each library were plated out to be screened for human clones.

Screening for Human Clones

Two replicas from each masterplate were produced using Hybond N+ filters (Amersham) and standard methods [22]. 50 ng human pool DNA plus recombinant plasmid DNA containing Alu- and Line-repetitive fragments were labelled with ³²P-dCTP by random priming. The labelled probes were preannealed with 100 µg unlabelled hamster DNA in 0.24 M Na-phosphate buffer for 2 h at 65 °C. The filters were hybridized with 5 × 10⁵ cpm probe/ml Church medium (7% SDS/0.5 M Na-phosphate buffer pH 7.2/1 mM EDTA) overnight at 65 °C. Filters were washed in 3 × SSC at room temperature, then in 2 × SSC/0.1% SDS, 1 × SSC/0.1% SDS, and 0.1 × SSC/0.1% SDS each for 30 min at 65 °C and exposed to Kodak X-OMAT films at –70 °C for 1 day.

Human clones were identified, rescreened and DNA was isolated from 3-ml overnight cultures. A protocol described by Pohl et al. [23], which has been modified, was used. A single plaque was picked with a Pasteur pipette, inoculated in 3 ml of NZCYM-broth medium (Dianova) and shaken at 37 °C and 300 rpm overnight in a 12-ml Falcon tube (2059). After adding 1/100 of chloroform the phage-lysed bacteria were pelleted. 3 µl each of DNase and RNase (10 mg/ml)

were added to the clear supernatant and then incubated for 30–60 min at 37°C. Afterwards 300 µl of pronase E buffer (300 mM NaCl/100 mM Tris pH 7.6/50 mM EDTA) and 30 µl pronase E (20 mg/ml, Boehringer) were added and incubated at 37°C for 2 h. The supernatant was kept on ice for 15 min and DNA was precipitated with 1 vol of isopropanol. After 15 min centrifugation at 16,000 rpm, the supernatant was discarded, the pellet air-dried and very well resuspended in 500 µl 2 M ammonium acetate, on ice. After 5 min centrifugation at 13,000 rpm the supernatant was transferred into a fresh tube and the DNA was precipitated with 1 vol isopropanol. The pellet was air-dried and resuspended in 30 µl of 1 × TE. About 1–3 µg of DNA were obtained.

Southern Blots and Hybridization with Whole Phage Clone DNA

Genomic DNA of somatic cell hybrids, human and hamster as controls was digested with *EcoRI*, electrophoresed in 0.8% agarose gels overnight at 35 V and blotted on Hybond N+ (Amersham) filters. 50 ng phage or cosmid clone DNA were labelled by random priming and preannealed with 100 µg human pool DNA as described above. Panel filters were prehybridized and hybridized in 50% formamide, 4 × SSC, 50 mM Na-phosphate buffer pH 7.2, 1 mM EDTA, 10% dextran sulphate, 1% SDS, 100 µg/ml denatured salmon sperm DNA and 10 × Denhardt's solution, overnight at 42°C. Filters were stringently washed as described above and exposed for 2–5 days.

Alu- and Line-PCR

The Alu primers 599 and 517 [13] and Alu 5' [14] as well as the Line primers L1Hs [13] were used either alone or in combination.

A 100-µl PCR reaction contained 1 µg of RH DNA, 0.1 µM primer, 200 µM dNTPs, 1 × Taq polymerase buffer (50 mM KCl, 10 mM Tris pH 8.3, 1.5 mM MgCl₂, 0.01% gelatine) and 2.5 U Taq polymerase (BRL or Cetus). Amplification conditions were as follows: 1 cycle denaturation at 95°C for 5 min, 35 cycles including 1 min of denaturing at 94°C, 1 min of annealing at 55°C and 4 min of extension at 72°C, and 1 cycle of final extension for 7 min at 72°C, in a Perkin-Elmer Cetus cyler.

The Alu-PCR products were checked on 1.5% agarose gels. PCR products obtained from one RH were pooled and used as one probe to hybridize two sets of chromosome-5-gridded cosmid filters (kindly provided by Dr. A.M. Frischauf). The filters were prehybridized in 7% PEG (MW 8,000) dissolved in 10% SDS with 200 µg/ml sheared human pool DNA, 1 µg/

ml plasmid DNA containing Alu/Line DNA and 100 µg/ml sheared salmon sperm DNA overnight at 65°C. 100 ng of the Alu-PCR products were labelled with ³²P-dCTP by random priming and the probes were preannealed with 300 µg human pool DNA plus 5 µg cloned Alu/Line DNA and hybridized to the filters overnight at 65°C. Filters were stringently washed and exposed for 1–2 days.

Results

Mapping of 111 BssHII Phage Clones Derived from a Size-Selected Somatic Cell Hybrid Endclone Library

The *BssHII* endclones were obtained as described in Material and Methods. We have chosen 111 *BssHII* endclones which either mapped on 5q or were not yet mapped. All of them were remapped to deletion hybrid panels containing the following somatic cell hybrids and control DNAs: PN/TS (chromosome 5), HHW1064 (del 5q11.2-q13.3), HHW213 (5pter-q11), IH99 (RH supposed to contain a fragment spanning the SMA locus), human and hamster (fig. 1). The location of all the clones is summarized in table 1.

9 out of 111 clones were mapped into the region 5q11.2-q13.3 (table 2). In order to check the uniqueness of the clones, single-copy fragments were isolated and hybridized to each other. All 9 clones were represented only once.

As expected, some of these clones contain well-conserved sequences, showing strong signals with hamster DNA. This proves once more the usefulness of clones flanking CpG islands for the isolation of transcribed sequences.

Construction of Chromosome-5-Specific Libraries Using RHs, and Isolation and Mapping of Human Clones

27 RHs obtained from Dr. E. Solomon have been extensively characterized with SMA flanking markers and most of them con-

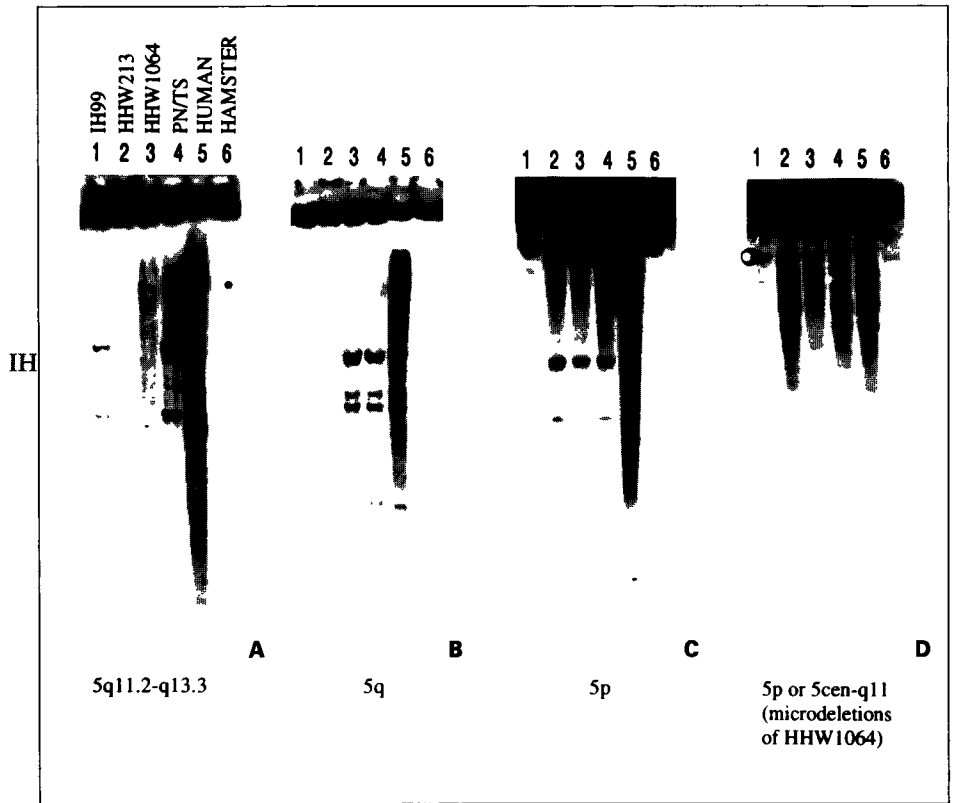


Fig. 1. Autoradiographies showing human clones mapped to different chromosome regions. The panel filters contain the following DNAs: (1) IH99, (2) HHW213, (3) HHW1064, (4) PN/TS, (5) human, (6) hamster. Clone mapped to 5q11.2-q13.3 (**A**), 5q (**B**), 5p (**C**), microdeletions of HHW1064 (5p or 5cen-q11) (**D**).

Cosmids

Table 1. Summary of all phage and cosmid clones isolated and mapped back to panels containing chromosome-5-specific somatic cell hybrids

| Location | <i>Bss</i> HII end-clone phage library from H64 | Phage library IH12 | Phage library IH132 | Cosmids Alu-PCR from RH | Total |
|-------------------------------|---|--------------------|---------------------|-------------------------|------------|
| 5q11.2-q13.3, in the deletion | 9 | 8 | 5 | 8 | 30 |
| 5q, outside the deletion | 59 | 53 | 43 | 15 | 170 |
| 5p | 9 | 12 | 19 | 1 | 41 |
| Microdeletions of HHW1064 | 1 | 2 | 8 | 1 | 12 |
| Chromosome 4 | 18 | – | – | – | 18 |
| Hamster | 14 | 23 | 25 | – | 62 |
| Total | 110 | 98 | 100 | 25 | 333 |

tain several small, often disrupted fragments. Two of 27 RHs were chosen for the construction of libraries and 10 RHs for the generation of inverted Alu-PCR products since they are all positive for several SMA flanking markers (table 3).

One genomic library was constructed from RH IH12 and the second one from IH132. In both cases high-molecular-weight DNA was partially digested with MboI and subcloned in the *Bam*HI site of the phage arms EMBL3 for IH12 and Lambda-Dash II (Stratagene) for IH132, respectively.

From the IH12 library, 98 clones were isolated and mapped back to panel filters (table 2). 8 clones were localized in the region 5q11.2-q13.3. From the IH132 phage library, 100 clones were isolated and mapped to panel filters. 5 of these map into the region 5q11.2-q13.3.

Based on all three chromosome-5-specific phage libraries, we isolated and mapped 308 clones, of which 228 were of human origin. A summary of these clones is listed in table 1.

22 phage clones (table 2) derived from the region 5q11.2-q13.3, 155 clones mapped to the remainder of chromosome 5q, 40 clones to 5p, and 11 clones to two microdeletions of the somatic cell hybrid HHW1064 (one on 5p and one on 5cen-q11). The relatively high number of clones mapping into the microdeletions of HHW1064 is unexpected, since these deletions are cytogenetically undetectable [J. McPhearson, pers. commun.].

Direct Isolation of Alu- and Line-PCR Products from Overlapping RHs

Alu- and Line-PCR was performed on 10 RHs (table 3) containing overlapping fragments of the SMA region and from two somatic cell hybrids (PN/TS and HHW1064) as controls. The PCR products were separated on 1.5% agarose gels and DNA fragments were compared to each other. On the basis of

fragment sizes, common fragments were identified, some of them varying strongly in their intensities. In order to define whether the various intensities of same-sized bands are due to supraposition of several different fragments

Table 2. New phage clones from the region 5q11.2-q13.3

| Current number | D number | Clone-name |
|--|----------|------------|
| Clones from the <i>Bss</i> HII endclone library | | |
| 1 | D5S212 | ECB35 |
| 2 | D5S213 | ECB38 |
| 3 | | ECB91 |
| 4 | D5S510 | ECB143 |
| 5 | D5S215 | ECB306 |
| 6 | D5S216 | ECB311 |
| 7 | D5S217 | ECB314 |
| 8 | | ECB331 |
| 9 | | ECB365 |
| Clones from the phage library IH12 | | |
| 10 | D5S508 | L12.16 |
| 11 | | L12.66 |
| 12 | | W5 |
| 13 | D5S507 | W15 |
| 14 | | P26 |
| 15 | | P27 |
| 16 | | P51 |
| 17 | D5S506 | P62 |
| Clones from the phage library IH132 | | |
| 18 | D5S509 | PH22 |
| 19 | | PH16 |
| 20 | | PH99 |
| 21 | | PH154 |
| 22 | | PH167 |
| Clones from the chromosome-5-gridded cosmid library identified by inverted Alu-PCR products from overlapping RHs | | |
| 23 | | Alu-C82 |
| 24 | | Alu-C18 |
| 25 | | Alu-C31 |
| 26 | | Alu-C38 |
| 27 | | Alu-C35 |
| 28 | | Alu-C40 |
| 29 | | Alu-C93 |
| 30 | | Alu-C72 |

Table 3. Characterization of 10 RHs with SMA flanking markers

| RH | pM4 | EF(TG/AG) | MIT-I105 | RB110/111 | pJK53 | YNCT | p105-153 |
|-------|-----|-----------|----------|-----------|-------|------|----------|
| IH159 | + | - | - | - | - | - | - |
| IH167 | + | + | - | - | - | - | - |
| IH8 | + | + | + | + | + | - | - |
| IH132 | + | + | + | + | + | - | - |
| IH58 | + | - | + | + | + | - | - |
| IH114 | - | + | + | + | + | - | - |
| IH88 | - | + | + | + | + | + | - |
| IH21 | - | + | + | + | + | + | + |
| IH12 | + | + | + | + | + | + | + |
| IH99 | + | + | + | + | + | + | + |

or whether they are due to amplification differences, the Alu-PCR product of one radiation hybrid was labelled and hybridized against the others. Clear common bands were observed in the autoradiographies for fragments of the same size in some cases, while other bands which seemed to be of the same size did not hybridize (fig. 2). 15 different bands were eluted (table 4) and hybridized either directly to panel filters or subcloned and hybridized afterwards. In general, the hybridization signals were better when subcloned fragments were used. However, none of the fragments mapped to the region 5q11.2-q13.3.

Alu-PCR from 4 RHs and a Somatic Cell Hybrid (HHW1064, Used as Negative Control) and Hybridization to Flow-Sorted Chromosome-5-Gridded Cosmid Libraries

In this approach a less labor-intensive strategy was used with very high efficiency. Using the Alu primers 599 and Alu 5' either alone or in combination, Alu-PCRs were performed from DNA of four chromosome-5-specific RHs with overlapping fragments in the SMA region (IH99, IH132, IH88 and IH21). Additionally, the same experiment was performed with the DNA of the somatic cell hybrid

Table 4. Isolated Alu-PCR products from various RHs by comparing common bands in EtBr-stained agarose gels

| | Estimated fragment length, kb | | | | | |
|---------|-------------------------------|-----|-----|-----|-----|-----|
| Alu 517 | 3.5 | 3.3 | 2.3 | 2.2 | 0.4 | 0.3 |
| Alu 559 | 1.8 | 1.6 | 1.2 | 1.0 | | |
| L1Hs | 3.0 | 0.9 | 0.8 | 0.5 | 0.3 | |

HHW1064 (used as a negative control), which has the region 5q11.2-q13.3 deleted.

Aliquots of the Alu-PCR products obtained from the three different PCR reactions of each RH were electrophoresed on a 1.5% agarose gel to check their quality. The remaining DNA was pooled and precipitated to remove free nucleotides. About 100 ng DNA of each pooled Alu-PCR product were labelled and hybridized to a set of two filters containing about 20,000 clones of a chromosome-5-specific gridded cosmid library (fig. 3).

The positive clones from each of the five hybridizations were identified, scored and compared to each other. 22 clones were isolated which match between two RHs, and 3 clones which match between three RH. All 25 clones were mapped back to somatic cell hy-

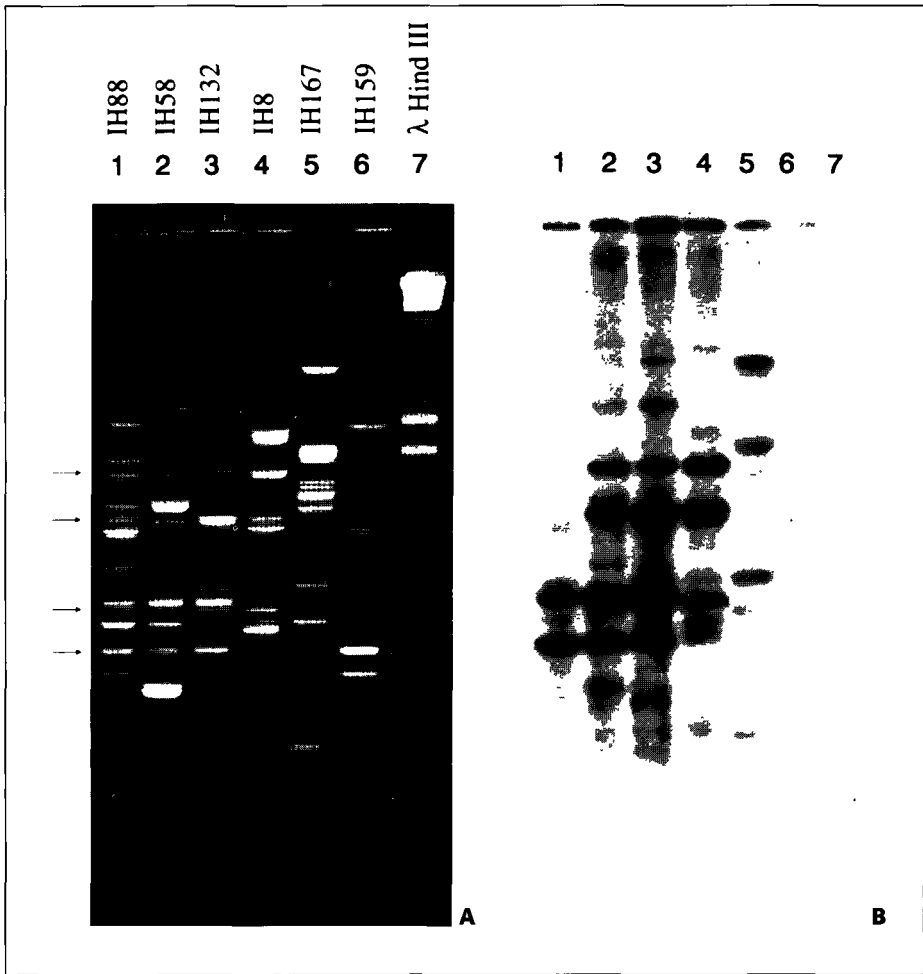


Fig. 2. Inverted Alu 599-PCR products from different RH separated on an EtBr-stained 1.5% agarose gel (**A**), and autoradiography after Southern blot hybridization with the Alu-PCR products of IH132 (**B**). Common bands are marked with an arrow.

brid panels (table 1); 8 cosmids were localized into the region 5q11.2-q13.3 (table 2). These 8 cosmids were digested with *EcoRI* and the restriction patterns compared in order to check for redundant clones. None of them were identical.

In addition, there were 23 clones which were positive for all four RHs and HHW1064 (used as negative control) and showed the strongest signals, which we did not isolate and

map back. It seems that there is a chromosome-5-specific repetitive sequence which is preferentially amplified and localized in the region 5q11.2-q13.3 as well as elsewhere on chromosome 5.

Using the different strategies, we isolated 30 new clones from the region 5q11.2-q13.3, which represent excellent material for further physical, genetic and transcriptional characterization of this region.



Fig. 3. Examples of autoradiographs after hybridization of gridded chromosome 5 cosmid library filters with Alu-PCR products of IH88 (**A**) and IH21 (**B**).

Discussion

The enrichment of the region 5q11.2-q13.3 with new markers may speed up chromosome walking and isolation of genes in general, and of the gene(s) at the SMA locus in particular. These clones are excellent landmarks for YAC screening and physical mapping of the region, as well as for isolating polymorphic markers. From several clones, polymorphic markers, sequence tagged sites (STSs) and YACs have already been isolated [24, 25, and unpubl. data].

Using different methods like the construction of various chromosome-specific libraries, direct isolation of Alu-PCR products from overlapping RHs or their hybridization to chromosome-specific gridded libraries, as well as different materials like somatic cell hybrids, RHs and selected libraries, we were able to isolate 30 new human clones from the region of interest. Comparing the different methods, the generation of new clones by Alu-PCR from overlapping RHs followed by hybridization to

chromosome-specific gridded libraries is the most rapid and efficient. In our case, the RHs contain many small, and often noncontiguous, fragments, which made them less useful for direct selection of Alu-PCR products as region-specific probes. Consequently, our attempt to directly generate DNA fragments from overlapping RHs by either comparing the fragment sizes in EtBr-stained gels or by hybridizing the Alu-PCR products of one RH to a Southern blot containing the Alu-PCR products of the other RHs was not successful; all fragments failed to map back to the region 5q11.2-q13.3. This might be due to supraamplification of certain chromosome-5-specific fragments, as has been shown by hybridization experiments to gridded cosmid filters with Alu-PCR products from HHW1064 and RHs.

Despite the above-mentioned disadvantages of our set of RHs, the use of Alu-PCR products from overlapping RHs for hybridization of chromosome-specific gridded libraries, followed by isolation and mapping of only those clones showing common signals with the Alu-

PCR products of at least two RHs, has been very successful: a yield of 32% of clones out of the region 5q11.2-q13.3 is rather remarkable.

The use of libraries containing selected clones close to CpG islands is of great importance for the isolation of genes [26–28]. In addition, CpG endclones can easily be combined with jumping and linking libraries in order to isolate the most important and useful segments of a given region. By mapping 9 *Bss*HII endclones into the region 5q11.2-q13.3, we isolated 9 putative starting points for genes. Unfortunately, the construction of libraries and the subsequent steps to identify region-specific clones are very tedious work. Additionally, this *Bss*HII endclone library has been constructed from size-selected fragments, which decrease the complexity of the library. Therefore, the construction of total genomic libraries from RHs was chosen as an additional source for the generation of randomly distributed clones from 5q11.2-q13.3.

The fact that we isolated 11 phage clones from the microdeletions of HHW1064 (5p or 5cen-q11) is unexpected. All three RHs, IH99, IH132, and IH12, contain fragments from these microdeletions; this is rather unusual, since they were not selected for these regions. The RH IH99 was used as a reference

to physically map the clones out of the deletion 5q11.2-q13.3 and closely localized to the SMA gene, while the other two RHs IH12 and IH132 were used as starting material for the libraries. One possible explanation might be that a certain region around the SMA gene is duplicated on 5p or 5cen-q11.

In conclusion, the combination of different methods and materials enabled us to select various clones (CpG flanking clones, randomly distributed genomic clones, Alu repeat flanking clones) and to enrich the region 5q11.2-q13.3 with 30 new clones.

These clones represent important material for the identification of polymorphic markers and sequence-tagged sites, isolation of YACs, construction of YAC contigs, and especially for the isolation of transcribed sequences.

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