A precise quantitation of gene number by saturation hybridization using cloned DNA

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ABSTRACT

This paper describes a precise method of gene titration as applied to the α- and β-globin genes in the mouse. The three salient features of the method are: (i) the use of saturation hybridization in probe cDNA excess, (ii) the use of highly purified cDNA probes prepared by preparative hybridization with cloned globin sequences (Longacre and Mach (1978) J. Biol. Chem. 253, 7500) and (iii) the use of cloned globin sequences to calibrate the system internally. The results indicate that there are two genes for α-globin and two genes for β-globin in the BALB/c mouse. The significance of these results are discussed in relation to other data regarding adult and embryonic globin genes.

INTRODUCTION

In many instances, it is important to know how many copies of a particular gene are present in a given genome. This has been generally measured by hybridization of a specific probe to cellular DNA under conditions which allow the reiteration frequency of the probe sequence (including cross hybridizing homologues) to be deduced. Under the conditions most frequently employed, a general notion of sequence reiteration is obtained by measuring the rate of probe hybridization (Cot analysis) (1). However, a precise number cannot be determined in this way especially for the so-called 'unique' genes in eukaryotes. Analysis of fractionated restriction endonuclease fragments in combination with cloning and further structural studies on purified genes provides another approach, but one which will not easily and immediately
establish the exact number of copies of a given gene. A simple procedure for the precise titration of gene number by saturation hybridization in probe cDNA excess (2) is described here and applied to the case of mouse α- and β-globin genes. It makes use of cloned globin plasmid DNA (3, 4) for the purification of cDNA probes by preparative hybridization (5) and for an internal calibration of the hybridization system.

With the procedure presented here we find two gene copies per haploid genome for both α- and β-globin. The implications of these results for the organization of the globin genes in the mouse genome are discussed.

MATERIALS AND METHODS

DNA and enzymes

\(^{3}H\)dCTP (20 Ci/mmol) was obtained from the Radiochemical Centre, Amersham, England. AMV DNA polymerase was kindly provided by Dr. J. Beard (Life Sciences Inc., St. Petersburg, Fla.). Single strand specific nuclease from Aspergillus oryzae (S1 nuclease) was purified according to the procedure of Vogt up to the DEAE cellulose step (6). Mouse and rabbit 9S globin mRNA was prepared as described (7). Recombinant plasmids carrying mouse α- or β-globin (pCR1 M4 and pCR1 M9 respectively), constructed from cDNA as described (4), were prepared by the cleared lysate procedure of Katz et al. (8), including fractionation on CsCl gradients in the presence of ethidium bromide followed by sucrose gradients (3). Embryonic or myeloma 173 DNA was prepared as described (7) and sonicated at 200 μg/ml at 30 sec intervals for 5 min with the large probe of a Branson sonicator. Mouse globin cDNA was synthesized and the α- and β-moieties purified by sulfhydryl-sepharose chromatography using mercurated recombinant plasmid DNA as described (5). Large molecular weight cDNA was isolated by alkaline sucrose gradient centrifugation as described (3). The cDNA size was analyzed by polyacrylamide gel electrophoresis in formamide and fluorography as described (5).
Hybridizations

Hybridization mixtures contained (in 200 μl) 10 mM Tris/HCl, pH 7.6, 0.6 M NaCl, 1 mM EDTA, 900 μg sonicated cellular DNA and increasing amounts of cDNA as noted. Hybridization was carried out at 68°C under paraffin oil for 48 h. Following hybridization, the samples were diluted into 2 ml of S1 buffer, incubated at 45°C with 5 μl S1 enzyme and precipitated in TCA. The backgrounds obtained by hybridizing the cDNA with 900 μg of sonicated E. coli DNA have been subtracted from each point. Backgrounds were generally about 0.5% of the input cDNA.

The quenching of the (3H)cDNA probes by the large amounts of cellular DNA used in these experiments was determined in two ways. First, increasing amounts of double stranded calf thymus DNA were TCA precipitated with a fixed amount of (3H) cDNA. Under these conditions about 66% of the (3H)cDNA cpm were quenched in the presence of 900 μg DNA (equivalent to that used in the titration experiments). Alternatively 900 μg of cellular DNA (embryo or myeloma) were hybridized and S1 digested as for saturation hybridization, but in the absence of (3H)cDNA probe. Immediately before TCA precipitation, equal amounts of (3H)cDNA were added to the hybridized, S1 digested cellular DNA or to 20 μg of carrier DNA. Under these conditions, about 50% of the (3H)cDNA cpm were quenched by the remaining cellular DNA in the simulated titration experiment. These results indicate that the actual amount of (3H)cDNA cpm hybridized in the saturation hybridization experiments is 2-3 fold higher than the raw values given in Figures 2-4. This significant quenching made it important to rely on internal calibration to quantitate the genes in cellular DNA (see Results).

RESULTS

Description of cDNA probes

Three cDNA probes were used in the study described here: (i) cDNA synthesized from mouse 9S globin mRNA (total globin cDNA), (ii) purified α-globin cDNA and (iii) purified 8-globin
cDNA (5). α- and β-globin cDNAs were purified using sulfhydryl-
sepharose chromatography with mercurated recombinant plasmid
DNA and in situ melting as described previously. Full sized
cDNAs in each preparation were selected after centrifugation
in alkaline sucrose. The sizes of these probes were analyzed
by polyacrylamide gel electrophoresis under denaturing
conditions and are shown in Figure 1. Unpurified globin cDNA
(slot b) consists of two diffuse bands which are about 600
and 650 nucleotides in length. The long purified α- and β-
cDNAs, which are shown in slots c and d, correspond respectively
to the faster and slower migrating majority bands seen in
the unpurified cDNA.

The effect of probe purity on saturation hybridization of
cellular DNA

A constant amount (900 μg) of sonicated mouse embryo DNA
was hybridized with increasing amounts of globin cDNA until
saturation as outlined by Bishop and Freeman (2). Two cDNA
probe preparations were used: (i) total globin cDNA (Figure 1,
slot b) and (ii) α- and β-cDNAs purified independently and
recombined in equal amounts (Figure 1, slots c and d). The
saturation hybridization curves obtained with these probes
are shown in Figure 2. The curve obtained with the unpurified
globin probe indicates that it contains additional sequences,
not present in the purified α- and β-probes. This result is
of interest since cDNA made from globin mRNA has frequently
been considered to consist almost exclusively of α- and β-globin
sequences. The important difference in the saturation curves
obtained with unpurified and purified globin probes illustrates
unequivocally the necessity of using purified probes whenever
a saturation hybridization is attempted.

Titration of globin genes in cellular DNA

Two cellular DNAs derived from mouse embryos or from a
mouse myeloma tumor (MOPC 173) were titrated with the purified
α- and β-globin cDNA probes (Figure 1, slots c and d) by
Figure 1. Electrophoretic analysis of $^{3}$H)cDNA probes. $^{3}$H)cDNA was synthesized from 9S globin mRNA and the α- and β-globin cDNAs were purified as described. cDNA larger than about 400 nucleotides was isolated in each case by centrifugation in 5-20% alkaline sucrose as described in Materials and Methods. 11,000 cpm of unpurified globin cDNA and 9,000 cpm each of purified α- and β-globin cDNA were precipitated and analyzed by acrylamide gel electrophoresis in 98% formamide as described in Materials and Methods. $^{3}$H)SV-40 DNA (5,000 cpm) restricted with Hind III (generous gift of P. Chambon) was used as a marker. Slot a, SV-40, Hind III marker; slot b, total 9S globin cDNA; slot c, purified β-globin cDNA; slot d, purified α-globin cDNA.

saturation hybridization. In each case, 900 μg of sonicated cellular DNA were hybridized in liquid with increasing amounts
Figure 2. Saturation hybridization of mouse embryo DNA with unpurified and purified globin cDNAs. Hybridizations were carried out with 900 μg sonicated embryo DNA as described in Materials and Methods. The cDNA probes were prepared as described in the legend to Figure 1 and in Materials and Methods. Hybridizations with purified probes (•——•) contained equal amounts of α- and β-cDNA which were purified independently (Figure 1, slots c and d) and recombined. Unpurified globin cDNA (Figure 1, slot a), (○—○).

of α- or β-globin cDNA until saturation. The extent of hybridization was measured by S1 nuclease digestion (see Materials and Methods). In addition, the saturation hybridization plateaus obtained with cellular DNA were internally calibrated with recombinant plasmid DNA carrying either α- or β-globin inserts. This was done by adding an amount of sonicated plasmid DNA equivalent to one or more globin genes per haploid genome to 900 μg of cellular DNA in the hybridization mixture already containing cellular DNA. The increase in the saturation plateaus observed when different amounts of globin plasmid DNA were included was therefore a measure of the number of globin gene equivalents represented by the plasmid DNA. It was then possible to calculate, in the same experiment, how many gene equivalents were present in mouse DNA by comparing the plateau values obtained with cellular DNA alone and cellular DNA plus one or
two globin gene equivalents.

The results of these experiments are shown in Figures 3 and 4 for α- and β-globin probes respectively. They show that the α- and β-probes hybridize to similar extents with either embryo or myeloma DNA. Furthermore, in each case, the initial plateau level of hybridization is doubled by the addition of two genome equivalents of globin DNA. The addition of one

![Figure 3. Titration and calibration of α-globin sequences in cellular DNA.](image)

A. Embryo DNA: 900 μg of sonicated embryo DNA was hybridized with increasing amounts of purified α-globin cDNA (Figure 1, slot d) and digested with S1 nuclease as described in Materials and Methods. Alternatively, for calibration, 7.2 ng of sonicated plasmid DNA containing an α-globin insertion (pCR1 M4) was added to the 900 μg of cellular DNA. This amount of plasmid DNA contained the equivalent of two α-globin cDNA complements per haploid genome in 900 μg of cellular DNA. The calculation of plasmid globin sequence equivalents assumes 1.8 x 10^{12} daltons for the molecular weight of the haploid mouse genome and 550 bases for both the inserted sequence (into pCR1 containing 11,000 bases) and the cDNA probe. Embryo DNA,●; embryo DNA plus two plasmid α-globin gene equivalents,○.

B. Myeloma DNA: As for A, above, except that 900 μg of sonicated MOPC 173 tumor DNA was hybridized with α-globin cDNA. In this case, either one (3.6 ng pCR1 M4) or two (7.2 ng pCR1 M4) α-globin gene equivalents were added to the cellular DNA. Myeloma DNA,▲; myeloma DNA plus one plasmid α-globin gene equivalent,△; myeloma DNA plus two plasmid α-globin gene equivalents, ■.
Figure 4. Titration and calibration of \( \beta \)-globin sequences in cellular DNA.

A. Embryo DNA: As in Figure 3A, except that increasing amounts of purified \( \beta \)-globin cDNA (Figure 1, slot c) were hybridized to cellular DNA. For calibration, 7.2 ng of sonicated plasmid DNA containing a \( \beta \)-globin insertion (pCR1 M9) was added to the cellular DNA. Embryo DNA, - - ; embryo DNA plus two plasmid \( \beta \)-globin gene equivalents, - - .

B. Myeloma DNA: As in Figure 3B with the same substitutions as in A above. Myeloma DNA, - - ; myeloma DNA plus one plasmid \( \beta \)-globin gene equivalent, - - ; myeloma DNA plus two plasmid \( \beta \)-globin gene equivalents, - - .

genome equivalent increases the plateau level of hybridization by an amount equal to half the plateau value of cellular DNA alone.

The obvious interpretation of these data is that there are two genes for \( \alpha \)- and two genes for \( \beta \)-globin per haploid genome. However, it should be noted that this number differs from the number which can be calculated from the measured amount of \( ({}^{3}H) \)cDNA hybridized to a given amount of cellular DNA. Given 1.8 x 10^{12} daltons as the mouse genome and a cDNA probe of about 600 base pairs, a single copy of globin gene would protect 1.8 x 10^{5} daltons of cDNA, or about 1/10^{7} of the genome DNA mass, or 90 pg for 900 \( \mu \)g of cellular DNA. From the specific activity of the cDNA (9.63 x 10^{6} cpm/\( \mu \)g) and correcting for quenching (see Materials and Methods), we calculate that the
225 cpm hybridized represents 70 pg, or less than one gene. There is, therefore, an important discrepancy between this calculation and measurements derived from an internal calibration of cellular DNA with cloned sequences (see Discussion).

Hybridization of purified α- and β-globin probes with divergent sequences

In order to investigate to what extent saturation hybridization as described here might permit the detection of non-identical but related genes, we have studied the hybridization of the purified globin probes with related globin sequences. α- and β-cDNAs (Figure 1, slots c and d) were hybridized to completion with sonicated plasmid DNA or 9S mRNA containing homologous (mouse) and heterologous (rabbit) globin gene sequences. The hybrids were then assayed by S1 nuclease digestion (% protection). As shown in Table I, the extent of DNA:DNA hybridization between mouse and rabbit globin gene sequences is only 30% of the hybridization observed between the identical homologous globin genes. This is equally true for α- and β-globin both of which show about 20% sequence divergence between the rabbit and mouse nucleotide sequences (9). When the extent of RNA:DNA hybridization was measured with the same globin sequences, 70% cross hybridization between mouse and rabbit sequences was observed. This indicates that, when assayed by S1 resistance, DNA:DNA hybridization is more discriminating in the measurement of divergent sequences than RNA:DNA hybridization.

DISCUSSION

This paper presents a procedure for the sensitive measurement of gene numbers. This procedure relies on the availability of recombinant DNA carrying the gene under study which is needed for the purification of the radioactive probe and for the internal calibration of the hybridization system. The ability to measure the gene numbers precisely
Table I. Hybridization of purified mouse globin cDNAs with homologous (mouse) and heterologous (rabbit) 9S mRNA and recombinant plasmid DNA

<table>
<thead>
<tr>
<th>mRNA or plasmid DNA</th>
<th>Mouse globin cDNA probes</th>
<th>8-globin (cpm hybridized)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse 9S RNA</td>
<td>a-globin (cpm hybridized)</td>
<td>4554 (100%)</td>
</tr>
<tr>
<td>Rabbit 9S RNA</td>
<td></td>
<td>3143 (69%)</td>
</tr>
<tr>
<td>pCR1 M10 (mouse a)</td>
<td></td>
<td>7295 (100%)</td>
</tr>
<tr>
<td>pCR1 R11 (rabbit a)</td>
<td></td>
<td>2409 (33%)</td>
</tr>
<tr>
<td>pCR1 M9 (mouse 8)</td>
<td></td>
<td>16005 (100%)</td>
</tr>
<tr>
<td>pCR1 R19 (rabbit 8)</td>
<td></td>
<td>4509 (28%)</td>
</tr>
</tbody>
</table>

1 ug of mouse or rabbit globin 9S mRNA was hybridized to 4,500 cp (0.45 ng) of purified a-globin cDNA (Figure 1, slot d) or to 5,000 cp (0.5 ng) of purified 8-globin cDNA (Figure 1, slot c) in 50 µl containing 10 mM Tris, pH 7.6, 0.3 M NaCl, 1 mM EDTA. Hybridization mixtures were incubated at 68°C for 6 h and digested in 1 ml of SI buffer with SI nuclease as described in Materials and Methods. Alternatively, 7,400 cp (0.74 ng) of purified a-globin cDNA was hybridized to 5 µg of sonicated pCR1 M10 (mouse a-globin insert) or pCR1 R11 (rabbit a-globin insert) and 16,000 cp (1.6 ng) of purified 8-globin cDNA was hybridized to 5 µg of pCR1 M9 (mouse 8-globin insert) or pCR1 R19 (rabbit 8-globin insert) for 18 h and digested with SI nuclease as noted above. The numbers in the Table are the average of duplicate determinations from which the SI resistance of the probes alone (0.5%) has been subtracted. To facilitate the comparison with heterologous complements, the SI resistance of the probe cDNAs with their homologous mouse complements has been arbitrarily set at 100%. However, in every case the real SI resistances in the homologous system were 98-100% of the input cDNA.

in a complex genome is an important complement to the detailed structural studies now made possible by the cloning of cellular genes. Four particular methodological features of the proposed procedure deserve special comment: (i) the use of saturation hybridization with probe sequences in excess rather than 'Cot' analysis with target cellular DNA sequences in excess, (ii) the importance of assaying the hybrids by SI nuclease digestion and not by hydroxylapatite chromatography, (iii) the requirement for pure radioactive
probe and (iv) the reliance on an internal calibration with cloned gene sequences for accurate quantitation.

(i) For hybridization under conditions of target cellular DNA excess (Cot), the cellular gene sequence should be in sufficient molar excess over the radioactive probe so that most or all of the probe will be hybridized at high Cot values. This target cellular DNA excess is not easily achieved in the case of 'unique' genes. Furthermore, the estimation of gene number by Cot analysis depends on the kinetics of hybridization of the probe and is deduced from the 50% hybridization point (Cot 1/2). This value is often difficult to determine accurately since it depends on having a well defined upper plateau, ideally at near 100% probe hybridization, which is rarely obtained due to probe impurity and insufficient target cellular DNA excess. These factors and the fact that Cot 1/2 is determined on a log scale render this method unsatisfactory for a precise quantitation although it does permit a general estimate of gene reiteration.

An even more important limitation of procedures based on the kinetics of hybridization is apparent when the problem of scoring multiple non-identical but closely related genes arises. Here, the kinetics of probe hybridization to several related but non-identical target genes is followed under conditions where each of the divergent genes has its identical complement in the cellular DNA.

It is known that the mismatch from divergent sequences slows the rate of hybridization (10). This effect of mismatch on rate will result in discrimination against the radioactive probe for hybridization to divergent gene sequences. As a consequence, these conditions of hybridization (Cot) may not allow the detection of multiple related but non-identical genes. With saturation hybridization this situation is avoided since the probe sequence is now in large excess over the homologous cellular target sequences and there is no significant competition by the cellular DNA strands. Because
the plateau level at hybridization saturation is an equilibrium measurement, there is no longer any dependence on relative rates of hybridization among divergent genes.

(ii) The objective of the titration experiments described here is to measure the amount of a specific gene sequence without reference to its continuous or discontinuous fine structure in the genome (reviewed by Chambon (11)). For this reason, hybridization must be assayed by S1 nuclease resistance which measures only the sequence target area defined by the radioactive probe whatever its organization. In contrast, hydroxylapatite chromatography or filter binding assays result in a variable tail effect, the magnitude of which is affected by gene discontinuity.

(iii) Inspite of its numerous advantages, saturation hybridization has one inherent weakness which has limited its use in the past. Even a small amount of contaminating sequences in the probe preparation can contribute disproportionately to the hybridization observed (2). It is thus necessary to use probes of higher purity than can be obtained by conventional purification procedures based on physio-chemical properties of mRNA or cDNA. The importance of probe purity is clearly illustrated in Figure 2 of this paper for the case of globin where significantly higher saturation plateaus are obtained with unpurified relative to plasmid purified probes. The availability of DNA cloned in recombinant plasmids for the purification or preparation of highly purified probes will considerably facilitate the use of the saturation hybridization technique.

The probes used in saturation hybridization experiments should also have a low level of intrinsic S1 nuclease resistance. Since the amounts of radioactive probe used are in large excess of the amount finally hybridized, the background S1 resistance needs to be low relative to the saturation plateau values in order to obtain accurate data.
The purified cDNA used in these studies had 0.5% or less intrinsic S1 resistance and this allowed reproducible and accurate estimation of plateau values at saturation. It should be noted that other probe sequences may have more secondary structure resulting in higher intrinsic S1 nuclease resistance which might limit their use in this procedure.

The availability of cloned globin gene sequences has permitted the calibration of the hybridization system in a way not previously possible. One or more haploid genome equivalents of cloned globin sequences are added to mouse cellular DNA (or to E. coli DNA) and a comparison of the resulting hybridization plateaus provided a means of interpreting a given plateau value from cellular DNA alone in terms of numbers of globin gene copies. Thus, by adding two haploid mouse genome equivalents of cloned alpha- or beta-globin sequence the hybridization plateau obtained with mouse cellular DNA alone was doubled. It can, therefore, be deduced that the BALB/c mouse has two copies each of alpha- and beta-globin per haploid genome.

The internal calibration proved to be very important since the alternative way of estimating gene numbers from the amount of probe hybridized by a given amount of cellular DNA corresponded to less than one haploid copy for each of the alpha- and beta-globin genes. It is not clear why there is such a large discrepancy between the two approaches. However, as discussed below, the data currently available from classical genetics and molecular cloning support the value determined using an internal standard. It appears therefore, that this method of calibration is a valuable safeguard against possible artifacts in the evaluation of gene number by saturation hybridization. Even when saturation hybridization is done using pure probes and internal standards, there are two general reservations which should be considered in the interpretation of hybridization plateaus.

(i) A hybridization plateau could either reflect a given
number of copies of the entire probe sequence or more extensive reiteration of only a part of the probe sequence. For example, 50 copies of a sequence corresponding to only one fifth of a gene would result in the same S1 resistance as 10 copies of the entire probe sequence. However, in the extreme case, the shape of the saturation curve might allow a distinction between these possibilities since a reiterated sequence will require a higher probe concentration to reach saturation.

(ii) In cases where multiple genes are related but not identical, there will be incomplete S1 nuclease protection of the probe by the divergent genes, resulting in an underestimation of the actual number of homologous sequences. For example, the hybridization plateau value observed for the globins could represent either two genes which are almost identical to the probe sequence or one identical gene and two related genes which hybridize to 50%.

The data presented in this paper are consistent with the presence of two genes for both α- and β-globin sequences in BALB/c mouse DNA. This interpretation is supported by data from other laboratories involving genetic studies, amino acid sequences and molecular cloning.

Amino acid sequences for the α-chains of hemoglobins from BALB/c mice revealed the presence of two forms differing at position 68 where either serine or threonine are found at relatively equal frequencies (12). Amino acid sequences of the α-globin polypeptides from C3H mice where three variable positions are involved, indicate that the two α-chain polypeptides are controlled by two α-chain structural genes and are not the result of translational ambiguity (13). An embryonic chain (x) which is structurally and functionally related to the α-chain has been shown to exist in BALB/c mice by Melderis et al. (14). However, these authors indicate that there is considerable divergence between the α-chains and the embryonic x chains in the mouse (at least 33% difference and probably more). Thus, only very limited, if any cross
hybridization would be expected between the x embryonic globin sequence and an α-globin probe. Therefore, the two genes we have measured are very likely the two forms of adult α-globin.

Amino acid sequence data have also shown that BALB/c mice contain two 6-globin polypeptides, a major (80%) and a minor (20%) form which differ from one another at six positions (15). Genetic evidence suggests that the two 6-chains are controlled by two separate but adjacent genes located in linkage group I of the mouse genome (16, 17). Recently, Tilghman et al. (18, 19) have cloned two different segments containing 6-globin from BALB/c DNA which presumably correspond to the 6-globin major and minor genes.

In addition, BALB/c mice have two types of embryonic 6-like ε-chains, εy and εz (reviewed by Steinheider et al. (20)). The amino acid composition of tryptic peptides has been obtained for these two embryonic forms (21) and compared with the adult 6-chains (15, 17) to establish a tentative amino acid sequence for both embryonic chains. Most amino acid substitutions in εy with regard to the adult 6-chain are concentrated at the aminoterminal and carboxyterminal ends with the central part of the chain being like the adult form. The reverse relationship is true for the εz and adult 6-chains. In this case, substitutions are concentrated at the center of the εz-chain whereas both the amino- and carboxyterminal ends appear very similar to the adult 6-chain.

The above data suggest that a nucleic acid probe to the adult 6-chain might be expected to cross hybridize with large regions of the two 6-like embryonic genes in addition to the two adult 6-major and minor genes. However, our saturation hybridization experiments using the purified 6-globin probe show no evidence for more than the two gene equivalents which are also observed with the α-globin probe.

The most likely explanation for our inability to detect more than two gene equivalents with 6-globin probe is that
there is much more sequence divergence than expected from the preliminary amino acid sequence data and therefore no cross hybridization to the embryonic gene.

However, a more interesting possibility is that the portions of the embryonic y and z genes that appear to be identical to the adult β-gene are actually coded by the same nucleic acid sequences. Thus, an adult β-gene may be divided into three parts which could be recombined with appropriate coding segments specific for the embryonic y and z genes by intervening sequences. Appropriate recombinations could generate under different circumstances either εy, εz or 6 from the same collection of sequences. In this regard, it is interesting to note that intervening sequences are found in the BALB/c adult β-globin structural genes at positions which are precisely in the transition regions suggested by the amino acid sequence data of Steinheider et al. (22, 23).

The method of gene quantitation described here is particularly sensitive for counting gene sequences present at a very low percentage of cellular DNA such as for the 'unique' genes of eukaryotes. For sequences smaller than globin, the sensitivity could be increased even further by using probes of higher specific activity. Other potential precision methods for estimating gene number such as counting bands on Southern gel blots (24) from total restricted cellular DNA may underestimate the gene number if there are intervening sequences with restriction sites or overestimate the number if a given fragment contains multiple copies or a given band is composed of distinct fragments. Even extensive cloning of a gene sequence might underestimate the number if the sequences were present on several identical restriction fragments.

This method has recently been used in a reexamination of the number of immunoglobulin genes which indicated the presence of considerably more variable region genes than had been measured in previous studies (manuscript in preparation). In addition, it should be useful for the rapid and accurate
detection of gene deletions or additions whenever a cloned
sequence is available as well as for the study of integrated
viral genomes.

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