
Representative in Vitro cDNA Amplification From Individual Hemopoietic Cells and Colonies

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A simple procedure is described for preparing microgram amounts of cDNA, suitable for cloning into libraries, from samples as small as a single cell. Synthesis of cDNA is primed with oligo(dT) and the resulting strands are tailed with poly(dA). The cDNA is then amplified using an oligo(dT)-containing sequence to prime the polymerase chain reaction. By limiting the size of the first cDNA strands, efficient amplification is achieved independently of size of the original mRNA transcripts. The method was applied to small numbers of hemopoietic cells of various lineages, as well as to single micromanipulated cells. The results established the fidelity and specificity of the amplified product, and showed that sensitivity was sufficient to detect moderate-to-low abundance messages in a single cell. Amplification of defined RNA mixtures confirmed that the procedure could detect low abundance sequences at least as rare as .025%, and that relative abundance was preserved during amplification.

INTRODUCTION

While the changing patterns of gene expression that underlie differentiation are a major focus of interest in modern biology, the heterogeneity of differentiating systems frequently poses a difficult technical challenge. Stage-specific expression can be studied by bulk extraction of mRNA or protein, but large numbers of cells are required in sufficient purity. An alternative approach for unpurified cells is to use in situ techniques for RNA or protein detection, but this presupposes that the cells of interest can be distinguished by unique visual or positional markers. Frequently, as with early embryonic development or the stem cells of cell renewal systems, none of these conditions can be met.

Techniques based on the polymerase chain reaction (PCR) (Saiki et al., 1988) have recently become available for analysis of genomic DNA and RNA transcripts in samples as small as a single cell (Gyllenstein et al., 1988; Rappolee et al., 1989). While

the approach removes the requirement for large numbers of cells, significant limitations remain. The procedure depends on the availability of specific primers, and is usually applied to the detection of only a single known sequence in a given preparation. Since the original cells are destroyed by the procedure, their identity can not be established with certainty. Further, it is difficult to identify patterns of co-expression of several genes within the same cell, analysis is limited to known genes for which primers are at hand, and the power of subtractive analysis can not be applied to the identification of previously unknown genes.

If, instead of expanding a single sequence, it were possible to amplify simultaneously the entire spectrum of messages present in a single cell, new and extremely powerful approaches to development and differentiation would become possible. The amplified preparations would provide all the flexibility that is currently available only with large samples of mRNA. The amplified material from one cell could be probed any number of times for the presence of different sequences. This would be crucial in providing an identifying profile of the cell type from

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which a given sample was derived. Further, once the origin of individual samples was determined, they could be used in subtractive protocols to identify genes that are differently expressed at different developmental stages.

Recently, the PCR technique has been adapted to amplification of cDNA sequences in which one of the priming sites is synthesized by addition of a homopolymeric tail using terminal transferase (Frohman et al., 1988; Berchtold, 1989; Loh et al., 1989; Ohara et al., 1989.). In one of the reports (Belyavsky et al., 1989), general cDNA libraries were created from a fraction of a 1,000 cell sample. However, these protocols depend on extraction or precipitation steps which make it difficult to apply them to very small samples. Moreover, there is a tendency

for longer messages to be significantly underrepresented in the amplified product (Belyavsky et al., 1989). In this communication, we describe a simple procedure for simultaneous amplification of sequences representing the majority of polyadenylated mRNAs present in a sample which overcomes the limitations of the earlier methods. First, all steps are performed in the original suspension without extraction or precipitation steps. As a result, the procedure can be applied reliably to multiple samples each as small as a single cell. Second, the initial cDNA transcripts are limited in size to a range in which subsequent amplification is maximally efficient. The result is an amplified product which is representative of the starting sample in terms of presence as well as relative abundance of specific sequences.

METHODOLOGY

cDNA Preparation



Up to 1 μ l of cells or RNA were added to 4 μ l (for samples of 1- 100 cells) or 8 μ l (for 100-1,000 cells and pure RNA) of prechilled 1st Strand Buffer containing 50 mM Tris HCl pH 8.3, 75 mM KCl, 3 mM MgCl₂, 2 μ M of each dNTP (A,T,C,G), 100 ng/ml (dT)24, 100 u/ml Inhibit Ace, 2,000 u/ml RNAGuard, and 0.5% NP-40. RNase inhibitors, dNTPs, and (dT)24 were kept as separate concentrated stocks and added to the remaining buffer components to produce the final 1st Strand Buffer which was then stored for no more than a few hours on ice before use. Samples were heated to 65°C for 1 min, cooled at 22°C for 3 min, and returned to ice. Following the addition of 100 units Moloney and 2 units avian reverse transcriptase, samples were incubated at 37°C for 15 min, heat-inactivated for 10 min at 65°C, and returned to ice. The low concentrations of nucleotides and primers, and the brief incubation with reverse transcriptase served to limit the length of the cDNA to 300-700 bases. The low concentration of (dT)24 primer also served to minimize its use as a template in the following tailing and amplification reactions.

Poly(A) Tailing




Tailing was initiated by adding an equal volume of 2 x Tailing Buffer containing 200 mM potassium cacodylate pH 7.2, 4mM CoCl₂, 0.4 mM DTT, 10 units terminal transferase, and 200 μ M dATP (in 100-fold excess over the dNTPs remaining after cDNA synthesis). All tubes were spun briefly to bring down condensation and then incubated for 15 min at 37°C. After heat-inactivation of the enzyme (10 min at 65°C), the cDNA was either amplified immediately or stored at - 70°C for later processing.

Amplification of cDNA




From each cDNA preparation, 4 μ l were diluted to a final volume of 50 μ l in 1 x Taq Buffer containing 10 mM Tris HCl pH 8.3, 50 mM KCl, 2.5 mM MgCl₂, 100 μ g/ml bovine serum albumin (BSA), 1 mM each




dNTP, 0.05% Triton X-100, and a total of 0.2 OD 260 units PCR primer (in later experiments, this was reduced to 0.100 units), and 5 units Taq polymerase. For the single-cell preparations the entire 8 µl sample was adjusted to 100 µl 1 x Taq Buffer including 10 units of Taq polymerase. The sequence of the PCR primer was CATGTCGTCCAGGCCGGTCTGGACAAAATATGAATTC-T(23). Samples were initially amplified for 25 cycles of 1 min at 94°C, 2 min at 42°C, and 6 min (plus 10 sec extension/cycle) at 72°C in a thermal cycler (Perkin Elmer Cetus). An additional 5 units of Taq polymerase were then added and a further 25 cycles carried out.

Preparation of RNA Mixtures




Total RNA was extracted from P388D₁ cells (Koren et al., 1975) purified on a CsCl₂ gradient and diluted to 60 ng/ml in buffer containing 100 u/ml Inhibit Ace, 2,000 u/ml RNAGuard, 0.5% NP-40, and 500 µg/ml glycogen. Rabbit globin RNA was then serially diluted in the above preparation.

Cloning Amplified cDNA



From each original PCR sample, 10 µl were adjusted to 50 µl Taq Buffer containing fresh nucleotides and oligo(dT+) at the same concentration as in the starting PCR reaction. The mixture was boiled for 2 min, annealed for 4 min at 42°C, mixed with 5 units Taq polymerase, and incubated 30 min at 72°C. After an ether extraction to remove residual mineral oil, samples were ethanol precipitated, washed with 70% ethanol, dried, and resuspended in 100 µl buffer (50 mM Tris HCl pH 8.0, 10 mM MgCl₂, 100 mM NaCl). Samples were then digested with EcoRI and spun twice through a CL6B spin column to remove liberated "tag" sequences. After a further ethanol precipitation each sample was resuspended in 50 µl 1 mM EDTA/10 mM Tris HCl pH 8.0 and 2 µl removed for ligation to 100 ng EcoRI digested and dephosphorylated pUC19 (Yanisch-Perron et al., 1985). cDNA libraries were produced from aliquots of this ligation and screened using standard colony hybridisation protocols (Maniatis et al., 1982).

Hybridisation and Probes



The lysozyme probe was a 500 bp mouse lysozyme M cDNA EcoRI fragment containing the 3' region up to and including the polyadenylation site (Cross et al., 1988). The rabbit β-globin probe was the entire gene isolated from the plasmid pβG (Leung and Miyamoto, 1989). The c-fms probe was a EcoRI-BglII cDNA fragment including the extreme 3' region of the mouse mRNA (Rothwell and Rohrschneider, 1987). Probes having specific activities of approximately 10⁸ cpm/ug were labelled using random oligo priming (Feinberg and Vogelstein, 1984). Hybridisation to the Gene-Screen filters was carried out for 18 hours in 50% formamide at 42°C according to the manufacturer's (DuPont) recommendations with a probe concentration of 10⁶ cpm/ml. The α-globin probe used in Fig. 2 was an oligonucleotide sequence (TAGCCACCACCCTGCCGATTTCAC) (Nishioka and Leder, 1979) located 150 bases from the putative polyadenylation site and was labelled using terminal transferase



(Eschenfeldt et al., 1987). To obtain a more sensitive and convenient α -globin probe, plasmid cDNA clones established from amplified erythroid colony cDNA were screened with a radiolabelled α -globin oligonucleotide. A positive clone was isolated and found to contain a 550 bp EcoRI fragment having a restriction digestion pattern identical to that predicted from the known α -globin sequence (not shown). This was used in all subsequent experiments. Southern blots were prepared by running 5 μ l aliquots of amplified cDNA on 1.5% Tris Borate agarose gels followed by bidirectional transfer to Gene-Screen filters [Smith and Summers, 1980]. Radiographic exposure was carried out at -70°C using DuPont Cronex Lightning Plus intensifying screens.

Cell Culture



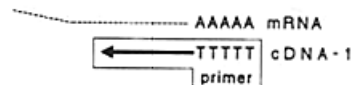
Mouse bone marrow cells were incubated in methyl cellulose containing IL-3 and erythropoietin as detailed elsewhere [Iscoe et al., 1980; Iscoe, 1984]. After 6 days, single lineage erythroid, neutrophil, and macrophage colonies were individually picked using finely drawn Pasteur pipettes. Each colony, in a volume of less than 1 μ l methyl cellulose, was placed without washing into 1st Strand Buffer for cDNA amplification. Cells from the P388D₁ mouse macrophage line [Koren et al., 1975] were diluted to 100 cells/ml and individually micromanipulated under direct vision into 1st Strand Buffer.

RESULTS

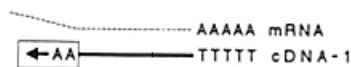
The Amplification Protocol

Briefly, cells are lysed in a small volume of first cDNA strand buffer, and all subsequent reactions are carried out in the same suspension without any extraction procedures. An overview of the biochemistry is shown in Fig. 1. A first strand of cDNA is generated on polyadenylated mRNA templates using reverse transcriptase and an oligo(dT)₂₄ primer. The conditions for this first step were designed to limit the length of the cDNA to about 300-700 bases. This was done to minimize selection against longer cDNAs during amplification with consequent distortion of relative sequence abundance. In a second step, a 3' oligo(dA) tail is added to the cDNA strand using terminal transferase. The resulting DNA is now defined at both ends and amenable to amplification by PCR using a single oligo(dT)-containing primer. Amplification proceeds first by generating the second cDNA strand on the first strand template using Taq polymerase and primer, and then by cyclic de-naturation and repetition of the same reaction following standard PCR procedures. The PCR primer contains, in addition to oligo(dT)₂₄, a unique 36 base sequence labelled X in Fig. 1. The additional sequence serves to enhance the specificity of hybridisation and provides convenient EcoRI restriction sites for later cloning. In contrast to the oligo(dT)-X primer, use of a pure oligo(dT) primer gives rise to

1 1st Strand cDNA



2 Poly(dA) Addition



3 PCR Amplification

1st Cycle



2nd Cycle

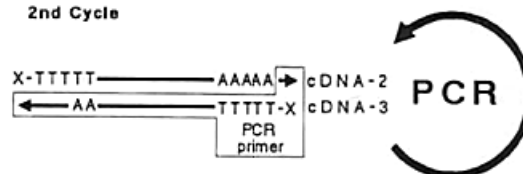


Figure 1. Sequence-independent amplification of total cDNA. Boxes enclose the reactions occurring at each step. The PCR primer contains oligo(dT) as well as a unique 36 base sequence labelled X (see Methodology.)

products which increase in length as amplification progresses. The result may be due to the ability of the homogeneous dT primer to hybridise to any position in the target poly(dA) sequences.

Fidelity of the Amplification Procedure Applied to Small Numbers of Hematopoietic Cells

Results of a test of the applicability of the technique to small numbers of hemopoietic cells are shown in Fig. 2. Mouse bone marrow cells were cultured in methylcellulose containing IL-3 and erythropoietin. Six days later, single lineage colonies containing either erythroblasts, neutrophils, or macrophages were identified by their characteristic ap-

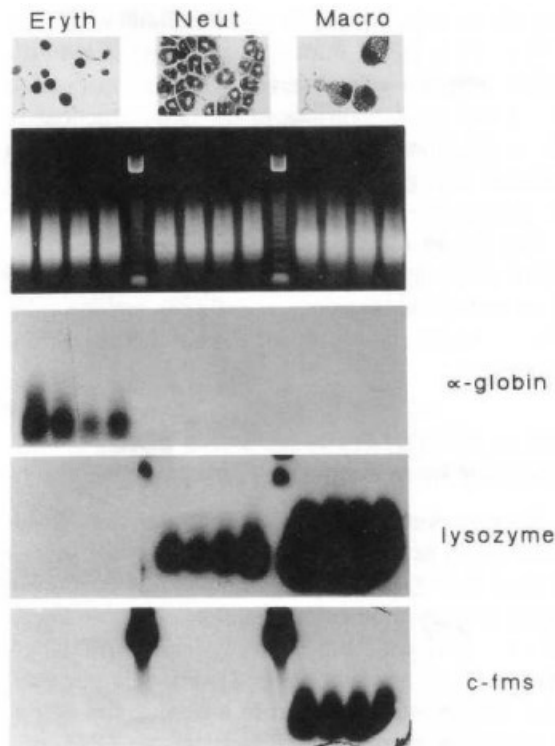


Figure 2. Amplification of total cDNA from individual hematopoietic colonies. Aliquots of amplified cDNA (4 μ l from a total of 50) prepared from erythroid (Eryth), neutrophil (Neut), and macrophage (Macro) colonies, four of each type, were run on a 1.5% agarose TBE gel and transferred to replica filters. Above each group of lineage-specific cDNAs is a photomicrograph (1000 x magnification) of cells from a colony actually used to generate one of the lanes. The top panel shows the ethidium stained gel prior to Southern transfer. Size markers (123 bp multiples) separate each group. The autoradiographs show hybridisation with the indicated radiolabelled probes. The hybridisation to the size marker in the lysozyme and c-fms panels is a result of residual plasmid contamination of the probes. Films were exposed for 6 (lysozyme) or 18 hours (c-fms, α -globin).

pearance and drawn up individually into pipettes. The size of the colonies ranged from 100 to 800 cells. A portion of each colony was spread on a glass slide, fixed, and stained for later confirmation of the lineage assignment. The rest of the colony was placed directly into 40 μ l 1st Strand Buffer. After a brief spin to remove debris and nuclei, 8 μ l were removed, converted into tailed cDNA, and processed to yield 1-5 μ g of amplified cDNA. Southern transfers were prepared from the amplified material and hybridised with the indicated probes. Each probe included a 3' sequence adjacent to the polyadenylation site.

Fig. 2 shows results obtained with four colonies of each type. The amount and size distribution of total amplified cDNA from each colony was similar (top panel). The hybridisation results were lineage-specific and were consistent with faithful amplification of the mRNA present in each of the distinct colony types: lysozyme sequences were detected only in cDNA from macrophages and neutrophils, α -globin only from erythroblasts, and c-fms only from macrophages. There was little variation between colonies of a given type, indicating that extent of amplification was consistent. Further, successful amplification was achieved with our procedure despite wide variation in initial size of mRNA transcripts ranging from 0.6 (α -globin) through 1.5 (lysozyme) to 4.5 kb (c-fms). The experiment therefore establishes that specific messages can be amplified without the use of sequence-specific primers, demonstrates the specificity and fidelity of the technique, and shows it to be relatively insensitive to initial transcript size.

Amplification of cDNA From Single Hemopoietic Cells

The ultimate test of the sensitivity of the method would be amplification of total cDNA from single cells. Results of an experiment performed on single micromanipulated P388D₁ macrophages are shown in Fig. 3. Individual cells were added directly to 1st Strand Buffer. Each sample was processed individually to yield amplified material comparable in quantity (1-5 μ g) to that obtained from 200 cells. A fraction of the amplified product was electrophoresed, and Southern transfers were probed for lysozyme and c-fms sequences. Hybridisation of both probes was observed with each sample (Fig. 3) whereas no signal was seen when α -globin was used as a probe (not shown). Similarly consistent results have also been obtained from single cells isolated from primary bone marrow cultures (not shown).

The result shows that both transcripts can be present simultaneously in an individual macrophage, a statement that could not be made from analysis of cell populations. Detection of c-fms sequence, whose abundance is estimated at 0.02% in these cells, shows that the technique is sensitive enough to detect moderate-to-low abundance messages even

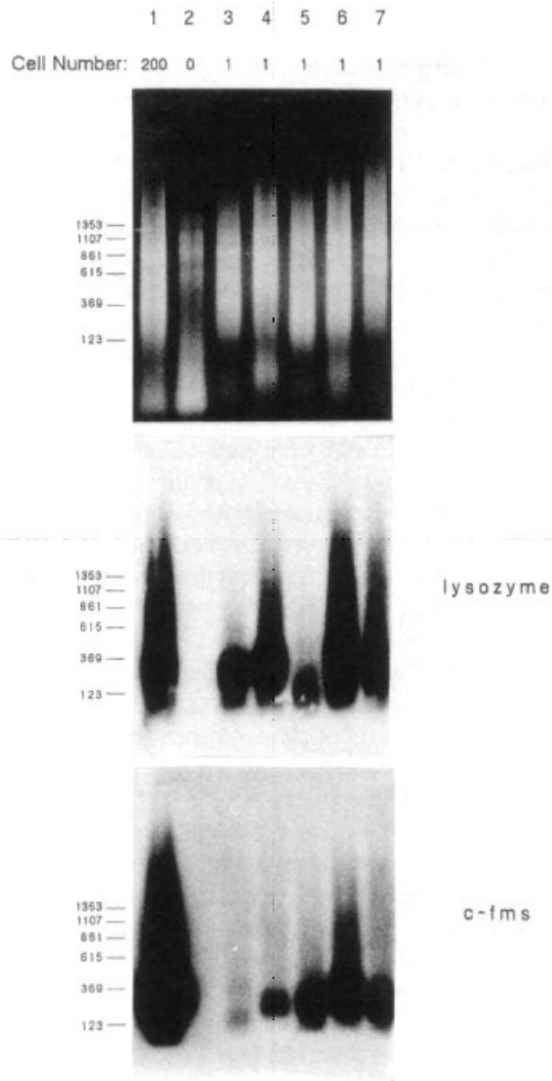


Figure 3. Amplification of total cDNA from single cells. Aliquots (8 μ l from a total of 100 μ l of amplified cDNAs prepared from single and 200 cell samples of the macrophage line P388D, were run on a 1.5% agarose TBE gel and transferred to replica filters. The number of cells in the starting preparation is indicated above each lane. The top panel shows the ethidium stained gel prior to Southern transfer. The bottom panels show hybridisation to the indicated probes. The position and size (base pairs) of molecular weight markers are indicated at the left side. Films were exposed for 18 (lysozyme) or 48 hours (c-fms).

in single cells, again without any requirement for sequence-specific primers.

Preservation of Relative Abundance During Amplification of RNA Mixtures

We next wished to establish whether relative abundance relationships would be preserved during amplification, and whether rare sequences could be detected in an RNA mixture. Since there is little in-

formation on the precise abundance of mRNAs present in bone-marrow colonies, or in single cells, we prepared mixtures of purified RNAs in defined ratios. Purified RNA from P38813₁ cells was distributed in aliquots of 240 pg, an amount representing about ten cells and containing about 5 pg of polyadenylated RNA. Purified rabbit globin mRNA (a mixture of α and β chains) was added to each sample in amounts ranging from 500 down to 5×10^{-4} pg. Taking into account the relatively small size of the globin message (about 40% of the average), this range corresponded to a globin message abundance of 99.5% to 0.025%. Each mixture was then individually amplified, and Southern transfers were prepared from a portion of the product. Fig. 4 shows the results of hybridisation with rabbit (β -globin and lysozyme probes. Lysozyme sequences were detected only where P388D₁ RNA was present, and β -globin sequences only in samples which initially contained added globin RNA. Further, a gradation in hybridisation intensity was observed which corresponded to the varying proportions of the two sequences in the mixtures. The difference in hybridisation intensity of β -globin between lanes 2 and 3 was readily apparent on shorter exposures (not shown).

The results establish that the procedure is capable of detecting rare sequences in a mixture at least down to an abundance level of 0.025%, and that relative abundance is preserved during the amplification process.

Generation of Representative cDNA Libraries From Amplified Samples

It was important to establish that the amplified material could be efficiently cloned. First, we wished to show that cDNA libraries could be generated from single primary hemopoietic colonies. Plasmid libraries were produced from samples of amplified cDNA from each colony type (Fig. 2). For this purpose, each sample was subjected to a final round of polymerisation to ensure that most of the DNA was double-stranded. After digestion with EcoRI to cleave within the PCR priming sequence, the samples were ligated into digested and dephosphorylated pUC19. Competent bacteria were transformed to yield 4×10^5 to 10^6 colonies/ μ g of insert. Of 52 randomly chosen colonies, 98% harboured plasmids with EcoRI inserts averaging about 400 bp in size (range 150- 1,000 bp). The results of hybridisation of each library to lineage-specific probes are shown in Table 1A, providing an estimate of the abundance of these sequences within the libraries.

In order to establish that the abundance of a sequence within an amplified cDNA library could reflect its abundance in the starting RNA, the amplified samples from the experiment shown in Fig. 4 were also cloned into plasmid libraries. Libraries prepared

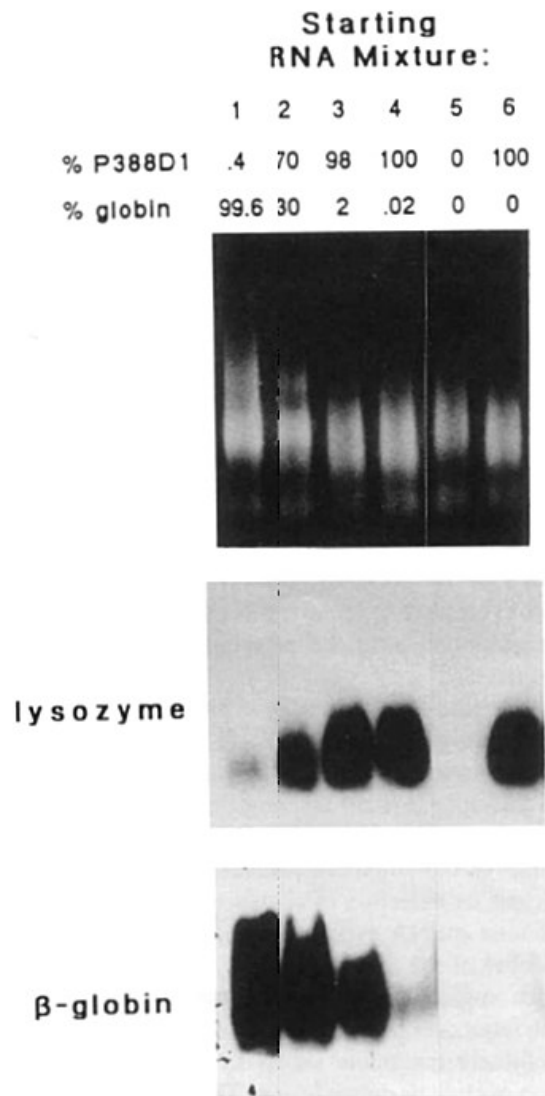


Figure 4. Amplification of RNA mixtures containing varying proportions of globin mRNA. Rabbit globin mRNA was mixed with total RNA isolated from P388D₁ cells to give the indicated molar percentages. After synthesis and amplification of total cDNA, 5 μ l aliquots were run on a 1.5% agarose TBE gel and transferred to nylon membranes. The top panel shows the ethidium stained gel prior to Southern transfer. The autoradiographs (6 hour exposure) show hybridisation to the indicated probes.

from each sample were screened with probes for rabbit β -globin and lysozyme. Although weak, cross-hybridisation of the β -globin probe to α -globin was sufficient to detect α -globin as well as β -globin clones. The frequencies of globin-positive colonies, presented in Table I B, correlated directly with the abundance of α - and β -globin in the starting RNA samples. The frequency of lysozyme-positive colonies was 5% in the P388D₁ sample alone and was similarly correlated with relative concentration of P388D₁ RNA in the starting mixtures (not shown).

The results show that plasmid libraries can be generated readily from the amplified material, and that

TABLE 1. A. Frequency of Lineage-Specific Sequences in Amplified cDNA Libraries From Single Hemopoietic Colonies

Colony type	Probe	Percentage of library positive	Total library clones screened
Erythroid	α -Globin	9.7	380
Neutrophil	Lysozyme	0.97	828
Macrophage	Lysozyme	5.0	585
	c-fms	0.05	1,977

B. Frequency of Globin Sequences in Amplified cDNA Libraries From RNA Mixtures Containing Varying Amounts of Globin mRNA

Percent abundance of globin mRNA in starting sample	Globin-positive clones	
	Percentage	Total clones screened
99.6	83	121
71.0	82	135
2.4	5.3	716
.025	0	1,384

abundance relationships can be correctly reflected within the resulting libraries.

DISCUSSION

The development of our protocol was guided by three main objectives. First, the method had to amplify simultaneously all mRNA transcripts in a sample independently of their sequence. Second, for relative abundance relationships to be preserved in the amplified product, the efficiency of amplification had to be relatively insensitive to initial transcript size. Finally, in order to achieve the sensitivity required for work with single cells, the protocol had to minimize both sample loss as well as risk of contamination with exogenous sequences.

In common with the method of Belyavsky et al. (1989), our approach achieves amplification independently of specific primers by taking advantage of the poly(A) sequence present in most mRNAs and adding a second homopolymeric site using terminal transferase. However, the Belyavsky procedure yielded an amplified product in which longer messages were severely underrepresented. In contrast, our method is relatively insensitive to initial transcript size: long (c-fms, 4.5 Kb), medium (lysozyme, 1.5 Kb), and short transcripts (α -globin, 650 bases) were all efficiently amplified (Fig. 2). We achieved this by designing the conditions for the reverse transcriptase reaction to limit the size of the first cDNA strand to 300-700 bases. PCR is therefore initiated on cDNA that is relatively uniform in size and within the range for which amplification is most efficient. Agarose gel analysis confirmed that the amplified product consisted mainly of molecules within this size range. Although some sequences,

such as lysozyme and c-fms, appeared to be present as relatively small fragments (Fig. 3), examination of a large number of amplified cDNA clones has shown their size distribution to be close to the bulk profile.

In keeping with the size restriction to 300-700 bases of 3' sequence, optimal detection of specific sequences requires probes which include sequence at or close to the extreme 3' ends of the native transcripts. In several instances we compared the efficiency of hybridisation of both 5' and 3' probes from the same gene. Hybridisation of 5' probes was consistently weak or undetectable. Some cDNA clones lack coding sequence in their terminal few hundred bases, and may include repetitive motifs. Even in such cases, it has generally been possible to prepare suitably specific 3' probes after sequence analysis and appropriate subcloning.

In order to achieve the required sensitivity for work with single isolated cells, it was necessary to eliminate any opportunity for sample loss. Our procedure is carried out by lysing one or more cells in a small volume of first cDNA strand buffer, and performing all subsequent reactions in the same suspension without any extraction procedures. This simplicity has the additional advantage of minimising opportunities for contamination by exogenous nucleic acids.

When cDNA is amplified using sequence-specific primers, the extreme sensitivity of the method can lead to the detection of transcripts that are too rare in the original samples to be biologically meaningful (Chelly et al., 1988; Sarkar and Sommer, 1989). This potential for misleading positives does not arise with our approach: all poly(A)⁺ RNA sequences in a sample are amplified and very rare transcripts in the starting material remain very rare in the amplified product. Furthermore, our approach is amenable to exact quantitation of transcript frequencies by comparison to amplified standards on

Southern transfers or by the cloning approach illustrated in Table 2.

Another potential artifact during PCR is the generation and amplification of non-specific sequences which are unrelated to the original cellular material. We have observed such products after PCR of control samples (Fig. 4, lane 5; Fig. 3, lane 2) which have received Taq polymerase, buffer, nucleotides, and primer, but no cellular templates. Tests of digestion of the material at restriction sites included within the primer seem to rule out simple oligomerisation of the PCR primer, and the problem has not been traceable to contamination of individual buffer ingredients, enzyme batches, or suppliers of Taq polymerase. However, the background is always diminished when a template is added and can be distinguished from specific PCR products on the basis of size (Fig. 3, lane 2). Moreover, it has not hybridised to specific probes and therefore appears not to have the potential to produce false positives.

The simple procedure described here will generate microgram amounts of cDNA representative of the entire spectrum of polyadenylated mRNAs in a sample as small as a single cell. Relative abundance relationships in the original sample remain intact in the amplified product. Sensitivity is sufficient for detection of sequences as rare as 0.02% of total mRNA even in single isolated cells. The fidelity of the method allows the cellular origin of each amplified sample to be deduced accurately from the sequences present. The amplified material is indefinitely renewable simply by further amplification of aliquots drawn from it. The cDNA sequences are readily cloned for generation of libraries. The material can be tested at any convenient time for presence of sequences hybridising to any chosen probe, since preparation of the amplified product does not depend on any foreknowledge of sequence. Further, it can provide an inexhaustible supply of reagent for subtraction procedures. The approach will be ideally suited to the investigation of previously intractable problems in development and stem cell biology.

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TABLE 2. Materials Used

Material	Source
RNAguard	Pharmacia
CL6B spin columns	Pharmacia
Inhibit Ace	5'-3' Inc.
dNTPs 100 mM solutions ^a	Boehringer/Mannheim
Avian RTase	Boehringer/Mannheim
Terminal transferase ^a	Boehringer/Mannheim
Glycogen	Boehringer/Mannheim
BSA molecular grade	Boehringer/Mannheim
Taq polymerase ³	Perkin-Elmer Cetus
Moloney RTase	Gibco-BRL
Tailing buffer (K. cacodylate / CoCl ₂ /)DTT)	Gibco-BRL
Rabbit globin RNA	Gibco-BRL
Gene-Screen Plus	DuPont
X-ray film	Kodak X-OMAT AR-5

^aReagent from other suppliers was less effective

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