molecular structure outside the chromatographic contact region, that is, either the exterior or interior, will go undetected unless this change alters the structure of the chromatographic contact region. Although liquid chromatographic systems are capable of remarkable discrimination, their greatest weakness is the prerequisite that differences must lie within the chromatographic contact region to be detected.

These conclusions have important implications in both preparative and analytical systems. For example, it would be possible to purify most of the lysozyme variants with a single monoclonal antibody column because the chromatographic contact region of the immunosorbent is narrowly targeted and amino acid variations are broadly distributed. HIC and IEC columns, in contrast, are less effective in the initial purification of lysozymes but can easily differentiate between most of the lysozyme variants.

The case of lysozyme variants illustrates that purification of a genetically engineered protein based only on immunosorbents is probably not wise in systems where expression errors, faulty post-translational processing, and chemical alterations during purification can be encountered. In particular, monoclonal antibodies will be ineffective in discriminating among these small, random variations within a structure. Analytical systems based on immunological assays will be plagued with the same problem. Although chromatographic modes such as IEC, HIC, and RPC are much less dramatic than immunosorbents, they are less expensive and can play an important role in molecular discrimination.

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High-Speed Chromosome Sorting

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Dual-beam high-speed sorting has been developed to facilitate purification of chromosomes based on DNA staining with the fluorescent dyes Hoechst 33258 and chromomycin A3. Approximately 200 chromosomes per second of two types can be sorted from a suspension of chromosomes isolated from human lymphoblasts while fluorescent objects (chromosomes, debris fragments, chromosome clumps, and nuclei) are processed at the rate of about 20,000 per second. This sorting rate is approximately ten times that possible with conventional sorters. Chromosomes of a single type can be sorted with a purity of about 90 percent. DNA from the sorted chromosomes is suitable for construction of recombinant DNA libraries and for gene mapping.

CHROMOSOMES HAVE BEEN RECOGNIZED FOR ALMOST A century as the fundamental organizational units of DNA and as the determinants of gene segregation during cell division. Much effort has been devoted to determination of the chromosomal locations of specific genes and to identification of disease-linked chromosome aberrations in humans. Over 1600 DNA sequences have now been mapped, at least provisionally, to chromosomes. In addition, the chromosomal locations of more than 50 disease-linked DNA sequences are known (*1, 2*). Efforts to understand the DNA sequence organization of chromosomes are now accelerating with the initiation of efforts to determine the complete genetic structure of the human genome (*2*). Much of this work can be facilitated by the ready availability of DNA from one or

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a few chromosome types. Such DNA can be used to determine the chromosomal location of specific genes or DNA sequences. In addition, it can be used for production of recombinant DNA libraries to facilitate selection of new DNA sequences whose chromosomal locations are known.

One approach to the production of such DNA was suggested by the discovery in 1975 (*3*) that chromosomes could be isolated from cells, stained with a DNA-specific fluorescent dye, classified by means of flow cytometry and purified by means of flow sorting. Since that time, significant improvements have been made by numerous groups in chromosome preparation and in flow cytometry and sorting (see *4–7* for recent reviews). Now, up to 20 different human chromosome types can be distinguished in a population of human chromosomes and purified by sorting. Chromosome types that cannot be sorted at high purity from chromosomes isolated from human cells (for example, human chromosomes 9 through 12) can be sorted from human-hamster hybrid cell lines containing one or a few human chromosomes (for example, only one of the 9 through 12 group). Sorted chromosomes have been used for production of recombinant DNA libraries and gene mapping. However, these studies have been limited by the amount of time required to sort usable quantities of chromosomal DNA. This limitation has been eased by our recent development of high-speed sorting for chromosome purification (*8*). In this article, we summarize the current status of chromosome purification by high-speed sorting, discuss the characteristics of chromosomes purified by sorting, and describe some of the biological studies in which chromosomal material purified by sorting has played an important role.

Chromosome Purification Technology

Chromosome isolation and staining. Chromosome isolation begins with the collection of mitotic cells. This is accomplished by treating cultured cells with colcemid to inhibit the progression of cells through mitosis. The mitotic cells are resuspended in a hypotonic buffer to swell them. The buffer is designed to facilitate mechanical membrane disruption and to preserve the integrity of the chromosomes once they are released from the cells. We commonly use a buffer containing magnesium sulfate [50 m*M* KCl, 5 m*M* Hepes, 10 m*M* $MgSO_4$, 3 m*M* dithioerythritol, and 0.25% Triton X-100 (*9*)]. However, a buffer containing polyamines (15 m*M* tris-HCl, 2 m*M* EDTA, 0.5 m*M* EGTA, 20 m*M* NaCl, 80 m*M* KCl, 14 m*M* β-mercaptoethanol, digitonin (0.1%), 0.2 m*M* spermine, and 0.5 m*M* spermidine) also has proved useful for chromosome stabilization (*10*). Mechanical disruption of the weakened cell membranes by needle shearing or by spinning in a vortex mixer releases the mitotic chromosomes into the buffer. Unfortunately, DNA fragments, chromosome clumps, and nuclei are also released by this process. These nonchromosomal objects must be recognized and rejected during sorting.

Isolated chromosomes are equilibrated with one or more DNA-specific fluorescent dyes for flow cytometry and sorting. Commonly used dyes include propidium iodide and ethidium bromide (specific for double-stranded nucleic acids with no base composition preference), Hoechst 33258, and 4,6-diamidino-2-phenylindole, abbreviated DAPI (DNA-specific, binding preferentially to DNA rich in adenine and thymine), and chromomycin A3 and mithramycin (DNA-specific, binding preferentially to DNA rich in guanine and cytosine). We routinely use the dyes Hoechst 33258 (Ho) and chromomycin A3 (CA3) together so that chromosomes can be distinguished by their total DNA content and their DNA base composition (*9, 11*).

Table 1. A comparison of the operating characteristics of conventional and high-speed sorters.

Operating pressure (psi)	Jet velocity (m/sec)	Droplet production frequency (sec^{-1})	Theoretical sort rate* (sec^{-1})	Actual rate achieved during sorting (sec^{-1})
		Conventional sorter		
15	10	30,000	125	15–35†
		High-speed sorter		
200	50	215,000	660	200

*Sorting rates are based on the assumptions that autosomes are purified from other human chromosomes and that chromosome preparation is ideal. †(*14*).

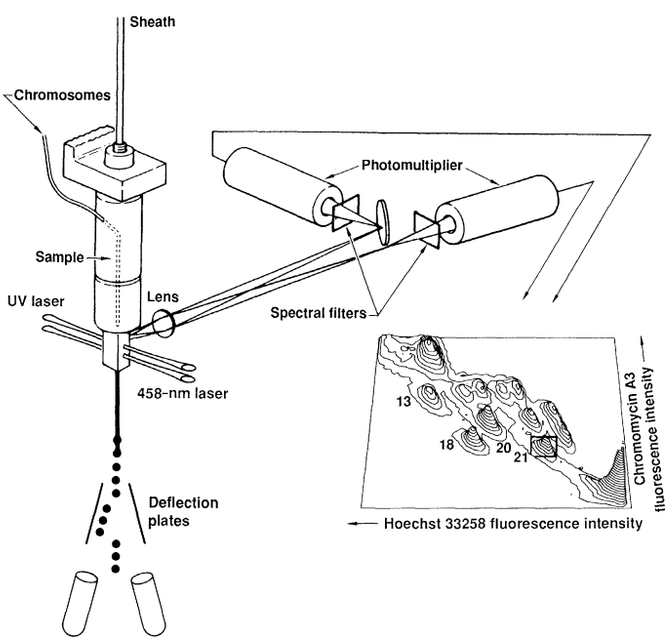

Fig. 1. Chromosomes stained with Hoechst 33258 (Ho) and chromomycin A3 (CA3) flow sequentially through two laser beams, one adjusted to 458 nm to excite CA3 and the other adjusted to the UV (351 + 363 nm) to excite Ho (*9, 11*). The Ho and CA3 contents of individual chromosomes are determined by measuring the intensities of fluorescence that are emitted as they pass the UV 458-nm and laser beams. These measurements are accumulated to form fluorescence intensity distributions (called flow karyotypes). The flow karyotype shown here was generated while human chromosome 21 was sorted at high speed from other human chromosomes. The peaks are produced by chromosomes of one or a few types. The identities of several peaks are indicated. This flow karyotype shows peaks only for chromosomes smaller than number 9. The approximate region from which chromosomes were sorted is indicated in the flow karyotype. The high-speed sorter is capable of measuring fluorescence intensities of approximately 20,000 objects per second. After fluorescence analysis, the chromosomes flow at 50 m/sec in a liquid stream in air to the point where this liquid jet breaks into droplets. Droplet formation is forced to occur at 215,000 objects per second by the action of a vibrating piezoelectric transducer attached to the flow chamber. The time between fluorescence measurement (laser beam crossing) and arrival at the end of the liquid jet is nearly constant so that the droplets that contain chromosomes having preselected fluorescence intensities can be electrically charged as they separate from the liquid column. Usually, one additional droplet is charged on either side of the droplet presumed to contain the desired chromosome, to compensate for slight differences in chromosome velocity through the flow system. Three-drop groups containing more than one fluorescent object are not sorted. This eliminates the possibility of sorting an unwanted chromosome or debris fragment along with a chromosome satisfying the sorting criteria. Droplets can be charged either positively or negatively so that two classes of chromosomes can be selected for sorting during the same run. All droplets traverse an electric field of several thousand volts where the charged droplets are separated from the uncharged droplets.

High-speed chromosome analysis and sorting. Stained chromosomes are classified and sorted at high speed as illustrated in Fig. 1. Approximately 200 chromosomes of two autosomal types can be sorted each second from a population of human chromosomes while about 20,000 fluorescent chromosomes and other objects are processed per second. This is approximately one order of magnitude faster than conventional sorting. Table 1 compares the operating and sorting characteristics of conventional and high-speed sorters.

High-Speed Sorting Results

Sorting purity. The purity with which a chromosome type can be sorted depends on the degree to which it can be distinguished from other chromosomes, DNA debris, and clumps of chromosomes during sorting. The purity of sorting also depends on the shape and location of the region defined in the bivariate flow karyotype from which chromosomes will be sorted. Figures 1 and 2 show flow karyotypes measured for chromosomes isolated from human, mouse, and human-hamster hybrid cells. Flow karyotypes shown in Fig. 1 and Fig. 2, b and c, were generated during actual high-speed sorting. These distributions show that some chromosomes are much more clearly resolved from their neighbors than others. The smaller human chromosomes are particularly well resolved (Fig. 1 and Fig. 2, a and b). The larger human chromosomes in some human-hamster hybrid cells are also well resolved (Fig. 2c). Individual mouse chromosomes, however, are poorly resolved (Fig. 2d). For sorting, a region is defined in the bivariate flow karyotype (see Fig. 1), and all objects with fluorescence intensities falling within the region are selected for sorting. Multiple-event sorts (that is, sorts in which desired chromosomes would be sorted along with other DNA-containing objects) are aborted to maximize sorting purity. The size and location of the sort region, the separation of the peaks, the measurement resolution achieved during chromosome analysis, the criteria established for multiple-event sort rejection, and long-term sorter stability all affect the purity of the sort.

Sort region selection and peak separation. The size of the sort region relative to the width of the peak for the chromosome to be sorted determines the sorting efficiency (that is, the fraction of objects of the desired type that fall within a sorting region) and the sorting purity. In general, the sorting efficiency increases and the purity of the sort decreases as the sort region size is increased. A rule of thumb for sorting (*12*) to produce 90% purity and 75% efficiency from a peak with a nearby neighbor of equal volume is that the separation of the two peaks must be $>2\sigma$, where σ is the standard deviation of the peaks. This rule suggests that human chromosomes 13, 18, 20, 19, 21, 22, and Y can be separated from other human chromosomes with a purity of better than 90% when measurement coefficients of variation (CVs) of 0.05 are maintained during sorting. (CV = standard deviation of the fluorescence intensities measured for one chromosome type divided by the mean fluorescence intensity for that chromosome type.) All chromosomes except chromosomes 9 through 12 can be separated with purity greater than 90% with a measurement CV of 0.02. Unfortunately, it has proved difficult to maintain measurement CVs better than about 0.03 to 0.05 while processing 20,000 objects per second through the high-speed sorter. This has made it difficult to sort human chromosomes 1 through 12 and X with high purity. Fortunately, these large chromosomes are well separated from the hamster chromosomes in some human-hamster hybrid cell lines on the basis of their Ho and CA3 contents and they can be sorted at high purity. Figure 2c, for example, shows a bivariate flow karyotype measured for a human-hamster hybrid line containing human chromosomes 4, 8, and 21. Chromosomes 4 and 8 are especially well resolved from each other and from the hamster chromosomes in this cell line so that they can be easily purified by high-speed sorting. However, many hybrid lines are not stable karyotypically, and may lose or rearrange the human chromosome of interest during cell culture. In practice, it is difficult to find hybrid lines carrying intact large human chromosomes that are suitable for sorting. Table 2 shows a list of relatively stable human-hamster hybrid cells containing intact human chromosomes that we have sorted at good to high purity using a high-speed sorter.

Debris and clumps. The purity with which chromosomes can be sorted is also affected by the presence of DNA debris and chromosome clumps in the sample. Debris result from the disruption of chromosomes and nuclei during the chromosome isolation process. Debris form a smooth continuum (ridge) that is high near the origin, runs along the same diagonal in the flow karyotype as the majority of the chromosomes and decreases rapidly with increasing Ho and CA3 content. Thus, sorts of small chromosomes with the same Ho/CA3 fluorescence intensity ratio as the debris continuum are most likely to be contaminated with debris. Human chromosome 21 is difficult to purify to better than 80 to 90% because of the debris continuum, even though this chromosome is usually well separated from other human chromosomes (see Fig. 1). Clumps

Table 2. Characteristics of human-hamster cell lines containing human chromosomes purified by sorting for DNA library construction. Chromosomes designated with + were measured to be larger than normal in the flow karyotype. Chromosomes enclosed in brackets were observed at an abnormally low frequency. Source locations are as follows: LLNL, Lawrence Livermore National Laboratory; ERCI, Eleanor Roosevelt Cancer Institute; NIH, National Institutes of Health; HGMCR, NIGMS Human Genetic Mutant Cell Repository, Camden, New Jersey.

Chromosome sorted	Hybrid line	Source	Human chromosomes detected by flow karyotyping	Human chromosomes detected by isoenzyme analysis or banding analysis or both
1	UV24HL10–12	L. Thompson, LLNL	1, 3+, 11, 13, 19	1, 3, 11, 13, 19
2	UV24HL5	L. Thompson, LLNL	2, X	2, X
3	314-1b	C. Jones, ERCI	3	3
4	UV20HL21–27	L. Thompson, LLNL	4, 8, [21]	4, 8, 21
5	640–12	C. Jones, ERCI	5, 9, 12	5, 9, 12
6	UV20HL15–33	L. Thompson, LLNL	6, 9, 13, [15], 20, [21]	6, 9, 11, 13, 15, 17, 19, 20, 21
7	GM131*	HGMCR	All	Not applicable
8	UV20HL21–27	L. Thompson, LLNL	4, 8, [21]	4, 8, 21
9	UV41HL4	L. Thompson, LLNL	6, 9, 13, 16, 18, Y	6, 9, 16, 18
10	762–8A	C. Jones, ERCI	10	10
11	UV20HL4	L. Thompson, LLNL	1, 4, 5, 6, 11, 14, 15, 16, 19, 21	1, 4, 5, 6, 11, 14, 15, 16, 19, 21
12	81P5D	S. O'Brien, NIH	12, 15+, X	12, 15, X
X	UV24HL5	L. Thompson, LLNL	2, X	2, X

*GM131 is an apparently normal human lymphoblastoid line; all the other lines are human-hamster.

resulting from incomplete separation of small chromosomes during isolation often have the same Ho and CA3 contents as the larger chromosomes. Chromosomes 1 and 2 are difficult to sort at high purity because of the presence of these clumps.

Multiple-event sort rejection. High-purity chromosome sorting requires rejection of all multiple-event sorts that would result in contamination of the sorted chromosomes with the other DNA-containing material such as debris fragments, cell nuclei, or chromosome clumps. This rejection is automatic in the high-speed sorter (or any other sorter) as long as very small fragments containing DNA (for example, fragments whose DNA contents are larger than 5% of the DNA content of the number 21 chromosome) and large fragments (for example, chromosome clumps and nuclei) are detected.

Analysis of sorting purity. The actual purities achieved during high-speed sorts of human chromosomes 4, 11, 16, 17, and 21 are presented in this section as an indication of the purities that are routinely achieved. Chromosome 4 is an example of a large human chromosome sorted from a human-hamster cell line containing only two other human chromosomes (numbers 8 and 21). Chromosome 4 is well resolved from all others. Chromosome 11 was also sorted from a human-hamster hybrid line. However, it was not well resolved from the hamster chromosomes, and, in addition, the line contained nine other human chromosomes. Chromosomes 16 and 17 were sorted from two different human cell lines. They were not well resolved in chromosomes isolated from one cell line and the sorting purity was low. In more recent sorts, a better cell line and a better chromosome preparation allowed these chromosomes to be more highly purified by sorting. Chromosome 21 was sorted from a human cell line in which it was well resolved from all other chromosomes but not from a small debris continuum. Three approaches have been used to estimate sorting purity: (i) mathematical analysis of the bivariate flow karyotype recorded during sorting, (ii) in situ hybridization, and (iii) molecular analysis of recombinant DNA libraries made from the sorted chromosomes. The results are summarized in Table 3. The DNA accumulated during these sorts was used by the National Laboratory Gene Library Project to construct recombinant DNA libraries (see *5*, *14*, and below).

Mathematical analysis of the flow karyotypes recorded during high-speed sorting gives estimates of the amount of contamination in the sort region from neighboring peaks, debris, and clumps. The flow karyotype is modeled mathematically as the sum of several bivariate normal distributions (one for each peak) and a smoothly varying function to approximate the continuum produced by debris and clumps. The parameters in the model (for example, peak volumes, means, and continuum magnitude) are varied by a least squares technique until the model best matches the data. The purity of the sort is then calculated from the model as the fraction of the events in the sort region that come from the normal distribution matched to the peak from which chromosomes were sorted. However, it is difficult to carry out this process in bivariate flow karyotypes, since the debris continuum is difficult to model mathematically. Thus, analysis is now performed on univariate Ho and CA3 distributions generated from small portions of the bivariate flow karyotype. Figure 3 shows the analysis of such a univariate distribution generated from the region including peaks 13 through 17 shown in Fig. 2b. The bivariate distribution was generated during high-speed sorting for chromosomes 16 and 17. The analysis shows the purity of the sort for these chromosomes to be 95 to 96%. The contamination in these sorts seems to come largely from the debris continuum (4 to 5%). Contamination from adjacent peaks appears minimal (about 1%). This technique showed the purity of the most recent sorts of chromosomes 4 and 21 to be about 95% and 75 to 80%, respectively (Table 3). The impurities in these sorts appeared to be due to debris and clumps having the same Ho and CA3 contents as the sorted chromosomes.

Sort purity also can be estimated from a fluorescence in situ hybridization technique (*5, 13, 14*). In this approach, a sample of sorted chromosomes is fixed on a microscope slide, denatured, and hybridized with a chemically modified DNA probe. The hybridized probe is detected with a fluorescent reagent that binds to the chemical modification. Whole genomic hamster DNA was used as the probe for human chromosomes collected during purification of chromosomes 4 and 11 from human-hamster cells (Table 3) and yielded an estimate of hamster contamination. Two human DNA probes, one complementary to centromeric sequences on chromosome 16 and the other complementary to centromeric sequences on chromosome 17, were used to estimate cross-contamination of the simultaneous sorts of chromosomes 16 and 17 from a human cell line (Table 3). This technique is limited to detection of contamination by chromosome-sized DNA fragments. It is not sensitive to small debris.

Molecular analysis of the DNA inserts in recombinant DNA libraries made with sorter-purified chromosomes provides the most

Table 3. Purity estimates for chromosome sorts and eight libraries.

Sorted chromosome	Library identification*	Purity (%) from analysis of chromosomes			Purity (%) from analysis of libraries	
		Flow karyotype†	In situ hybridization with total hamster DNA‡	In situ hybridization with chromosome-specific DNA‡	Plaque hybridization with total hamster DNA	Unique sequence mapping
4	LL04NS01	High			83§	100‖
4	LL04NS02	95	95		95	
11	LL11NS01	Fair¶	65		48§	63#
16	LL16NS02	Low	Not applicable		Not applicable	23, 33**
16	LL16NS03	95	Not applicable	90	Not applicable	
17	LL17NS01	Low	Not applicable		Not applicable	50††
17	LL17NS02	96	Not applicable	90	Not applicable	
21	LL21NS02	75	Not applicable		Not applicable	80‡‡

*Name of the National Laboratory Gene Library Project library made from these sorted chromosomes. The chromosome type is underlined. †Qualitative information was determined by visual inspection of the flow karyotypes. Quantitative information was determined by the computer analysis technique described in the text. ‡Chromosomes 4 and 11 were sorted from hybrid cells. Purity for these sorts was estimated by hybridization with hamster DNA. Chromosomes 16 and 17 were sorted from human cells. Purity for these sorts was estimated by hybridization with chromosome-specific repeat sequence DNA. §(*15*). ‖(*37*). ¶A sort of much higher purity has been made recently from chromosomes isolated from the human-hamster hybrid cell line J1 (obtained from C. Jones, Eleanor Roosevelt Cancer Institute). The only human chromosome in this line is number 11 so that there is no contamination from other human chromosomes. The hamster contamination is about 25%. Construction of a new library from these chromosomes is under way. #The hybrid line used for the number 11 library construction contains ten human chromosomes, so impurities come from both hamster and human chromosomes. Thus, the fraction of clones mapping to chromosome 11 is (0.45) (0.63) or 28% according to the molecular analysis of the library (*38*). **(*39*). ††(*40*). ‡‡(*41*).

complete characterization of sort purity. An estimate of the fraction of hamster DNA in sorts of chromosomes 4 and 11 from human-hamster hybrid cells was made by hybridizing radioactively labeled hamster and human DNA to plaque lifts made from library clones growing on agar plates. The fraction of hamster inserts in the library was estimated from the fraction of plaques that hybridize to hamster and not human DNA (*15*). The extent of hamster contamination was 5 and 45% in sorts of chromosomes 4 and 11, respectively (Table 3). The contamination was higher in the sort of chromosome 11, since this human chromosome was less clearly resolved from nearby hamster chromosomes (*15*). Complete information can be obtained by analyzing inserts from randomly picked clones to determine (i) whether they contain human or hamster DNA, (ii) whether the inserts are repetitive or unique, (iii) insert size, (iv) whether insert is single or multiple, and (v) the chromosome location of each unique sequence. However, this is a time-consuming process. Current information about the fraction of clones carrying sequences from the desired chromosome in libraries made from DNA accumulated during sorts for chromosomes 4, 11, 16, 17, and 21 is listed in Table 3.

Table 3 shows that the sorting purity is about 90% or better when the sorted chromosomes are well resolved from other chromosomes and background. However, the purity may be considerably lower if measurement resolution is not good. High-purity chromosome sorting can be accomplished routinely if the instrumental resolution is good (CV $\leq$ 0.05), cells are selected in which the chromosome of interest is well resolved from other chromosomes, and chromosomal debris and clumping are minimized during chromosome isolation.

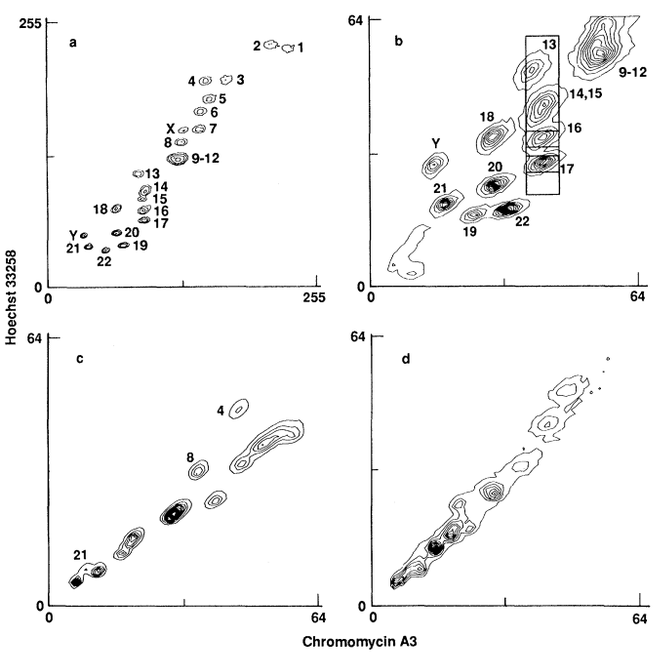

Fig. 2. Bivariate Ho versus CA3 flow karyotypes measured for chromosomes isolated from several cell lines. (**a**) A flow karyotype measured for human chromosomes at low speed and very high resolution on Livermore's dual-beam flow cytometer. All chromosomes except 9 through 12 are well resolved. The chromosomes responsible for each peak are indicated. (**b**) A flow karyotype measured for human chromosomes smaller than number 9 during high-speed sorting for chromosomes 16 and 17. The rectangular box defines the analysis region referred to in Fig. 3. (**c**) A flow karyotype measured during high-speed sorting for chromosomes from a human-hamster hybrid cell line carrying human chromosomes 4, 8, and 21. The identities of the human chromosome peaks are indicated in the figure. (**d**) A flow karyotype measured for a mouse cell line showing unusually distinct separation of the chromosomal peaks. For each panel, amplifier gains were adjusted to utilize the full range of the measurement scale.

Sorting rate. The rate, r, at which chromosomes can be sorted is given by the equation $r = Rfe^{-Rt}$, where f is the fraction of the objects in the population that satisfy the sorting criteria, R is the rate at which objects are sensed by the system, and t is the time required for the formation of three drops. This equation assumes (i) that three droplets are charged for each sorting event, (ii) that objects are randomly spaced along the flow axis (that is, they obey Poisson statistics), (iii) that all multiple-object sorts are aborted, and (iv) that the time to measure a chromosome is considerably less than t.

This equation predicts that the maximum sort rate occurs when the average throughput rate $R = 1/t$. However, 0.63 of the desired chromosomes will be rejected at this rate. In high-speed sorting, R is approximately 20,000 objects per second, and t is 1.4×10^{-5} seconds. This keeps the flow karyotyping resolution acceptably high (Ho and CA3 CVs about 0.03 to 0.05), and the predicted sorting efficiency about 0.75. Under these conditions, this equation predicts that approximately 660 chromosomes of one type can be sorted each second from a population of human chromosomes, if one assumes that all chromosomes of one type fall in the sorting region and that no debris, chromosome clumps, or nuclei are present. In practice, up to 75% of the objects sensed by the system are not single chromosomes so that the actual sorting rate for a single chromosome type is only about 200 per second. This rate is approximately ten times as high as the rate that can be achieved from conventional sorters (Table 1) if one assumes similar sorting and multiple-event rejection criteria for both instruments.

The order-of-magnitude increase in sorting rate of high-speed sorting compared to conventional sorting is especially important if microgram quantities of chromosomal DNA are required. The actual time required to accumulate a microgram of chromosomal DNA depends on the size of the chromosome to be purified and on its relative frequency in the population from which it is to be sorted. The actual sorting rates indicated in Table 1 suggest that accumulation of microgram quantities of human chromosomes 1, 21, and Y require approximately 3, 15, and 30 hours, respectively, with high-speed sorting and approximately 10 times as long with conventional sorting. Purification of this amount of material borders on being impractical with conventional sorting.

Characteristics of sorted chromosomes. The DNA in chromosomes purified by sorting is subjected to a variety of agents and effects that may cause damage. These include: mechanical shearing during chromosome isolation and during passage through the flow system, exposure to DNA-damaging agents such as fluorescent dyes and intense ultraviolet (UV) light, and exposure to nucleases liberated during chromosome isolation. These phenomena probably do cause some damage. However, the putative damage does not interfere with the cloning of DNA from sorted chromosomes into λ insertion vectors since recombinant DNA libraries have now been produced from sorter-purified DNA for every human chromosome type (see below). Large DNA (>150 kb) is required for cloning into λ replacement vectors or cosmids. The $MgSO_4$ chromosome isolation procedure does not yield DNA of this size, whereas the polyamine isolation procedure does (*16*). The utility of DNA extracted from chromosomes isolated by the polyamine procedure and purified by sorting for production of large-insert recombinant DNA libraries was demonstrated by Lebo *et al.* (*17*) who produced a partial-digest Mbo I library in EMBL-4 for human chromosome 1 and by Deaven *et al.* (*18*) who produced a partial-digest Sau 3A library in Charon 35 for the human X chromosome.

There is little information about damage induced in the DNA from sorted chromosomes at the DNA sequence level. Thus, it is uncertain whether the dye–DNA–UV light interaction causes damage that may lead to DNA sequence alterations. Living hamster cells and murine bone marrow cells stained with Ho and passed through

the UV beam from a sorter show no reduction in survival (*19, 20*). Thus, the induced damage is not so large that it cannot be repaired in mammalian cells. However, Libbus *et al.* (*21*) have reported evidence of chromosome aberrations induced in mammalian sperm stained with Ho and purified by sorting. Definitive information about DNA damage during sorting must await DNA sequence analysis of the inserts in recombinant DNA libraries made from sorted chromosomes.

Biological Studies with Sorted Chromosomes

Recombinant DNA library production. The high degree to which chromosomes can be purified by sorting makes sorter-purified DNA especially suitable for the production of recombinant DNA libraries. Several groups have now successfully used sorted chromosomes as the source of DNA for production of libraries for human chromosomes 1 (*22*), 7 (*23*), 13 (*24*), 15 (*25*), 21 (*26*), 22 (*26*), X (*27*), and Y (*28*), mouse chromosomes X (*29*) and Y (*30*), and hamster chromosomes 1 and 2 (*31*). In addition, the National Laboratory Gene Library Project, a joint effort of the Lawrence Livermore and Los Alamos National Laboratories, has produced recombinant DNA libraries for all 24 human chromosomes with sorter-purified DNA (*5, 14, 32*). All but a few of the libraries produced at Livermore were made from chromosomal DNA purified by high-speed sorting.

Charon 21A is the vector used in the National Laboratory Gene Library Project. It is an insertion vector that has two cloning sites (Hind III and Eco RI), accepts insert sizes up to 9.1 kb, and grows well in *Escherichia coli* strain LE392. The Hind III site was used in preparing the Livermore libraries and the Eco RI site was used in preparing the Los Alamos libraries. The cloning efficiency obtained in practice is 10^6 to 10^7 independent recombinants per microgram of starting chromosomal DNA. This project has now produced libraries containing five chromosomal equivalents for almost all human chromosomes. Thus, the statistical probability of finding any DNA sequence from a specific chromosome in either the Hind III or Eco RI library is about 0.90 (*33*). Experience has shown that libraries of this size can be produced from approximately 0.1 μg of sorted chromosomal DNA. This amount of DNA can be obtained from approximately 5×10^5 average-sized chromosomes. Theoretically, this number of chromosomes can be obtained in less than an hour of high-speed sorting. Of course, the total elapsed sorting times are usually longer in practice. In addition, successful cloning may require several attempts. Thus, we routinely sort several million chromosomes of each type. This usually requires 1 to 2 days.

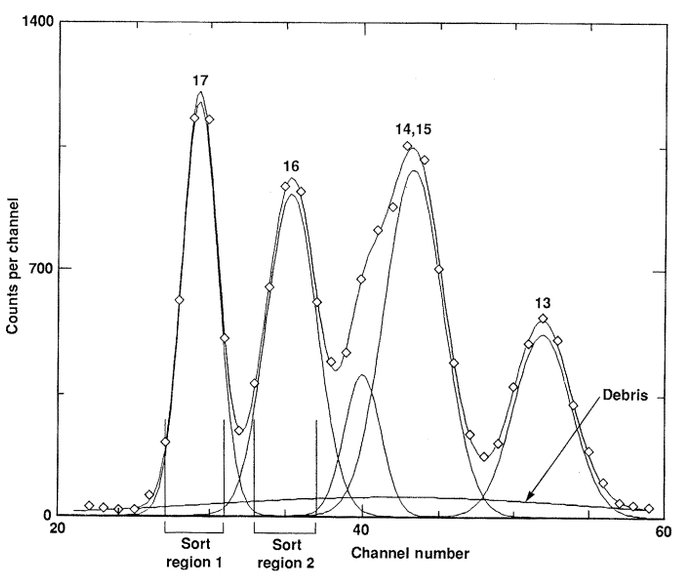

Fig. 3. Quantitative flow karyotype analysis to determine sort purity. This univariate Ho fluorescence distribution was generated from the data in the boxed region of Fig. 2b. The data are shown as points. Individual normal distributions fit to each peak are shown as solid lines. The slowly varying line underlying the peaks is a normal distribution used to approximate the debris continuum. The regions from which chromosomes 16 and 17 were sorted are indicated in the figure.

Construction of large-insert libraries may require considerably more DNA, since DNA that has been partially digested with a restriction enzyme is used. Partial digestion produces a wide range of DNA fragment sizes and only a fraction of these are in the right size range for large-insert λ vectors (approximately 20 kb) or cosmid vectors (approximately 40 kb). Thus, much of the DNA is wasted. Experiments to determine the minimum amount of DNA required for the reliable production of complete, large-insert libraries are now under way.

Gene mapping. Gene mapping is usually accomplished by analyzing the pattern of hybridization of a candidate DNA sequence to a panel of rodent-human hybrid cells carrying various human chromosomes or fragments thereof (*1*). The chromosomal location of the sequence is indicated by the finding that the sequence consistently hybridizes to DNA from hybrids containing one chromosome type. Hybrid cell panels that allow mapping to most human chromosomes are readily available. However, hybrid panels that allow subchromosomal mapping are harder to obtain. In addition, chromosome loss may occur in hybrid panels with continued culture because of karyotypic instability so that careful monitoring is required.

Chromosome sorting offers an alternative to mapping against hybrid panels (*17*). In this approach, assignment of nucleic acid sequences to chromosomes and subregions thereof can be accomplished by hybridization to sorted chromosomes. Twenty to fifty thousand chromosomes of each type are sorted onto small, well-separated regions on nitrocellulose filters. The DNA on the filters is denatured and hybridized to a radioactively labeled sample of the nucleic acid sequence to be mapped. Binding of the radioactively labeled probe to the sorted chromosomal material is detected autoradiographically. Probes present in genomic DNA in more than one copy per cell will hybridize to a few or all of the spots formed by sorted chromosomes. Unique sequence probes, however, hybridize only to the DNA from the chromosome carrying the sequence homologous to the probe DNA. Unique sequence probes can be further localized along a chromosome by sorting DNA from rearranged chromosomes that carry a portion of the chromosome to which the probe originally hybridized. Cell lines carrying a broad spectrum of cytogenetically well-characterized chromosomal rearrangements are readily available (for example, from the Human Genetic Mutant Cell Repository, Camden, New Jersey). In a recent review, Lebo noted that several dozen unique sequence probes have now been mapped by this method (*17*). High-speed sorting may contribute to the gene mapping effort by reducing the time required to produce filters containing DNA samples from each human chromosome.

Conclusion

High-speed sorting has been used to enrich each human chromosomal type. The purity of sorts exceeds 90% when efforts are made (i) to keep the instrumental resolution high, (ii) to select cell lines in which the chromosome to be sorted can be easily resolved from other chromosomes, and (iii) to minimize chromosome clumping and debris formation during chromosome isolation. Furthermore,

the DNA from isolated chromosomes has high molecular weight. Thus, sorter-purified DNA is suitable for gene mapping and for production of recombinant DNA libraries. However, additional development may be required if considerably larger amounts of DNA are required for such applications as analysis of large restriction fragments by pulsed-field gel electrophoresis (*34*) and cloning in yeast (*35*). Increases in the sort rate may come from several areas: (i) use of velocity sedimentation to enrich the chromosome of interest before sorting (*36*), (ii) production of hybrids or other cell lines in which the frequency of the chromosome of interest is high (for example, human-muntjac hybrids to which the muntjac contributes only three chromosome types), (iii) further increases in the droplet production rate in the high-speed sorter, and (iv) increased distinctness in chromosome staining so that high-purity sorting can be accomplished while objects are processed at rates of over 20,000 per second. Taken together, these developments eventually may lead to another order of magnitude increase in the rate at which chromosomes can be purified by sorting.

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Magnetoencephalography and Epilepsy Research

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Magnetoencephalography is the detection of the magnetic field distribution across the surface of the head, which is generated by a neuronal discharge within the brain. Magnetoencephalography is used in clinical epilepsy to localize the epileptogenic region prior to its surgical removal. A discussion of the instrumentation based on the superconducting quantum interference device that is used for detecting the magnetic field distribution, the analytical techniques, current research, and future directions of magnetoencephalography in epilepsy research is presented.

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MAGNETOENCEPHALOGRAPHY (MEG) IS THE MEASUREment of the extracranial magnetic fields produced by electrical currents within the brain. These spontaneous magnetic fields are about one-billionth the strength of the earth's magnetic field and are measurable only with a superconducting quantum interference device (SQUID). The electrical currents arise from ion movements produced by changes in the electrical potential of cell membranes of neurons in the brain (Fig. 1). The changes in the membrane potential and ion movements usually begin at one end of the neuron called the dendrite, and compensatory ion movements occur throughout the neuronal cell body, creating a current dipole. We will refer to the current within the cell as the source current and the return current outside the cell as the volume current. The neurons of interest in MEG are the pyramidal cells, which are oriented perpendicular to the brain surface in a thin outer layer of the brain called the cortex, where many such neurons are aligned parallel to one another. The surface of the brain has many