

# Multicolor fluorescence *in situ* hybridization for the simultaneous detection of probe sets for chromosomes 13, 18, 21, X and Y in uncultured amniotic fluid cells

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## ABSTRACT

**The most frequent aneuploidies in newborns involve the autosomes 13, 18 and 21 as well as both sex chromosomes. Fluorescence *in situ* hybridization readily allows the detection of numerical chromosomal aberrations throughout all stages of the cell cycle. Using a multicolor fluorescence *in situ* hybridization approach based on combinatorial probe labeling and digital imaging microscopy we demonstrate the simultaneous visualization of probe sets specific for chromosomes 13, 18, 21, X and Y. This approach enables one to evaluate aberrations of multiple chromosomes in a single hybridization experiment using metaphase chromosomes and interphase nuclei from a variety of cell types, including lymphocytes and amniocytes.**

## INTRODUCTION

The majority of aneuploidies in newborns involve chromosomes 13, 18, 21, X and Y. Aneuploidies involving these 5 chromosomes can account for up to 95% of all liveborn chromosomal abnormalities which are accompanied by birth defects and 67% of all chromosomal abnormalities if balanced translocations are included (1). Classical cytogenetic banding techniques have been extremely valuable in the identification, characterization and diagnosis of these chromosomal aberrations. For prenatal diagnostic applications, advances in culture methods and staining techniques have decreased the time required to carry out a full karyotype analysis to approximately seven days under the best circumstances, although turn around times of greater than two weeks are common. Thus, there is a perceived need for rapid methods for detecting the major aneuploidies. To meet this need we have developed probe sets (based primarily on cosmid contigs) specific for these 5 chromosomes, and FISH hybridization protocols specific for uncultured amniocytes. In previous studies we have shown sensitive and accurate prenatal aneuploidy detection in uncultured amniocytes using this approach (2). However, in these prior studies each probe was detected using biotin/avidin-FITC. Thus, analysis of 5 chromosomes required 5 replicate hybridizations. Simultaneous detection of multiple chromosomes would have several advantages, including increased efficiency, smaller sample requirements, and potential for analysis of a larger number of chromosomal abnormalities. Further, if

non-invasive methods of fetal sampling are successfully developed, simultaneous detection of clinically relevant chromosomes will almost certainly be necessary.

Numerous refinements have been made in non-radioactive *in situ* hybridization techniques over the last few years (3-5). Improvements in sensitivity, in particular of FISH routinely allow the detection of single-copy sequences (6-10). The development of alternative probe labeling chemistries and companion detection schemes enables the simultaneous visualization of multiple probes each labeled with a distinct hapten (11, 12). Combinatorial labeling schemes can further enhance the number of unique chromosomal loci that can be enumerated within a single multicolor hybridization experiment while minimizing the number of different probe haptenization and detection systems required (13, 14). In addition, the recent introduction of fluorophore-labeled nucleotides permits the direct production of fluorescent DNA probes which circumvents immunological detection procedures used in conventional indirect detection formats (15). It was also shown that interphase cytogenetics allows for chromosome enumeration during all phases of the cell cycle (16-18).

Digital imaging devices, like the CCD camera, are available for rapid image acquisition and such devices, controlled by appropriate computer hardware and software, improve the overall detection sensitivity and offer sophisticated methods for multiple probe visualization and analysis (14).

In the present paper we have combined two of the recent improvements in fluorescence *in situ* hybridization to extend the clinical application of FISH analysis. We used combinatorial probe haptenization and digital imaging with a cooled CCD camera in conjunction with custom computer software for the simultaneous visualization of specific probe sets for chromosomes 13, 18, 21, X and Y. This protocol can be applied to the analysis of both metaphase chromosomes and interphase nuclei, including the condensed chromatin characteristic of uncultured amniotic fluid cells.

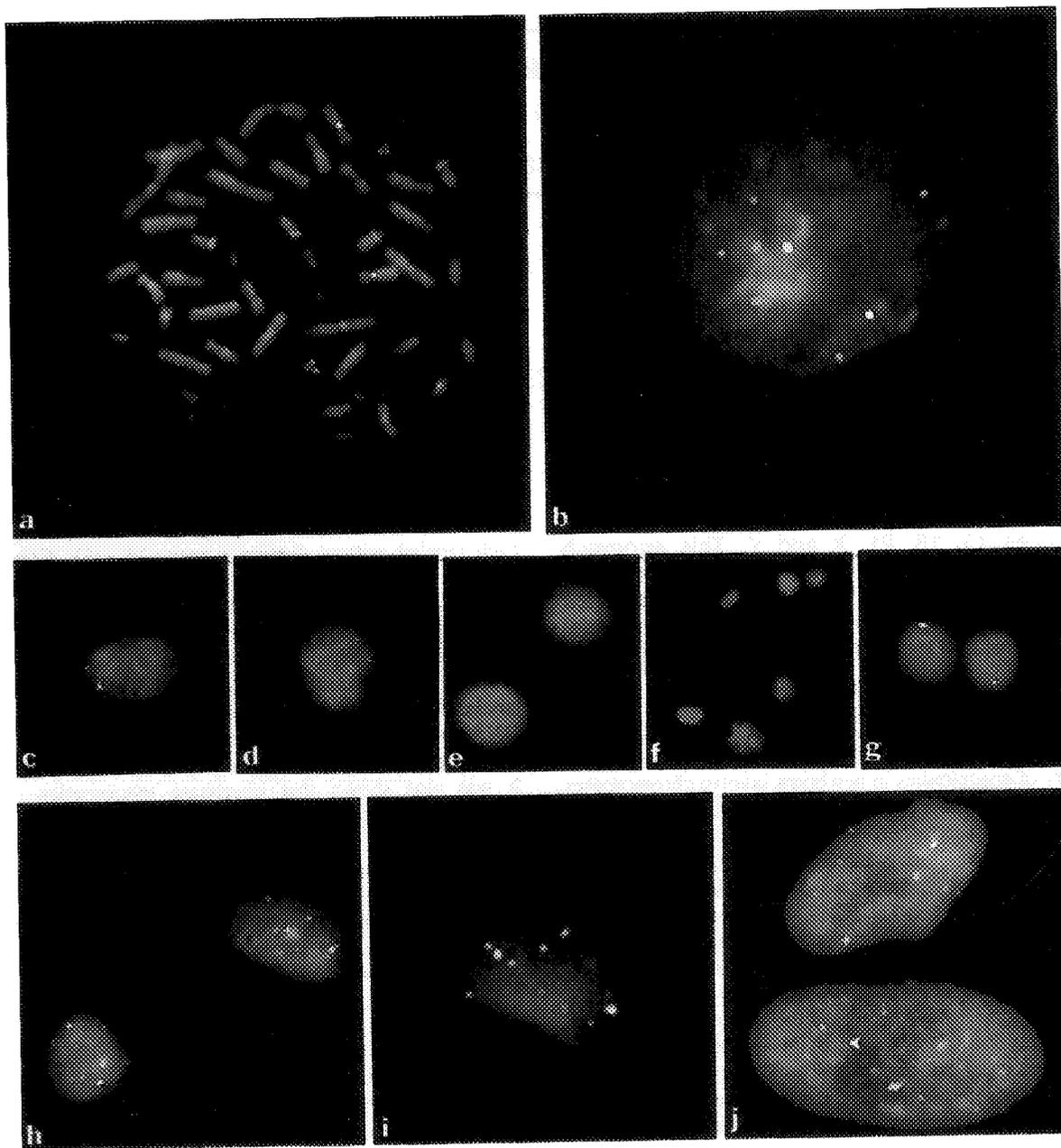
## RESULTS

In order to simultaneously assess the ploidy of the five most important chromosomes with respect to prenatal diagnosis for aneuploidy syndromes, we labeled chromosome specific probe sets for chromosomes 13, 18, 21, X and Y singly and in

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combination. Probes which are combinatorially labeled (e.g. with biotin-11-dUTP and digoxigenin-11-dUTP) and are visualized with a mixture of different fluorescent dyes (e.g. fluorescein and rhodamine) will appear at identical positions in images acquired

with specific filter sets for the dyes employed. Thus, combinatorially labeled probes can be distinguished from singly labeled probes, thereby increasing the number of simultaneously detectable targets (13–14). The probes we used for the detection

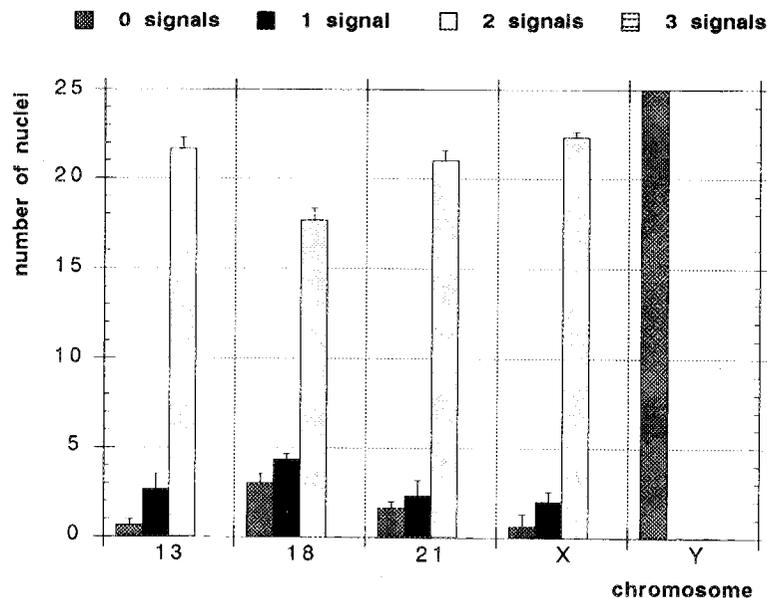


**Figure 1.** **a:** Simultaneous hybridization of probe sets for chromosomes 13, 18, 21, X and Y to a metaphase spread from a normal female. The hybridization signal for chromosome 13 is pseudocolored in red, 18 in violet, 21 in green, and the X chromosome in yellow. Note that the probe for the autosomes reveal signals on both chromatids, whereas the X chromosome specific probe displays a single spot due to the large size of the repeat sequence. **b:** Hybridization of the same probe set to an interphase nuclei derived from peripheral blood lymphocytes from a healthy male. Two signals for each autosome can be observed. The pseudocoloring scheme is as in 1; the Y specific repeat is pseudocolored white. For each of the sex-chromosome specific probes, one signal is observed. **c–g.** Single hybridization experiments with biotinylated probe sets for chromosomes 13 (1c), 18 (1d), 21 (1e), X (1f) and Y (1g) on uncultured amniotic fluid cells. In the majority of the cells, two signals were detected for the X chromosome and all autosomal probes. The Y specific probe gave one signal, indicating 47, XXY. **h.** Hybridization of the combined probe sets to the same slide preparation as in 1c–g. The pseudocoloring is the same as in Figure 1b. In addition to two X chromosome specific signals (yellow), one Y specific hybridization (white) is detected, again confirming a 47, XXY karyotype. **i.** Hybridization of the combined probe sets to uncultured amniotic fluid cells. Two signals for chromosomes 13 and 21 are seen (red and green, respectively), whereas three signals for chromosome 18 (pink) are counted, indicating trisomy for this autosome. In addition, two X chromosome and one Y chromosome specific signals are visible. The karyotype is 48, XXY, +18. **j.** Example of the hybridization of the combined probe sets to cultured amniotic fluid cells. Chromosomes 13 and 18 specific probes reveal two signals each. Both sex chromosomes reveal one signal each. The chromosome 21 specific probe sets (green), however, display three signals, indicating trisomy for chromosome 21. Note that in the cultured cells, the signals sometimes appear as doublets, indicating DNA replication at the hybridization site.

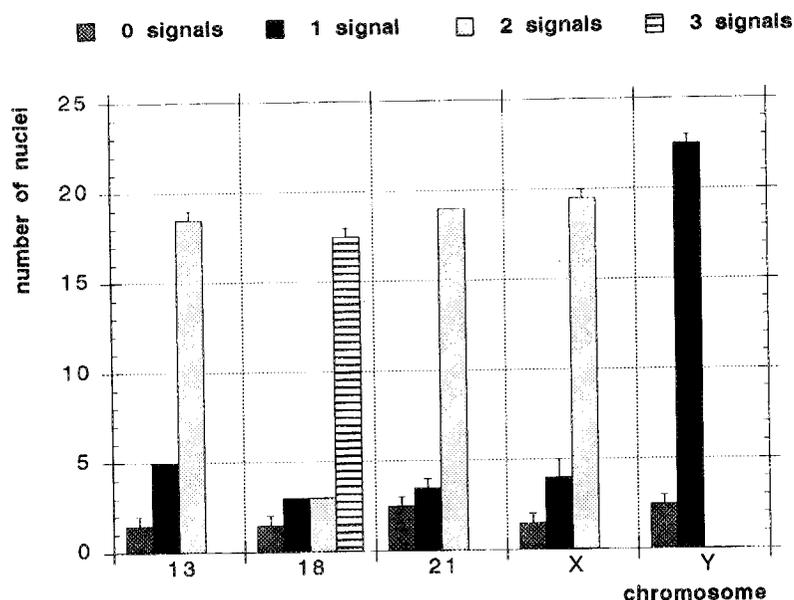
of each of the autosomes are cosmid contigs (2), which result in a reliably strong and well defined signal. The chromosome 13 probe set was labeled with digoxigenin, the cosmids for chromosome 18 with biotin and the chromosome 21 probe with DNP, all conjugated to dUTP. In some experiments we used fluorescein-12-dUTP instead of DNP-dUTP to circumvent the longer indirect immunological detection procedure. The repetitive probes for the sex chromosomes (see Materials and Methods) were labeled with a combination of two reporter molecules: the cloned X chromosome specific repeat with biotin and digoxigenin, the Y probe with digoxigenin and DNP (alternatively with fluorescein-12-dUTP). The biotinylated probe sequences were detected with Ultralite 680™ conjugated to streptavidin (infrared fluorescence), digoxigenin labeled probes were detected with anti-digoxigenin coupled with rhodamine (red fluorescence) while DNP labeled sequences were visualized using a rat antibody against DNP and a secondary fluorescein conjugated antibody against rat IgG (green fluorescence). When FITC-dUTP was used instead of DNP-dUTP the fluorescent hybridization signal was imaged directly after the posthybridization washes. According to the experimental design, we expected four distinguishable hybridization signals on female metaphases and interphase nuclei, and five on male specimens. Separate source images for each fluorophore were acquired using a CCD camera and pseudocolored to facilitate the easy detection and discrimination of the probe sets. Figure 1a demonstrates the hybridization of the combined probe sets on a normal female metaphase. The chromosome 13 probe is pseudocolored in red, chromosome 18 sequences in pink, chromosome 21 in green, and X with yellow. The four tagged chromosomes can be unambiguously identified. Figure 1b shows the hybridization of a male interphase nucleus. Again chromosome 13 sequences are colored in red, 18 in pink, 21 in green, the X-chromosome in yellow, whereas the Y-chromosome specific repeat clone is displayed in white. Using FITC-dUTP instead of DNP-dUTP, two additional peripheral

blood lymphocyte specimens were successfully hybridized with the combined probe sets. These results clearly validate the chromosomal specificity of the probe set to be used for the enumeration of the five chromosomes within interphase nuclei.

In order to evaluate the utility of this technique for prenatal diagnostic applications, the probe set, both singly and combined, was hybridized to four different uncultured amniotic samples. Initially, the FISH results obtained using individual members of the probe set were compared to those with the combined probe set. Six replicate slides were prepared from one of the amniotic fluid samples. Figure 1c–g shows the hybridization results of independent experiments using only one probe per *in situ* hybridization. Two green fluorescent spots occur in the images for the autosomal probes, respectively; here the nuclei from uncultured amniocytes were counterstained with propidium iodide. Hybridization of the Y probe reveals one signal, whereas the hybridization of the X repeat results in two signals, indicating a karyotype of 47, XXY. The sixth replicate was then hybridized with the combined and combinatorially labeled probe sets (Figure 1h). The karyotype of this amniotic sample could be assessed as 47, XXY in the single *in situ* hybridization experiment. Results obtained by the simultaneous multichromosome analysis were entirely consistent with the results from the five independent hybridizations. Figure 1i shows another example of a chromosomally abnormal amniotic cell nuclei. In this experiment the combined and combinatorially labeled probe set revealed two signals for chromosomes 13 (pseudocolored in orange) and 21 (green), respectively, indicating a normal ploidy for these chromosomes. Chromosomes 18 (pink), however, revealed three signals, indicating triploidy. In addition to the autosomal aberration, two X chromosomes were detected (yellow) along with one Y specific signal (white), revealing a 48, XXY + 18 karyotype. Two additional amniotic fluid samples revealed a normal ploidy for the chromosomes 13, 18 and 21, and a male and a female specific hybridization pattern with the probes for



**Figure 2.** Detection efficiency on peripheral blood lymphocytes. In order to determine the detection efficiency of the combined probe sets on peripheral blood lymphocytes, three different hybridization experiments were evaluated. The chromosome specimens were derived from two different preparations. 25 nuclei were counted, respectively. For each probe, the average value is given.



**Figure 3.** Detection efficiency on uncultured amniotic fluid cells. Two separate hybridizations to the same specimen were evaluated. 25 nuclei were counted, respectively. For each probe, the average value is given.

chromosomes X and Y. The ploidy of the chromosomes analyzed in these experiments was confirmed later by conventional cytogenetic analysis.

The combined probe sets were also hybridized to short term cultures of amniocytes, which had been grown on a coverslip. The enumeration for the probed chromosomes could be performed reproducibly and unambiguously after one hybridization experiment. Two different samples were investigated. The first showed a normal ploidy for the autosomes (13, 18 and 21) and revealed two X specific hybridization signals (data not shown). The other cultured amniotic fluid samples (Figure 1j) gave three green pseudocolored spots in the majority of the nuclei indicating trisomy for chromosome 21, whereas signals for chromosomes 13, 18, X and Y were as expected for a normal male. This assessment was then confirmed by single probe *in situ* hybridizations and classical banding techniques (data not shown).

The hybridization efficiency with each of the five chromosome specific probe sets was evaluated by enumerating the number of hybridization signals present within 25 metaphase spreads or interphase nuclei from either peripheral blood lymphocytes, cultured amniocytes or uncultured amniotic fluid cells. Specific examples are given in Figures 2 and 3. The detection efficiency observed with metaphase spreads, methanol/acetic acid fixed lymphocyte nuclei and cultured amniotic fluid cells was comparable (data not shown). Three different hybridizations were counted. In normal female lymphocytes (Figure 2), over 80% of the cells examined exhibited the expected diploid number of chromosome 13, 21 and X signals, however, only 68% of the cells gave two signals for chromosome 18. The chromosome probe, which had the smallest contig length, was detected with the Ultralite 680™ infrared fluorophore, and gave the weakest emission of all the fluorescent detections. As a result, the detection efficiency was decreased. We have recently noted (unpublished results), that another infrared fluorophore, Cy5 (Jackson ImmunoResearch Laboratories, West Grove, PA) gives much

stronger signals than Ultralite 680™. The detection efficiency of chromosome 18 using the Cy5 fluorophore is similar to the other chromosomes (> 80%). The overall detection efficiency was slightly decreased when uncultured amniotic fluid cells were enumerated. Here, 25 nuclei were enumerated from two independent hybridizations to the same amniotic fluid sample. Nevertheless, when examining the specimen with the 48, XXY, trisomy 18 karyotype (Figure 3), 68% and 76% of the cells exhibited aneuploidy of chromosomes 18 and X, respectively. Another general observation was that the detection efficiency of the autosomal chromosomes was slightly less than that of the gonosomes.

## DISCUSSION

We have presented a method for the simultaneous analysis of chromosomes 13, 18, 21, X and Y on metaphase and interphase chromosomes and applied it to the analysis of short term cultures of human peripheral blood lymphocytes and, of greater prenatal diagnostic importance, to uncultured amniocytes. Since the number of suitable probe labeling and fluorescence detection systems still remains relatively low, we used a combinatorial labeling strategy to distinguish the five probe sets. Hybridization signals were recorded by digital imaging microscopy, and analyzed using custom computer software which enabled the assignment of each detectable hybridization event to a specific probe set. A composite image containing all of the chromosome-specific hybridization signals, each uniquely identified by pseudocoloring, was generated from the set of individual fluorescence images. As the number of probe labeling methods and companion detection schemes increases, we anticipate that co-hybridization of multiple probe sets, such as the type used in this study, can be distinguished even without combinatorial probe labeling. However, combinatorial probe labeling schemes offer the opportunity to expand the number of unique loci that can be analyzed simultaneously well beyond the number of probe

labeling/detection systems. This is attractive for prenatal applications since the most common chromosomal defects, both aneuploidies and translocations, could be assessed simultaneously in a single hybridization.

We have shown that fluorescein-12-dUTP can be readily used in the labeling format described here. Direct fluorophore labeling of probes is advantageous for diagnostic procedures since it obviates the immunological detection steps which are time consuming and sometimes troublesome, i.e. by increasing non-specific fluorescence background. The major limitations of directly labeled probes are (1) their decreased sensitivity of detection compared to indirectly labeled probes and (2) different fluorescent nucleotides for labeling are only now being commercialized. Since the reduced sensitivity, about 10–15% that of indirectly labeled probes, can be compensated for in large part by the use of sensitive light gathering devices such as the CCD camera, it is clear that digital imaging microscopy will facilitate future applications of multicolor FISH.

The detection efficiency on metaphase spreads, interphase nuclei from peripheral blood lymphocytes and cultured amniotic fluid cells was in the range of 68% to 92%, with slight variations observed depending on the probe set used and the labeling and detection format (Figures 2 and 3). For uncultured amniotic fluid cells, the detection efficiency was in the range of 68% to 90%, again depending on the probe and the labeling schemes. The most difficult fluorophore to image was the infrared dye Ultralite 680™, even though it was used in the most efficient haptenization format (biotin/avidin system). However, those conditions will improve as additional filter sets and alternate dyes emitting in the infrared spectrum, such as Cy5, become commercially available. Based on the comparison of the detection efficiency we have now extended the chromosome 18 contig to 109 kb, to obtain equivalent detection efficiencies across all the autosomal probe sets.

All of the probes used in this study generated strong and distinct hybridization signals; each autosomal probe set was comprised of a cosmid contig of at least 50 kb while each of the sex chromosome specific probes identified a centromeric repeat on the respective chromosome.

Alternative probe sets have been used for prenatal aneuploidy detection, namely chromosome specific centromere repeat clones (19) and composite probe sets for chromosome painting (20–23). Centromere repeat probes are well suited for interphase analysis of aneuploidies, because of their brilliant hybridization signal capabilities, but they present some problems in clinical utilization. Chromosome specificity of the repetitive probes may be sensitive to the hybridization conditions while the signal size is affected by pericentromeric heteromorphisms. With respect to the prenatal applications reported here, chromosome-specific cloned centromeric repeats do not exist for either chromosomes 13 or 21 although a single centromeric repeat probe does exist which will hybridize to both chromosomes 13 and 21 (24). Finally, the centromeric location of this probe type does not allow for the identification of Robertsonian translocations, thus limiting the detection of Down's syndrome. Composite probe sets for chromosome painting yield rather extended and diffuse hybridization signals in interphase nuclei, often producing overlapping chromosome domains. Overlap of the hybridization domains is exacerbated by the nucleolar organization of the short arms of the D and G group, resulting in close proximity of the probes. In fact, Kuo and coworkers (23) reported low percentages, 10–50%, of trisomic amniocytes showing three

Table 1. Combinatorial labelling scheme.

nucleotide final conc. in $\mu$ M	chromosome				
	13	18	21	X	Y
biotin-11-dUTP		50		21	
digoxigenin-11-dUTP	40			30	20
DNP-11-dUTP			40		30
dTTP	10	5	10	5	5
dATP, dCTP, dGTP	50	50	50	50	50

hybridization signals when composite probe sets of high complexity were employed. The hybridization efficiency decreased further when uncultured amniocytes were analyzed. This problem is compounded when several composite probe sets are co-hybridized for simultaneous enumeration. Although painting probes will detect large translocations, responsible for some cases of Down's syndrome, subtle translocations are beyond the detection sensitivity. The spatial resolution of the hybridization signals generated by the autosomal probes used in the present study (cosmid contigs) facilitates simultaneous enumeration of multiple chromosomes, and the strong and highly focal size of the hybridization signal is more amenable to automated image analysis for chromosome enumeration. Automation would reduce personnel time and therefore cost, while eliminating the subjective character of manual evaluation.

The development of semi-automated FISH analysis systems is in its infancy. However, many of the components (CCD camera, image analysis software, color monitor, personal computer with hard drive) needed for such a device exist already as individual entities. More rapid analysis will be possible by further automating the system. Improvements could be achieved using an automatic slide loader, slide scanning drive and image analysis software which allows for nuclei and hybridization signal recognition. As the requirement and use of such systems expands the manufacturing cost and retail price should decrease which will make the technology more available to interested institutions and individual users.

The combination of multicolor FISH combinatorial probe labeling, digital imaging microscopy, and defined probe sets and optimized clinical sample handling methods can extend the diagnostic limits of molecular cytogenetics. When utilized as described in this report, it is possible to carry out a rapid (<48 hours) and simultaneous enumeration of the five most common aneuploidies of newborns using 1 ml or less of amniotic fluid. These results demonstrate the expanding capability of molecular cytogenetics and promote the accelerated application of these molecular tools to other challenging diagnostic problems.

## MATERIALS AND METHODS

**Probe DNA.** The probes used in this study consisted of cosmid contigs for the autosomes 13, 18 and 21 and cloned repeats specific for chromosomes X and Y. The characterization of probes for chromosomes 13, 18, 21 and X has been described (2). The relationship between the repetitive element present in the

chromosome X-specific cosmid used in these studies and the alpha satellite sequences located at the X centromere (25–27) is unknown. The cloned Y repeat is pDP96 (provided by Dr David Page, Whitehead Institute Biomedical Research, Cambridge, MA), a subclone of the alpha satellite repeat present in the cosmid Y97 (28). Briefly, the initial starting clones for each autosomal contig were D13S6, MBP and D21S71 for chromosomes 13, 18 and 21, respectively. The cosmid contigs were expanded as necessary to achieve a total sequence complexity of at least 50 kb or greater; the chromosome 21 probe set is a four cosmid contig containing 80 kb of non-overlapping DNA, the chromosome 18 probe set is a two cosmid contig of 50 kb of non-overlapping DNA, while the chromosome 13 probe set is a three cosmid contig containing about 65 kb of non-overlapping DNA.

**Samples.** Peripheral blood lymphocytes from three different individuals (male and female) were expanded in short term cultures using standard cytogenetic practices. Six different amniotic fluid samples were analyzed by both FISH and conventional cytogenetic analysis. Two of the six samples were cultured prior to FISH while the remaining four were analyzed without culturing as described below. Slides containing interphase and/or metaphase chromosomes from lymphocytes or amniocytes (cultured or uncultured) were hybridized simultaneously with the combined and combinatorially labeled probe set. In addition, six replicate slides were made from one of the uncultured amniotic fluid samples. For comparative purposes, each of the five replicates was hybridized simultaneously with the combined and combinatorially labeled probe set. For cytogenetic analysis, all six amniotic fluid samples were cultured to allow for a complete chromosome enumeration.

**Slide preparation.** Metaphase spreads and interphase nuclei were prepared from peripheral blood lymphocytes according to standard techniques. The amniocytes were prepared as described (2). Briefly, uncultured amniocytes in PBS were dispensed onto 3-aminopropyltriethoxysilane-coated slides at 37°C at a concentration of approximately  $5 \times 10^3$  cells/slide. Subsequently, the cells were processed *in situ* by the addition of KCl to 50 mM and incubated at 37°C for 15–30 min. The hypotonic solution was carefully decanted and replaced by 100  $\mu$ l of 30% 3:1 fix (methanol:acetic acid) and 70% 75 mM KCl for 5 min at room temperature. This solution was carefully decanted and fresh 3:1 fix was dropped onto the slide from a height of 60 cm. Excess fix was decanted and the slide were dried for 5 min at 60°C. Conditions which generated nuclei capable of supporting *in situ* nick translation (29) generally resulted in good hybridization results.

**Probe labeling.** The chromosome specific probes were labeled by nick translation. The following reporter molecules were used: biotin-11-dUTP (Sigma Chemical Co., St Louis, MO), digoxigenin-11-dUTP (Boehringer Mannheim, Indianapolis, IN), DNP-11-dUTP (Novagen, Madison, WI) and fluorescein-12-dUTP (Boehringer Mannheim, Indianapolis, IN). The combinations and final concentrations of the nucleotide analogs used are given in Table 1. When fluorescein-12-dUTP was used as an alternative for DNP-dUTP the final concentrations were maintained.

**In situ hybridization and detection.** 60 ng of each cosmid contig or cloned repeat was precipitated simultaneously in the presence of 15  $\mu$ g human competitor DNA, 5  $\mu$ g of salmon sperm DNA and 5  $\mu$ g yeast tRNA, and resuspended in 10  $\mu$ l of 50% formamide, 2 $\times$ SSC and 10% dextran sulfate. The probe DNA was denatured at 75°C, 5 min, and the repetitive sequences allowed to preanneal for 60 min at 37°C. Metaphase spreads, interphase nuclei and amniocytes were denatured in 70% formamide, 2 $\times$ SSC at 80°C for 2 min (3 min for amniocytes), and dehydrated through an ethanol series (70, 90, 100%) for 5 min each. The preannealed probe solution was applied to the slide, overlaid with a coverslip and sealed with rubber cement. After overnight incubation at 37°C, posthybridization washes and a blocking step (30 min, 37°C, in 4 $\times$ SSC containing 3% bovine serum albumin) the biotinylated probe sequences were detected with the infrared dye Ultralite 680™, conjugated to streptavidin (Ultradiagnostics Corp., Seattle, WA). Digoxigenin labeled sequences were detected with an anti-digoxigenin Fab fragment, conjugated to rhodamine (Boehringer Mannheim, Indianapolis, IN). DNP labeled sequences were detected indirectly with a monoclonal rat anti DNP antibody (Novagen, Madison, WI) and a secondary step with goat anti-rat IgG coupled with fluorescein (Sigma Chemical Co., St Louis, MO). When probes were labeled with fluorescein-dUTP, no immunocytological detection was necessary for the visualization of the green fluorescence. DNA was counterstained with DAPI. The slides were embedded in DABCO to reduce fluorescence photobleaching.

**Digital imaging microscopy.** Images were taken with a Zeiss epifluorescence microscope equipped with a cooled CCD camera (Photometrics PM512, Tucson, AZ), which was controlled by an Apple Macintosh computer. Gray scale images

were captured with filter sets for DAPI, fluorescein, rhodamine and Ultralite™ 680. Gray scale images were pseudocolored and merged using computer software developed by Timothy Rand. This software is available through the Office of Cooperative Research, Yale University, 246 Church St, New Haven, CT 06510. Photographs were taken with Kodak 100 HC color slide films. Details of the image acquisition and the image processing protocols are described elsewhere (14).

**Detection efficiency.** The detection efficiency was determined by enumerating the number of hybridization signals (0, 1, 2 and 3) present within 25 metaphase chromosomes or interphase nuclei from either peripheral blood lymphocytes, cultured amniocytes or uncultured amniotic fluid cells. Efficiencies were determined for metaphase chromosomes of peripheral blood lymphocytes, interphase nuclei of uncultured amniocytes and interphase nuclei of cultured amniocytes. The results using nuclei from cultured peripheral blood lymphocytes and cultured amniocytes are graphically presented while the others are described in the text.

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## ABBREVIATIONS

FISH, fluorescence *in situ* hybridization; FITC; fluorescein isothiocyanate; CCD, charge coupled device; PBS, phosphate buffered saline; dUTP, deoxyuridine-triphosphate; DNP, dinitrophenol; SSC, standard saline citrate; DAPI, 4',6'-diamidino-2-phenylindole; DABCO, 1,4-diazabicyclo(2,2,2)-octane.

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