

Running Headline: BAC POOLS FOR FISH

DNA Probe Pooling for Rapid Delineation of Chromosomal Breakpoints*

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*Parts of this work have been presented at HCS2005, Nordwijkerhout, The Netherlands, April 27-30, 2005.

Abstract

Structural chromosome aberrations are hallmarks of many human genetic diseases. The precise mapping of translocation breakpoints in tumors is important for identification of genes with altered levels of expression, prediction of tumor progression, therapy response, or length of disease-free survival as well as the preparation of probes for detection of tumor cells in peripheral blood. Similarly, *in vitro* fertilization (IVF) and pre-implantation genetic diagnosis (PGD) for carriers of balanced, reciprocal translocations benefit from accurate breakpoint maps in the preparation of patient-specific DNA probes followed by a selection of normal or balanced oocytes or embryos. We expedited the process of breakpoint mapping and preparation of case-specific probes by utilizing physically mapped bacterial artificial chromosome (BAC) clones. Historically, breakpoint mapping is based on the definition of the smallest interval between proximal and distal probes. Thus, many of the DNA probes prepared for multi-clone and multi-color mapping experiments do not generate additional information. Our pooling protocol described here with examples from thyroid cancer research and PGD accelerates the delineation of translocation breakpoints without sacrificing resolution. The turnaround time from clone selection to mapping results using tumor or IVF patient samples can be as short as three to four days.

Key words: translocation, chromosome aberration, cytogenetics, thyroid cancer, IVF, PGD, fluorescence in situ hybridization (FISH), bacterial artificial chromosome (BAC), DNA probes.

Introduction

Congenital anomalies including balanced and Robertsonian translocations and chromosomal inversions occur in as much as 1.4% of the general population, and were observed at even higher rates among infertile couples and patients with recurrent abortions (Subrt 1980; Peng et al. 2006). For example, Stern and colleagues reported balanced translocations in 0.6% of all infertile couples, 3.2% of couples that failed over 10 IVF cycles, and 9.2% among infertile couples experiencing three or more consecutive first-trimester abortions (Stern et al. 1999).

The most commonly observed consequence of balanced reciprocal translocations in carriers without clinical disease symptoms is an increased fraction of germ cells with an abnormal chromosome complement. This has been attributed to disturbed homologue pairing during meiosis or precocious chromatid separation (Srb et al. 1965; Kalousek et al. 2000). When translocations alter the expression of genes relevant to early human development, disturbed embryogenesis may also lead to primary infertility or repeated miscarriages (Subrt 1980; Munné 2002).

During the course of IVF, PGD can now be offered to affected couples as an alternative to prenatal diagnosis and medically-indicated termination of pregnancies with chromosomally-unbalanced fetuses. If there is a sufficient number of fertilized normal embryos available for transfer, PGD also provides an efficient option to put an end to a familial disease (Munné 2002). However, the greatest benefit of PGD is the reduction of spontaneous abortions (Verlinsky et al. 2004). On the other hand, the observed increases in pregnancy rates after PGD among couples carrying non-Robertsonian translocations lag behind expectations (Munné et al. 2000; Munné 2002).

Precise localization of chromosomal breakpoints is also an important milestone in the identification of tumor-related genes and preparation of tumor-specific DNA probes. Work in our laboratories focuses on the activation of proto-oncogenes among them receptor-type tyrosine kinase (rtk) genes and their aberrant pattern of expression in tumors of the thyroid gland. In the papillary type of thyroid cancer (PTC), for example, the activation of the rtk genes *ret* or NTRK-1 is often the consequence of a chromosomal translocation in which the 3'-end of the gene containing the catalytic domain is fused in frame to the 5'-end of a constitutively expressed gene (Hermann et al. 1991; Pierotti et al. 1992; Jossart et al. 1995,1996; Beimfohr et al. 1999; Greco et al. 2004). To determine translocation breakpoints, the conventional cytogenetic methods, i.e., chromosome banding procedures, are challenged when delineating subtle chromosome rearrangements, particularly for *de novo* abnormalities in newborns. Fortunately, fluorescence *in situ* hybridization (FISH), a technique for the analysis of chromosomal aberrations, is sensitive and specific enough to elaborate these objectives. To meet the needs of PGD or tumor research, an increase in the number of recombinant DNA libraries such as FISH-mapped, large insert clones now allow almost every laboratory to prepare individualized FISH probes in-house and circumvent the limitations of commercial probe availability (Stumm et al. 2006). Initially, we used yeast artificial chromosome (YAC) probes spaced more-or-less evenly in 8-15 megabasepair (Mbp) intervals along the target chromosomes (Cassel et al. 1997; Fung et al. 1998, 2001; Liehr et al. 2002; Zitzelsberger et al. 2002). The target interval was narrowed through repeated cycles of clone selection and hybridizations until a clone had been found that spanned the breakpoint (Fung et al. 1998). Although this

proved to be a straightforward approach for breakpoint mapping in some patients (Cassel et al. 1997; Munné et al. 1998; Weier et al. 1999), the precise determination of breakpoint locations often became a time consuming process plagued by YAC clone chimerisms (Selleri et al. 1992; Shizuya et al. 1992) or errors in the published physical maps (Fung et al. 1999).

The BAC clones, on the other hand, show a much reduced fraction of chimeric clones and have been used to maintain DNA fragments of several hundred kb (Shizuya et al. 1992; Thorsen et al. 2005). The popularity of BACs as probes in cytogenetic analyses or for generation of high-resolution physical maps and preparation of DNA sequencing templates can be attributed to their relative stability, ease of handling and large DNA insert-to-vector size ratio (Kim et al. 1996; Osoegawa et al. 2001; Liehr et al. 2002; Carreira et al. 2007).

Our present study took advantage of a further advantage of BAC clones over YACs for breakpoint mapping: once retrieved from the -80°C freezer, BAC clones grow much faster than YACs. This should reduce the length of each mapping cycle compared to the use of YACs, thus accelerating the *in situ* delineation of chromosomal translocation breakpoints and preparation of breakpoint-specific DNA probes (Fung et al. 1998).

Furthermore, we decided to use sets of overlapping BAC clones forming 'contigs' or 'pools' instead of single recombinant clones, since this minimizes the rates of so-called 'FISH failures' (Munné et al. 1994; D'Alton et al. 1997; Plastira et al. 2006) or uninformative results (Sampson et al. 2004). The present article describes the strengths of BAC clone pooling strategies expediting probe preparation for PGD and the identification of candidate regions for gene expression studies in PTC tumors.

Materials and Methods

Preparation of metaphase cells

Metaphase spreads were made from short-term cultures of an anonymous normal male donor's white blood cells as described (Fung et al. 2002). Lymphocytes from a PGD patient (T-0512) were grown for 72 h in RPMI 1640 (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin and 2% phytohaemagglutinin (PHA, HA-15; Abbott Molecular Inc., Des Plaines, IL). Cells were blocked in mitosis during a 1 h treatment with colcemid (0.12 µg/ml, Invitrogen), harvested, and incubated in 75 mM KCl for 15 min at 37°C (Bayani and Squire 2004). The cells were then spun down, and approximately 10^7 cells were incubated in 5 ml of freshly prepared fixative (acetic acid/methanol, 1:3 (vol.:vol.)). The fixation step was repeated twice, before the cells were dropped on ethanol-cleaned microscope slides. Slides were aged for a minimum of 1 week in ambient air at 20°C, then sealed in plastic bags and stored at -20 °C until used.

For breakpoint delineation in cancer cells, we used the childhood papillary thyroid cancer cell line S48TK6 (Zitzelsberger et al. 1999; Weier et al. 2006). This tumorigenic cell line is a subclone of a primary culture of cancer cells (S48TK) prepared from a thyroid cancer that arose following the 1986 nuclear accident in Chernobyl, Ukraine. Since the line S48TK6 continued to undergo karyotype changes, the cells were once more cloned using limiting dilution. This gave rise to the tumorigenic cell line S48TK6A4. Colcemid-arrested, tumor cell metaphase spreads were prepared from cell lines S48TK6 and its derivative, S48TK6A4, as described (Zitzelsberger et al. 1999). Gross chromosomal changes in the S48TK6 metaphases were characterized by means of G-

banding, comparative genomic hybridization (CGH) and Spectral Karyotyping (SKY) (Zitzelsberger et al. 1999; Weier et al. 2006).

Cytogenetic analysis of PGD patient metaphase cells

Prior to our study, cells from the 31-year old female IVF patient T-0512 who was suspected to carry a translocation were analyzed by G-banding to define the chromosomes involved in the translocation and the approximate location of the breakpoints.

The karyotype information 46,XX, t(4;13)(q21.3;q21.2) suggesting a balanced, reciprocal t(4;13)(q21.3;q21.2) was available, before probe preparation commenced.

Preparation of DNA probes and DNA labeling

The general scheme for the selection and optimization of breakpoint-specific probes for PGD has been described previously (Cassel et al. 1997). Using information in publicly available databases (<http://genome.ucsc.edu/> and <http://www.ncbi.nlm.nih.gov/gquery/gquery.fcgi>), we selected BAC clones that map to the estimated breakpoint interval as well as to adjacent chromosome bands. The BACs were provided by the Human Genome Center, California Institute of Technology, Pasadena, CA. For initial mapping of clones, BAC DNA was isolated using an alkaline lysis DNA extraction protocol (Birnboim and Doly 1979; Weier et al. 1995). The isolation of DNA from individual clones was done from 10 ml bacterial cultures grown overnight in Luria-Bertani (LB) medium (Sambrook et al. 1989) containing 12.5 µg/ml chloramphenicol (Sigma, St. Louis, MO)(Lu et al. 2008). Briefly, cell pellets resuspended in 10 ml of phosphate-buffered saline (PBS) were treated with 50µg/ml lysozyme (Sigma, stock is 50mg/ml in 10 mM Tris, pH 7.5) and lysed in sodium hydroxide (0.2 N NaOH, 1 % SDS). After neutralization by addition of 3 M NaOAc and pelleting of bacterial DNA, the BAC DNA was

precipitated in isopropanol, washed once in 70% cold ethanol, and resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). Finally, the DNA was extracted once with phenol:chloroform, precipitated with isopropanol, and resuspended in 20-40 μ l sterile water. The DNA concentrations were determined by staining with Hoechst 33342 and fluorometry using a TKO100 fluorometer (Hoefer, San Francisco, CA)(Weier et al. 1995). The BAC-derived probe DNA (typically 1-2 μ l of DNA in a 10 μ l reaction) was labeled via random priming following the instruction of the kit manufacturer (BioPrime Kit, Invitrogen, San Diego, CA) (Jossart et al. 1996, Fung et al. 2000). For non-isotopic, indirect labeling biotin-14-dCTP or digoxigenin-11-dUTP was incorporated into the DNA. The preparation of DNA probes for BAC pools was performed in essentially the same way with the following modification: individual BACs were grown overnight in 10 ml of LB broth containing 20 μ g/ml chloramphenicol. Then, 5 ml of each culture was combined in the desired pool, the cells were spun down, resuspended in 10 ml PBS containing 50 μ g/ml lysozyme and DNA was isolated and labeled as described above. Probes for SKY analyses were purchased from Applied Spectral Imaging (Carlsbad, CA) and used according to the manufacturer's instructions (Zitzelsberger et al. 1999, 2002).

In situ hybridization

For FISH, 1 μ l of each probe, 1 μ l of human COT-1™ DNA (1 mg/ml, Invitrogen), 1 μ l of salmon sperm DNA (10 mg/ml, Invitrogen), and 7 μ l of the hybridization master mix (78.6% formamide (Invitrogen), 14.3% dextran sulfate in 1.43x SSC, pH 7.0 (20x SSC is 3 M sodium chloride, 300 mM tri-sodium citrate)(Lu et al. 2008) were thoroughly mixed and denatured at 76 °C for 10 min. Next, the hybridization mix was incubated at 37 °C for 30 min allowing the COT-1™ DNA to pre-anneal with the probes. In parallel, the

slides were denatured for 4 min at 76 °C in 70% formamide/2x SSC, pH 7.0, dehydrated in 70%, 85%, and 100% ethanol for 2 min each step, and allowed to air dry. The hybridization mix was then pipetted onto the slides, covered with a 22x22 mm² coverslip and sealed with rubber cement. The slides were incubated overnight in a moisture chamber at 37 °C. After removing the rubber cement, the slides were immersed in 2x SSC at 21°C until the coverslips slid off. Subsequently, the slides were washed twice in 50% formamide/2x SSC at 45°C for 10 min each followed by two washes in 2x SSC at 21°C. Slides were then incubated in PNM (5% nonfat dry milk (Carnation), 1% sodium azide in PN buffer (0.1 M sodium phosphate buffer, pH 8.0, 1% Nonidet-P40 (Sigma))) for 10 min at 21°C. Bound probes were detected with either fluorescein-conjugated avidin DCS (Vector, Burlingame, CA) or anti-digoxigenin-rhodamine (Roche Molecular Systems, Indianapolis, IN) as described (Weier et al. 1995). Finally, the slides were mounted in 4',6-diamidino-2-phenylindole (DAPI) (0.5 µg/ml; Calbiochem, La Jolla, CA) in antifade solution (Weier et al. 1995).

Image acquisition and analysis

Fluorescence microscopy was performed on a Zeiss Axioskop microscope equipped with a filter set for simultaneous observation of Texas Red/rhodamine and FITC, and a separate filter for DAPI detection (ChromaTechnology, Brattleboro, VT). Images were collected using a cooled CCD camera (VHS Vosskuehler, Osnabrueck, FRG). Further processing of the images was done using Adobe Photoshop® software (Adobe Inc., Mountain View, CA).

Results

Probe Mapping

Prior to the hybridization of DNA probes to patient samples or tumor cells, all probes were tested on normal male metaphase spreads to ensure sufficient signal strength, correct cytogenetic map positions and absence of chimerism (Selleri et al. 1992).

BAC contigs for PGD

Cells from an anonymized PGD case (T-0512) reported to carry a reciprocal $t(4;13)(q21.3;q21.2)$ (Fig.1) were provided to us by the referring clinicians after standard G-banding karyotype analysis. Initially, we selected 60 BAC clones from the Roswell Park Cancer Institute (RPCI) RP11 library (Oseogawa et al. 2001) spread out over the following intervals: chromosome 4: 79.7 Mbp - 91.3 Mbp (from clone RP11-57L13 at 4q21.2 to clone RP11-350B19 at 4q23) and chromosome 13: 57.4 Mbp - 66.8 Mbp (from clone RP11-16M6 at 13q21 to clone RP11-21B13 at 13q23)(data not shown). Hybridization results to normal metaphase spreads or to patient metaphase cells were unusually poor: 20 of 60 clones failed to produce informative hybridization signals. Clones that gave analyzable signals allowed us to narrow the breakpoint region on 4q22.1 to an interval distal of clone RP11-2I7 at 89.6 Mbp – 89.8 Mbp, but proximal to the map position of BAC clone RP11-115D19 at 90.7 Mbp – 90.9 Mbp (data not shown). The FISH mapping experiments of individual BAC clones for chromosome 13 were plagued by hybridization failures, but allowed us to narrow the breakpoint region to in-between the proximal clone RP11-16M6 (at ~57.4 Mbp)(data not shown) and the three

BAC clones RP11-10M21, RP11-138D23 and RP11-346A3, which map into the interval 66.165 Mbp - 66.753 Mbp (Table 1).

These results prompted us to change our mapping strategy, replace individual clones with selected contiguous sets of BAC's and prepare pools of labeled DNA probes. For the long arm of chromosome 13, we prepared eight BAC pools termed Pool 13 -1 to Pool 13-7 comprised entirely of clones from the RPCI RP11 library (Table 1). The clone pools 13-1 to 13-6 cover part of the long arm of chromosome 13 more-or-less evenly from band q21.2 to band q21.33, while Pool 13-7 is a distal reference probe comprised of 2 clones which map in band 13q22.3 between 77.3 Mbp and 77.5 Mbp (Table 1).

In our first BAC pool FISH experiment, we combined biotin-labeled DNA from pools 13-1, 13-3, 13-5 and 13-7 with digoxigenin(dig.)-labeled probe made from pools 13-2, 13-4 and 13-6. Hybridization of these 'superpool' DNA probes to normal male metaphase spreads and detection with avidin-FITC and rhodamine-conjugated anti-dig. antibodies showed strong, specific signals on both homologues of chromosome 13 without noticeable cross-hybridization to other chromosomes (Fig.2A). Due to close proximity of biotin- and dig.-labeled probes, which were detected with green- and red-fluorescent reagents, respectively, the superimposed FISH signals appear yellow in the pseudo-*RGB* pictures in Fig. 2.

Hybridization of the same combination of chromosome 13-specific probe pools to metaphase cell from T-0512 showed strong hybridization signals on the normal chromosome 13 and the der(13) as well as on the der(4)(Fig.2B). All three hybridization domains showed green as well as red fluorescence. Thus, the first BAC pool hybridization confirmed the results obtained with individual clones, i.e., the interval

covered by pools 13-1 to 13-6 extends onto both sides of the breakpoint region. Since both derivative chromosomes in Fig.2B showed red and green signals, the breakpoint must lie between Pools 13-2 and 13-6, i.e., between 60.6 Mbp and 67.8 Mbp.

For delineation of the breakpoint on the long arm of chromosome 4 in patient T-0512, we chose nine BAC clones that cover the region between 89.5 Mbp and 90.7 Mbp (Table 2). These nine BAC probes were combined in two pools as shown in Table 2: Pool 4-1 is a five BAC contig centered around the clone RP11-2I7, which was known to be proximal of the chromosome 4-specific breakpoint. Pool 4-2 binds distal of Pool 4-1 and covers the interval from 90.3 Mbp to 90.7 Mbp on the long arm of chromosome 4, i.e., slightly proximal of the above mentioned clone RP11-115D19 that was mapped distal of the breakpoint region. Pools 4-1 and 4-2 cover unique, non-overlapping chromosome regions of about 796 kb and 399 kb, respectively. The chromosome 4-specific probe pools were labeled with digoxigenin.

The following experiments were designed to determine the breakpoint locations relative to the BAC pools, and to optimize probes. In a second hybridization of BAC pools to patient metaphase spreads, we combined the two dig.-labeled pools for chromosome 4 (4-1 and 4-2) with a combination of four biotinylated DNA probes prepared from Pools 13-4, 13-5, 13-5.5 and 13-6 (Table 1). The chromosome 4- and 13-specific probes were detected in red and green, respectively. The images in Fig.2 C-F summarize the results of this hybridization. All four chromosomes of interest (4, 13, der(4), der(13)) can be identified by their DAPI banding pattern (Fig. 2C) and the red, green or yellow hybridization signal (Fig. 2D-F).

As a rule of thumb, in this hybridization scheme, the normal homologues show hybridization domains in a single color (either red (chr. 4) or green (chr.13)) (Munné et al. 1998; Weier et al. 1999). The color of signal domains on the derivative chromosomes depends on whether a probe binds proximal of the breakpoint (i.e., no translocation of probe target and signals are found on the normal and its derivative chromosome) or distal (probe target being translocated). If probe binding extends significantly on both sides of the breakpoint (i.e., it 'spans' the breakpoint region), probe signals will be found on both derivative chromosomes. The image in Fig.2D shows three red signals: one on the normal chromosome 4 and two on derivative chromosomes der (4) and der(13) as expected for a probe pool that spans the breakpoint on chromosome 4. We also noted that the signal on the normal copy of chromosome 4 was very strong and signals on the derivative chromosomes were approximately of equal strength (Fig.2D). The green fluorescent signal were found exclusively on the normal copy of chromosome 13 and the der (4) (Fig.2E). Thus, all biotinylated probes from Pool 13-4 to Pool 13-6 bound distal of the breakpoint on chromosome 13.

Having learned that the breakpoint on chromosome 13 lies proximal of Pool 13-4 (Fig.2E), but within or distal of Pool 13-2 (Fig.2B), we decided to map Pool 13-3. Dual color FISH using a combination of biotinylated Pool 13-3 DNA and the two dig.-labeled pools for chromosome 4 showed the expected signals on the normal non-rearranged copies of chromosomes 4 and 13 (Fig.2G). Red and green signals were found on both derivative chromosomes clearly indicated that Pool13-3 spans the breakpoint on chromosome 13 in T-0512. However, we noted that the green signal on the der(4) chromosome was very faint, while the green signal on the der(13) was strong. Thus,

only a small fraction of probe contained in Pool 13-3 bound distal of the breakpoint, and most of this pool bound proximal.

In summary, only three overnight FISH experiments with BAC pools and patient metaphase spreads allowed us to narrow the breakpoint position to a 1.1 Mbp interval between 62.5 Mbp and 63.6 Mbp on chromosome 13. The next step in the PGD probe preparation process was probe optimization: since the chromosome 4-specific DNA probe contigs was split more or less evenly (Fig.2D), we decided to design a chromosome 13-specific BAC pool probe that will be split asymmetrically by the translocation, thus allowing unambiguous identification of derivative chromosomes in interphase cell nuclei. This was achieved easily by combining the previously prepared biotinylated probe from Pool 13-3 with DNA probes prepared from Pools 13-4, 13-5, 13-5.5 and 13-6. This set of probes covers an interval from 62.5 Mbp to 67.8 Mbp. The FISH result showed that signals from biotinylated chromosome 13 probes were split into two differently sized parts: the signals derived from pool 13-3 BAC's binding to the proximal long arm of chromosome 13 were weaker than those of probes that covered the distal part (Fig.2H). Thus, the der(4) chromosome showed stronger green signals than the der(13) (Fig.2H).

This set of hybridization probe which extents differently on the proximal and distal sites of the chromosome 13-specific breakpoint and a simple dual color probe detection scheme allows classification of all chromosomes involved in this translocation in interphase cell nuclei (Fig.2I).

BAC contigs for characterization of chromosome rearrangements in the PTC cell line S48TK

Conventional karyotyping using G-banding and SKY analysis of cell line S48TK had indicated several derivative chromosomes carrying chromosome 1-derived material (Zitzelsberger et al. 1999)(Fig.2J). Comparative genomic hybridization indicated extra copies of the proximal long arm of chromosome 1 (Weier et al. 2006). To initiate the characterization of rearrangements involving the long arm of chromosome 1, we prepared BAC pools P1-1 and P1-2 (Table 3). Each of the two BAC pools is comprised of three minimally overlapping BAC clones which according the NCBI Map Viewer (<http://www.ncbi.nlm.nih.gov/projects/mapview/>) bind to DNA sequences at the border between bands 1q12 and 1q21 or band 1q22, respectively (Figure 3), i.e., proximal of the neurotrophic tyrosine kinase receptor, type 1, gene (NTRK1 or TRK-A) (Weier et al. 1995). According to the information provided by the Human Genome Reference DNA Sequence, Mapviewer build 35.1, pools P1-1 and P1-2 cover about 516 kb and 354 kb, respectively, of unique sequence.

Hybridization of a biotinylated probe for pool P1-1 in combination with a dig.-labeled probe for pool P1-2 onto normal chromosomes 1 showed the green and red signals in the expected positions (not shown). Hybridization onto metaphase chromosome prepared from line S48TK revealed a marker chromosome carrying about 2-3 copies of the target segment 1q21-1q22 (Fig.2K).

Discussion

Preimplantation genetic analysis is a laboratory procedure to identify chromosomally abnormal embryos among morphologically normal embryos, and thus increase the chances of nidation and successful pregnancy (Munné et al. 1994; Braude et al. 2002; Munné 2002; Verlinsky et al. 2004). Typically, no more than one or two blastomeres are removed for PGD from embryos on day three or four after insemination (Munné 2002; Sampson et al. 2004). Interphase cell analysis is an important component of PGD, since blastomeres following biopsy can be found in any stage of the mitotic cell cycle.

Chromosome-specific DNA repeat probes, which are commercially available for all human chromosomes, are suitable to detect numerical chromosome aberrations in individual or single interphase nuclei (D'Alton et al. 1997; Weier et al. 2005; Stumm et al. 2006). The majority of these commercial DNA probes are alpha satellite DNA repeats, which bind at or near the chromosomal centromeres (Waye et al. 1987; Baumgartner et al. 2006). In the absence of structural chromosome aberrations, the DNA repeat probes are the first choice of probes for chromosome enumeration, due to their ease of use, short hybridization times and typically bright signals.

For reciprocal translocation carriers, however, the prevalence of unbalanced gametes carrying a partial aneuploidy is estimated to range from 50% to 70% (Scriven et al. 1998; Braude et al. 2002; Sampson et al. 2004). Centromeric probes are likely to miss most partial aneuploidies. A number of FISH approaches for the analysis of blastomeres have been proposed, such as translocation probes sets binding distal of the translocation breakpoints and allow scoring of chromosome arms (Pehlivan et al. 2003; Sampson et

al. 2004) or the multi-color banding of chromosomes in interphase nuclei using probes prepared by chromosome micro-dissection (Iourov et al. 2007).

Several years ago, we proposed to prepare DNA probes or probe contigs comprised of YACs that span individual translocation breakpoints (Cassel et al. 1997). This was a rather time consuming process going through repeated cycles of clone selection and mapping. High-quality non-chimeric probes had to be selected and every cycle took at least 7-10 days (Fung et al. 1998). With time constraints in IVF programs, often little time was left for probe optimization once a breakpoint had been mapped (Fung et al. 1999).

The aim of the present study was to expedite the process of mapping translocation breakpoints by using BACs and DNA probe pooling strategies. Developed initially as sequencing templates for the International Human Genome Project (Oseogawa et al. 2001), several BAC libraries are now available for the human genome and allow the rapid preparation of probes for virtually any region of the human genome. Thus, BACs are being used more and more frequently for preparation of DNA microarrays (Fiegler et al. 2003) or cytogenetics analyses (Liehr et al. 2002; Toennies et al. 2007; Baldwin et al. 2008).

The probe preparation process for the t(4;13) case presented in this report used contigs or pools of BAC clones to minimize, if not eliminate, hybridization failures or so-called uninformative experiments (Pehlivan et al. 2003; Sampson et al. 2004). With translocation breakpoints roughly determined by G-banding, large numbers of BACs can be selected from in-house libraries and assembled in probe pools even before initiation of the IVF cycle. As the t(4;13) example shows, only few overnight

hybridizations will be required to localize the breakpoint to a single pool and optimize the probe for single interphase cell analysis. The small number of cells and the brief time frame (i.e., hours) available for PGD requires probes, which performed in FISH experiments with virtually 100% efficiency. Spanning probes can be designed to reach this benchmark and have the great advantage of being able to detect accurately all possible chromosome segregations as well as being able to distinguish normal and balanced constitutions. This is expected lead to more reliable PDG procedures, reduce the number of failed embryo transfers and make interphase PGD more affordable for infertile couples.

A second objective of the present study was to evaluate the use of BAC pools in the cytogenetic analysis of tumor cells. We chose cell line S48TK, a cell line established from a papillary thyroid tumor which arose in a child following the 1986 nuclear accident in Chernobyl, USSR, because these cell carry a large number of unbalanced translocations, some of which involve chromosome 1 (Lehmann et al. 1996; Zitzelsberger et al. 1999). The long arm of chromosome 1 is of particular interest, since it harbors the NTRK1 proto-oncogene, which is activated through recombination in a number of human solid tumor types among them childhood PTC (Kozma et al. 1988; Beimfohr et al. 1999). For the example presented in Figure 2K, we chose two BAC contigs which bind proximal of NTRK1, because our CGH studies suggested extra copies of the proximal long arm of chromosome 1 in S48TK (Weier et al. 2006). Interestingly, recurrent amplifications of the region 1q21-1q22 have also been reported in panels of sarcomas, bladder and breast cancers as well as hepatocellular carcinomas (Forus et al. 1998; Meza-Zepeda 2002). The hybridization results (Fig.2K) confirm the

complex nature of the chromosome 1q21-q22 amplicon in S48TK, which seems to be comprised of several, potentially incomplete copies of the region flanked by our probe contigs P1-1 and P1-2. Two other chromosomes in the metaphase spread shown in Fig.2K showed hybridization signals as expected, i.e., P1-1 binds proximal of P1-2. Given the different sizes of hybridization signal domains in Fig.2K, it is reasonable to speculate that hybridization of single BAC clones or even smaller probes such as cosmids might have missed a number of these copies.

In summary, the preparation and hybridization of pools of BAC clones requires slightly more up-front effort, but compared to FISH using single clones, it greatly increases hybridization efficiencies, minimizes hybridization failures, expedites breakpoint mapping without sacrificing resolution and increase the sensitivity to detect small rearrangements.

Acknowledgments

This work was supported in parts by NIH grants CA80792, CA88258, CA123370, and HD44313, and a grant from the Director, Office of Energy Research, Office of Health and Environmental Research, U.S. Department of Energy, under contract DE-AC02-05CH11231. JFW was supported in part by grant HD45736 from the National Institute of Health and a grant from the UC Discovery Program, which also supported AB. We acknowledge editorial help provided by E. Lowe, and support from staff at Reprogenetics providing us with metaphase spreads and initial mapping data. Ideograms were kindly provided by D. Adler, Ph.D., Dept. of Pathology, University of Washington. We like to express our thanks to the scientists at the Human Genome Center, California Institute of Technology, Pasadena, whose generosity has made these studies possible.

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Literature Cited

- Baldwin EL, May LF, Justice AN, Martin CL, Ledbetter DH (2008) Mechanisms and Consequences of Small Supernumerary Marker Chromosomes: From Barbara McClintock to Modern Genetic-Counseling Issues. *Am J Hum Genet* 82:398-410
- Baumgartner A, Weier JF, Weier H-UG (2006) Chromosome-specific DNA repeat probes. *J Histochem Cytochem* 54:1363-1370
- Bayani J, Squire JA (2004) Preparation of cytogenetic specimens from tissue samples In Bonifacino JS ed. *Current Protocols in Cell Biology*, Suppl 23. New York, John Wiley & Sons, Inc., 22.2.1-22.2.15
- Beimfohr C, Klugbauer S, Demidchik EP, Lengfelder E, Rabes HM (1999) NTRK1 re-arrangement in papillary thyroid carcinomas of children after the Chernobyl reactor accident. *Int J Cancer* 80:842-847
- Birnboim HC, Doly J (1979) A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucl Acid Res* 7:1513-1523
- Braude P, Pickering S, Flinter F, Ogilvie CM (2002) Preimplantation genetic diagnosis. *Nat Rev Genet* 3:941-953
- Carreira IM, Mascarenhas A, Matoso E, Couceiro AB, Ramos L, Dufke A, Mazauric M, Stressig R, Kosyakova N, Melo JB, Liehr T (2007) Three unusual but cytogenetically similar cases with up to five different cell lines involving structural and numerical abnormalities of chromosome 18. *J Histochem Cytochem* 55:1123-1128
- Cassel MJ, Munné S, Fung J, Weier H-UG (1997) Carrier-specific breakpoint-spanning DNA probes for pre-implantation genetic diagnosis [PGD] in interphase cells. *Hum Reprod* 12:101-109

D'Alton ME, Malone FD, Chelmos D, Ward BE, Bianchi DW (1997) Defining the role of fluorescence in situ hybridization on uncultured amniocytes for prenatal diagnosis of aneuploidies. *Am J Obstet Gynecol* 176:769-776

Fiegler H, Carr P, Douglas EJ, Burford DC, Hunt S, Scott CE, Smith J, Vetrie D, Gorman P, Tomlinson IP, Carter NP (2003) DNA microarrays for comparative genomic hybridization based on DOP-PCR amplification of BAC and PAC clones. *Genes Chrom Cancer* 36:361-374

Forus A, Berner JM, Meza-Zepeda LA, Saeter G, Mischke D, Fodstad O, Myklebost O (1998) Molecular characterization of a novel amplicon at 1q21-q22 frequently observed in human sarcomas. *Br J Cancer* 78:495-503

Fung J, Munné S, Duell T, Weier H-UG (1998) Rapid cloning of translocation breakpoints: from blood to YAC in 50 days. *J Biochem Mol Biol Biophys* 1:181-192

Fung J, Munné S, Garcia J, Kim U-J, Weier H-UG (1999) Reciprocal translocations and infertility: molecular cloning of breakpoints in a case of constitutional translocation t(11;22)(q23;q11) and preparation of probes for preimplantation genetic diagnosis (PGD). *Reprod Fert Dev* 11:17-23

Fung J, Weier H-UG, Goldberg JD, Pedersen RA (2000) Multilocus genetic analysis of single interphase cells by spectral imaging. *Hum Genet* 107:615-622

Fung J, Munné S, Weier H-UG (2001) Detection of chromosome translocation products in single interphase cell nuclei. In Darzynkiewicz Z, Chrissman HA, Robinson J.P, eds. *Methods in Cell Biology*, Vol. 64, Part B, Cytometry, 3rd ed. San Diego, Academic Press, 98-117

Fung J, Weier H-UG, Pedersen RA, Zitzelsberger HF (2002) Spectral Imaging Analysis

of Metaphase and Interphase Cells. In Rautenstrauss B, Liehr T eds. FISH Technology. Heidelberg, Springer Verlag, 363-387

Greco A, Roccato E, Pierotti MA (2004) TRK oncogenes in papillary thyroid carcinomas. In Farid NR, ed. Molecular basis of thyroid cancer, Cancer Treatment and Research. Boston, Kluwer Academic Publishers, 207-219

Herrmann MA, Hay ID, Bartelt DH, Ritland SR, Dahl RJ, Grant CS, Jenkins RB (1991) Cytogenetic and molecular genetic studies of follicular and papillary thyroid cancers. Journal Clinical Invest 88:1596-1604

Iourov IY, Liehr T, Vorsanova SG, Yurov YB (2007) Interphase chromosome-specific multicolor banding (ICS-MCB): A new tool for analysis of interphase chromosomes in their integrity. Biomol Eng 24:415-417

Jossart GH, Greulich KM, Siperstein AE, Duh Q, Clark OH, Weier H-UG (1995) Molecular and cytogenetic characterization of a t(1;10;21) translocation in the human papillary thyroid cancer cell line TPC-1 expressing the ret/H4 chimeric transcript. Surgery 118:1018-1023

Jossart GH, O'Brien B, Cheng J-F, Tong Q, Jhiang SM, Duh Q, Clark OH, Weier H-UG (1996) A novel multicolor hybridization scheme applied to localization of a transcribed sequence (D10S170/H4) and deletion mapping in the thyroid cancer cell line TPC-1. Cytogenet Cell Genet 75:254-257

Kalousek DK (2000) Pathogenesis of chromosomal mosaicism and its effect on early human development. Am J Med Genet 91:39-45

Kim UJ, Shizuya H, Kang HL, Choi SS, Garrett CL, Smink LJ, Birren BW, Korenberg JR, Dunham I, Simon MI (1996) A bacterial artificial chromosome-based framework contig

map of human chromosome 22q. *Proc Natl Acad Sci USA* 93:6297-6301

Kozma SC, Redmond SMS, Fu XC, Saurer SM, Groner B, Hynes NE (1988) Activation of the receptor kinase domain of the trk oncogene by recombination with two different cellular sequences. *EMBO J* 7:147-154

Lehmann L, Zitzelsberger H, Kellerer AM, Braselmann H, Kulka U, Georgiadou-Schumacher V, Negele T, Spelsberg F, Demidchik E, Lengfelder E, Bauchinger M (1996) Chromosome translocations in thyroid tissues from Belarussian children exposed to radioiodine from the Chernobyl accident, measured by FISH-painting. *Int J Radiat Biol* 70:513-516

Liehr T, Weise A, Heller A, Starke H, Mrasek K, Kuechler A, Weier H-UG, Claussen U (2002) Multicolor chromosome banding (MCB) with YAC/BAC-based probes and region-specific microdissection DNA libraries. *Cytogenet Genome Res* 97:43-50

Lu CM, Wang M, Greulich-Bode K, Weier JF, Weier HUG (2008) Quantitative DNA fiber mapping. In Liehr T, ed. *Springer FISH Lab Manual*. Heidelberg, Springer Verlag, 269-291

Meza-Zepeda LA, Forus A, Lygren B, Dahlberg AB, Godager LH, South AP, Marenholz I, Lioumi M, Flørenes VA, Maelandsmo GM, Serra M, Mischke D, Nizetic D, Ragoussis J, Tarkkanen M, Nesland JM, Knuutila S, Myklebost O (2002) Positional cloning identifies a novel cyclophilin as a candidate amplified oncogene in 1q21. *Oncogene* 21:2261-2269

Munné S, Grifo J, Cohen J, Weier H-UG (1994) Mosaicism and aneuploidy in arrested human preimplantation embryos: a multiple probe fluorescence in situ hybridization (FISH) study. *Am J Hum Genet* 55:150-159

Munné S, Fung J, Cassel MJ, Márquez C, Weier H-UG (1998) Preimplantation genetic analysis of translocations: case-specific probes for interphase cell analysis. *Human Genetics* 102:663-674

Munné S, Sandalinas M, Escudero T, Fung J, Gianaroli L, Cohen J (2000) Outcome of preimplantation genetic diagnosis of translocations. *Fertil Steril* 73:1209-1218

Munné S (2002) Preimplantation genetic diagnosis of numerical and structural chromosome abnormalities. *Reprod Biomed Online* 4:183-196

Osoegawa K, Mammoser AG, Wu C, Frengen E, Zeng C, Catanese JJ, de Jong PJ (2001) A bacterial artificial chromosome library for sequencing the complete human genome. *Genome Res* 11:483-496

Pehlivan T, Rubio C, Rodrigo L, Remohi J, Pellicer A, Simon C (2003) Preimplantation genetic diagnosis by fluorescence in situ hybridization: clinical possibilities and pitfalls. *J Soc Gynecol Investig* 10:315-322

Peng HH, Chao AS, Wang TH, Chang YL, Chang SD (2006) Prenatally diagnosed balanced chromosome rearrangements: eight years' experience. *J Reprod Med* 51:699-703

Pierotti MA, Santoro M, Jenkins RB, Sozzi G, Bongarzone I, Grieco M, Monzini N, Miozzo M, Herrmann MA, Fusco A, Hay ID, Della Porta G, Vecchio G (1992) Characterization of an inversion on the long arm of chromosome 10 juxtaposing D10S170 and RET and creating the oncogenic sequence RET/TPC. *Proc Natl Acad Sci USA* 89:1616-1620

Plastira K, Maher E, Fantes J, Ramsay J, Angelopoulou R (2006) Using BAC clones to characterize unbalanced chromosome abnormalities in interphase cells. *Eur J Med*

Genet 49:235-246

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning: A Laboratory Manual.

2nd ed. New York, Cold Spring Harbor Laboratory Press

Sampson JE, Ouhibi N, Lawce H, Patton PE, Battaglia DE, Burry KA, Olsen SB (2004)

The role of preimplantation genetic diagnosis in balanced translocation carriers. Am J

Obstet Gynecol 190:1707-1713

Scriven PN, Handyside AH, Ogilvie CM (1998) Chromosome translocation: segregation modes and strategies for preimplantation genetic diagnosis. Prenat Diagn 18:1437-1449

Selleri L, Eubanks JH, Giovannini M, Hermanson GG, Romo A, Djabali M, Maurer S,

McElligott DL, Smith MW, Evans GA (1992) Detection and characterization of "chimeric" yeast artificial chromosome clones by fluorescent in situ suppression hybridization.

Genomics 14:536-541

Shizuya H, Birren B, Kim UJ, Mancino V, Slepak T, Tachiiri Y, Simon M (1992) Cloning

and stable maintenance of 300-kilobase-pair fragments of human DNA in Escherichia coli using an F-factor-based vector. Proc Natl Acad Sci USA 89:8794-8797

Srb AM, Owen RD, Edgar RS (1965) General Genetics, 2nd ed. San Francisco, W.H.

Freeman and Co

Stern C, Pertile M, Norris H (1999) Chromosome translocations in couples with in vitro fertilisation implantation failure. Hum Reprod 14:2097-2101

Stumm M, Wegner R-D, Bloechle M, Eckel H (2006) Interphase M-FISH applications using commercial probes in prenatal and PGD diagnostics. Cytogenet Genome Res

114:296-301

Subrt I (1980) Reciprocal translocation with special reference to reproductive failure.
Hum Genet 55:303-307

Thorsen J, Zhu B, Frengen E, Osoegawa K, de Jong PJ, Koop BF, Davidson WS,
Hoyheim B (2005) A highly redundant BAC library of atlantic salmon (*Salmo salar*): an
important tool for salmon projects. BMC Genomics 6:50

Toennies H, Pietrzak J, Bocian E, MacDermont K, Kuechler A, Belitz B, Trautmann U,
Schmidt A, Schulze B, Rodriguez L, Binkert F, Yardin C, Kosyakova N, Volleth M,
Mkrtchyan H, Schreyer I, von Eggeling F, Weise A, Mrasek K, Liehr T (2007) New
immortalized cell lines of patients with small supernumerary marker chromosome:
towards the establishment of a cell bank. J Histochem Cytochem 55:651-660

Verlinsky Y, Cohen J, Munné S, Gianaroli L, Simpson JL, Ferraretti AP, Kuliev A (2004)
Over a decade of experience with preimplantation genetic diagnosis: a multicenter
report. Fertil Steril 82:292-294

Waye JS, Durfy SJ, Pinkel D, Kenwrick S, Patterson M, Davies KE, Willard HF (1987)
Chromosome-specific alpha satellite DNA from human chromosome 1: hierarchical
structure and genomic organization of a polymorphic domain spanning several hundred
kilobase pairs of centromeric DNA. Genomics 1:43-51

Weier HU, Rhein AP, Shadravan F, Collins C, Polikoff D (1995) Rapid physical mapping
of the human trk protooncogene (NTRK1) to human chromosome 1q21-q22 by P1 clone
selection, fluorescence in situ hybridization (FISH), and computer-assisted microscopy.
Genomics 26:390-393

Weier HUG, Munné S, Fung J (1999) Patient-specific Probes for Preimplantation Genetic Diagnosis (PGD) of Structural and Numerical Aberrations in Interphase Cells. *J Assist Reprod Genet* 16:182-189

Weier HUG, Tuton TB, Chu LW, Ito Y, Lu C-M, Baumgartner A, Zitzelsberger HF, Weier JF (2006) Molecular cytogenetic characterization of a human thyroid cancer cell line. *Cytogenet Genome Res* 114:284-291

Weier JF, Weier H-UG, Jung CJ, Gormley M, Yan Zhou Y, Chu LW, Genbacev O, Wright AA, Fisher SJ (2005) Human cytotrophoblasts acquire aneuploidies as they differentiate to an invasive phenotype. *Dev Biology* 279:420-432

Zitzelsberger H, Lehmann L, Hieber L, Weier H-UG, Janish C, Fung J, Negele T, Spelsberg F, Lengfelder E, Demidchik E, Salassidis K, Kellerer AM, Werner M, Bauchinger M (1999) Cytogenetic changes in radiation-induced tumors of the thyroid. *Cancer Res* 59:135-140

Zitzelsberger HF, O'Brien B, Weier H-UG (2002) Multicolor FISH techniques for the detection of inter- and intrachromosomal rearrangements. In Rautenstrauss B, Liehr T eds. *FISH Technology*. Heidelberg, Springer Verlag, 408-424

Figure Legends

Figure 1. Schematic diagram of the karyotypic abnormalities in a case reported as $t(4;13)(q22.1;q21.3)$. The normal homologues of chromosome 4 and 13 are shown in A). Breakpoints are indicated by thick horizontal lines at the approximate breakpoint position. The two derivative chromosomes $der(4)$ and $der(13)$ are shown in B). The open and hatched boxes represent the breakpoint-spanning probe contigs for chromosome 4 and 13, respectively. In our experiments, the chromosome 4- and 13-specific probes were detected in red and green, respectively. Please note that the translocation separates proximal and distal parts of the chromosome 13-specific DNA probe contig of different sizes.

Figure 2. Hybridization of BAC pools for delineation of chromosome breakpoints in human cells. A) Hybridization of chromosome 13-specific BAC pools to metaphase cells from a normal male donor demonstrates exclusive binding to the target region on the long arm of chromosome 13. The image shows a pseudo-RGB picture of bound probes on DAPI counterstained metaphase chromosomes. The insert shows the DAPI channel (blue fluorescence with arrows pointing at the 2 homologues of chromosome 13). B-I) Hybridization of BAC pool-derived DNA probes to patient (T-0512 cells carrying a $t(4;13)(q22.1;q21.3)$). Arrows in these panels point at normal chromosomes, while arrowheads point at derivative chromosomes. B) Hybridization of seven pools for chromosome 13 generates signals on the normal homologue and the $der(13)$ (center) as well as on the $der(4)$ (in the upper right) indicating that probes bind proximal and distal of the breakpoint on chromosome 13. C-F) Combined hybridization of a

chromosome 4-specific BAC pool (red) and pools 13-4 to 13-6 (green) shows hybridization pattern expected for a red probe pool spanning the chromosome 4 breakpoint, but the green probe pool binds distal of the breakpoint on chromosome 13. DAPI, red, and green fluorescence images are shown in C, D, and E, respectively. Panel F shows the pseudo-RGB picture. G) FISH results showing pattern generated by hybridization of the chromosome 4-specific probe pool (red) in combination with the breakpoint spanning pool 13-3 (green). H-I) Hybridization of the extended probe set for chromosome 13 (green) and chromosome 4 (red) to metaphase and interphase cells. Arrows in H) point at the normal chromosomes, and arrowheads indicated the red+green - labeled derivative chromosomes. J) SKY analysis of metaphase chromosomes from the post-Chernobyl childhood thyroid cancer cell line S48TK indicates several abnormal chromosomes carrying genetic material derived from chromosome 1 (yellow). K) Hybridization of chromosome 1q-specific BAC probe pools indicates a marker chromosome (arrowhead) with complex rearrangements including amplification of the proximal part of the long arm of chromosome 1. The arrows point at metaphase chromosome which show the normal order of signals, i.e., pool P1-1 (green) bind proximal of pool P1-2 (red). The insert shows an enlarged picture of the abnormal chromosome (left: FISH results, right: inverted DAPI image).

Figure 3. Ideogram of human chromosome 1. The arrows point at the map position of the two BAC pools for tumor cell analysis (P1-1, P1-2).

Table 1. BAC pools for breakpoint delineation on chromosome 13q

Clone	Band	Pool	Position (Mbp)*	BAC size (bp)
RP11-524F1	13q21.2	13-1	58.618 – 58.785	166436
RP11-26P21	13q21.2	13-1	59.004 – 59.210	206407
RP11-218B22	13q21.2	13-1	59.241 – 59.395	154212
RP11-442F12	13q21.2	13-1	59.389 – 59.599	209794
RP11-430I3	13q21.2	13-1	59.599 – 59.662	63441
RP11-350G11	13q21.31	13-2	60.619 – 60.721	102564
RP11-310K10	13q21.31	13-2	60.719 – 60.882	163231
RP11-432J3	13q21.31	13-2	60.881 – 60.940	59272
RP11-210L5	13q21.31	13-2	60.938 – 61.113	176085
RP11-543A19	13q21.31	13-2	61.112 – 61.178	66386
RP11-179D6	13q2131	13-2	61.179 – 61.264	85563
RP11-429G17	13q21.31	13-2	61.262 – 61.426	164831
RP11-71L7	13q21.31	13-2	61.426 – 61.530	103728
RP11-527N12	13q21.31	13-3	62.520 - 62.700	178323
RP11-282D7	13q21.31	13-3	62.699 - 62.806	106534
RP11-320N6	13q21.31	13-3	62.806 - 62.945	139649
RP11-67L17	13q21.31	13-3	62.945 - 63.070	125693
RP11-473M10	13q21.31	13-3	63.070 - 63.233	163218
RP11-394A14	13q2131	13-3	63.235 - 63.409	174801
RP11-520F9	13q21.31	13-3	63.408 - 63.481	73811
RP11-205B18	13q21.31	13-3	63.480 - 63.638	158460

RP11-261A1	13q21.31	13-4	64.362 - 64.528	166490
RP11-211D10	13q21.31-q21.32	13-4	64.560 - 64.742	182321
RP11-379K8	13q21.32	13-4	64.787 - 64.966	179790
RP11-229I7	13q21.32	13-4	64.967 - 65.062	96045
RP11-326D19	13q21.32	13-4	65.063 - 65.222	159919
RP11-223F20	13q21.32	13-4	65.232 - 65.400	168897
RP11-298H15	13q21.32	13-4	65.416 - 65.575	160015
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RP11-10M21	13q21.32	13-5	66.165 - 66.378	114290
RP11-138D23	13q21.32	13-5	66.378 - 66.543	164604
RP11-576O3	13q21.32	13-5	66.544 - 66.683	139881
RP11-346A3	13q21.32	13-5	66.683 - 66.753	71774
RP11-531B22	13q21.32	13-5	66.751 - 66.834	82944
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RP11-164E20	13q21.32	13-5.5	66.883 - 67.067	184374
RP11-520F22	13q21.32	13-5.5	67.006 - 67.147	141913
RP11-51P14	13q21.32	13-5.5	67.144 - 67.174	28761
RP11-562L19	13q21.32	13-5.5	67.174 - 67.254	81586
RP11-248N6	13q21.32	13-5.5	67.255 - 67.416	162695
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RP11-338L17	13q21.33	13-6	67.492 - 67.560	68556
RP11-157F14	13q21.33	13-6	67.558 - 67.682	123887
RP11-520F24	13q21.33	13-6	67.682 - 67.841	159235
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RP11-318G21	13q22.3	13-7	77.297 - 77.480	183337
RP11-122N18	13q22.3	13-7	77.314 - 77.489	175282

* The Human Genome Reference DNA Sequence was completed in April 2003. Unique position information is estimated from Mapviewer build 35.1 at <http://www.ncbi.nlm.nih.gov/mapview/>.

Table 2. BAC pools for breakpoint delineation on chromosome 4q22.1

Clone	Pool	Position (Mbp) ^a	BAC size (bp)
RP11-10L7	4-1	89.542-89.653	112291
RP11-466G12	4-1	89.652-89.841	189402
RP11-2I7	4-1	89.840-89.942	163551
RP11-496N17	4-1	89.941-90.052	111908
RP11-502A23	4-1	90.168-90.338	171273
RP11-84C13	4-2	90.337-90.448	112003
RP11-173C9	4-2	90.447-90.567	120662
RP11-549C16	4-2	90.566-90.737	172195
RP11-79M20	4-2	90.572-90.736	165894 ^b

^a Unique positions were estimated from Mapviewer build 35.1 at

<http://www.ncbi.nlm.nih.gov/mapview/>.

^b BAC size was determined via BLAST search at

http://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Web&PAGE_TYPE=BlastHome using the BAC end sequences.

Table 3. BAC pools for breakpoint delineation on chromosome 1

Clone	Band	Pool	Position (Mbp) ^a	BAC size (bp) ^b
RP11-37N10	1q12-q21	P1-1	142.777-142.944	166807
RP11-315I20	1q12-q21	P1-1	142.928-143.134	206466
RP11-71P2	1q12-q21	P1-1	143.125-143.293	167389
RP11-262A11	1q22	P1-2	152.356-152.532	176285
RP11-243J18	1q22	P1-2	152.441-152.599	158208
RP11-299D6	1q22	P1-2	152.522-152.710	188752

^a Unique positions were estimated from Mapviewer build 35.1.

^b BAC sizes are from the clone database at <http://genome.ucsc.edu/>.





