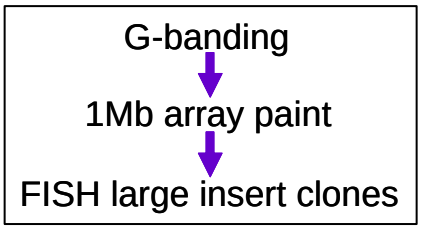
3 Results: Investigation of chromosome rearrangements by array painting, comparative genomic hybridisation and PCR analysis

3.1 Introduction

Approximately 0.087% of all live births have a balanced reciprocal translocation (Jacobs 1981) leading to a 6.1% risk of a serious congenital abnormality (Warburton 1991). Routine Geimsa banding analysis of a patient's karyotype can identify the chromosomes involved in the rearrangement if the chromosomes involved are altered in size or band content. After successful identification of the chromosome translocation by karyotyping, the breakpoints have conventionally been mapped either by fluorescence *in situ* hybridisation using genomic insert clones or by isolating the derivative chromosomes in somatic cell hybrids and extracting the genomic DNA for restriction enzyme digestion. The digested DNA can be separated on agarose gels and blotted onto nitrocellulose membranes so that DNA fragments that span the breakpoints can be identified by Southern blotting. These fragments can then be cloned and sequenced to obtain the breakpoint junction sequence. This approach is lengthy and of the many different translocation breakpoints present in the population, fewer than fifty* have been sequenced and published proving the need for a more rapid technique for breakpoint isolation. This chapter demonstrates how DNA microarrays and PCR can be used to more efficiently map and amplify chromosome rearrangement breakpoints rapidly for sequencing.

*(Bodrug et al. 1987; Bodrug et al. 1991; Giacalone and Francke 1992; Arai et al. 1994; Budarf et al. 1995; van Bakel et al. 1995; Toriello et al. 1996; Holmes et al. 1997; Ishikawa_Brush et al. 1997; Kehrer-Sawatzki et al. 1997; Krebs et al. 1997; Kurahashi et al. 1998; Roberts et al. 1998; Yoshiura et al. 1998; Ikegawa et al. 1999; Holder et al. 2000; Matsumoto et al. 2000; Millar et al. 2000; Bonaglia et al. 2001; Nothwang et al. 2001; Willett-Brozick et al. 2001; Duba et al. 2002; McMullan et al. 2002; Spitz et al. 2002; Sugawara et al. 2002; Vervoort et al. 2002; David et al. 2003; Hill et al. 2003; Jeffries et al. 2003; Nimmakayalu et al. 2003; Shoichet et al. 2003; Gotter et al. 2004; Rodriguez-Perales et al. 2004; Bocciardi et al. 2005; Borg et al. 2005; Klar et al. 2005; Schule et al. 2005; Velagaleti et al. 2005; Haider et al. 2006; Mansouri et al. 2006; Tagariello et al. 2006)



Flow chart describing the progress of breakpoint mapping throughout each stage of this chapter.

The three chromosome rearrangement patients described in this chapter have been studied previously to identify translocation spanning clones (Gribble et al. 2005). Briefly, initial identification of constitutional apparently balanced reciprocal translocations by Geimsa banding analysis at Wessex Regional Genetics Laboratories was followed by array painting and array CGH using a 1Mb resolution microarray. Array painting resolved each translocation breakpoint to an interval of approximately 1Mb, and clones in these regions were selected from the human genome project 'Golden Path' clone set for FISH analysis. Hybridisation of clones across these regions to patient metaphase spreads revealed a change in the localisation of the FISH probe from one derivative chromosome to the other; sequential clones hybridised to one derivative chromosome, showed a split signal on both derivative chromosomes or hybridised to the other derivative chromosome. The translocation breakpoint was contained within the clone showing a hybridisation signal on both derivative chromosomes. Details of the karyotypes, translocation spanning BAC clones and any additional complexity identified can be seen in Table 3.1.

Translocation karyotype	Spanning clones	Accessioned position (bp)	Additional complexity
46,XY,t(2;7)(q37.3;p15.1)	RP11-680O16	2; 236,390,125 to 236,562,868	dup(3)(p26.3;p26.3)pat
	CTA-471E18	7; 30,853,334 to 31,010,213	
46,XX,t(3;11)(q21;q12)	RP11-529F4	3; 130,599,785 to 130,768,338	none found
	RP11-855O10	11; 61,100,132 to 61,285,897	
46,XY,t(7;13)(q31.3;q21.3)	RP11-384A20	7; 121,121,492 to 121,279,437	inv(11)(p15.3;p15.5)pat
	RP11-360I23	13; 70,947,118 to 71,124,154	

Table 3.1 Previously published translocation spanning clones for the three patients whose breakpoints were previously investigated using array based methodologies (Gribble et al. 2005). Clone positions were taken from Ensembl Build 35.

In order to refine the breakpoint containing regions further and sequence across the translocation breakpoints of these patients a number of different array based methodologies have been investigated and the findings reported below. In addition, further complexity within the patients' chromosomes has been investigated in this chapter using array CGH as previous studies have shown that 'balanced' rearrangements are often accompanied by cryptic imbalances either at the breakpoints or elsewhere in the genome (Kumar et al. 1998; Astbury et al. 2004; Patsalis et al. 2004).

3.2 Summary of methods

During the final stages of the sequencing of the human genome a fosmid library was used to assess the quality of the reference sequence. The library was created using DNA from an individual not used in generating the reference sequence. As part of the quality control process, both ends of 750,000 fosmid inserts were sequenced and the data generated used to map each fosmid relative to the reference sequence (IHGSC 2004). We used this fosmid resource to construct increased resolution microarrays to investigate chromosome rearrangement breakpoints. Whilst this fosmid resource is highly redundant (750,000 fosmids represent, on average, a 10 times coverage of the human genome), the fosmid map is not contiguous along every chromosome and gaps are still present. Array painting using flow sorted derivative chromosomes from the patient onto these fosmid microarrays mapped the breakpoints at an increased resolution compared with the spanning BAC clones previously identified. Array CGH using DNA from the patient against a reference DNA onto these fosmid microarrays probed the surrounding area for any cryptic imbalance.

Once the breakpoint area had been refined by array painting on the custom-made fosmid microarray, STS PCR was used to further narrow down the breakpoint region. Primer pairs were designed at intervals to unique regions across the genomic region of interest and derivative chromosomes were used as template DNA. A switch from a positive result on one derivative chromosome to a positive result on the other derivative delineates the breakpoint. In practice, two

rounds of PCR were sufficient to refine the breakpoint sufficiently for the translocation junction to be amplified by Long Range PCR. The first round of STS PCR was performed using primer pairs spaced approximately every 10-20Kb, and once a breakpoint spanning region had been identified, a further round of primer pairs could be designed every 1-3Kb within it, so in principle refining the breakpoint to within a 1-3Kb region.

To amplify a product containing the translocation junction sequence by LR PCR, primers were designed at intervals across the refined regions. The forward primers from one chromosome were paired with the reverse primers from the other chromosome and genomic DNA from the patient used as template (Figure 3.1).

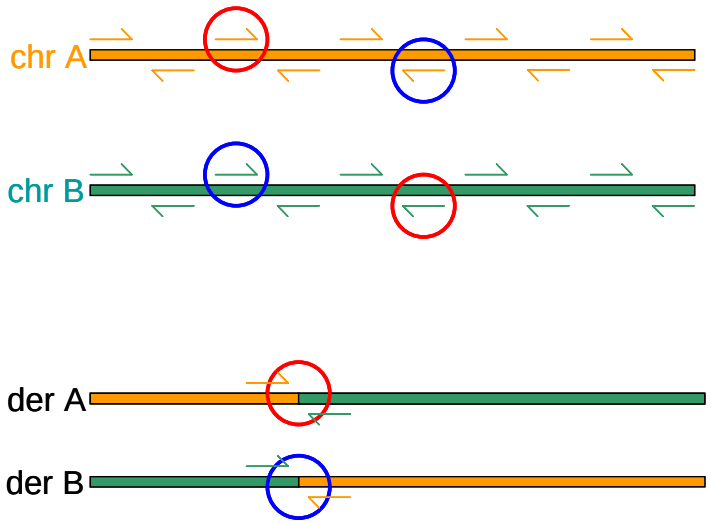
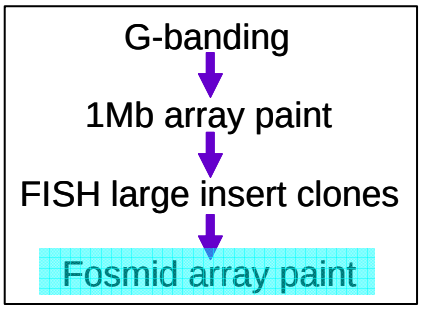


Figure 3.1 Diagrammatic representation of the principle behind long range PCR. Pairing a forward primer designed to one chromosome with the reverse primer designed to the other chromosome will amplify across the derivative chromosome junction.

After successful amplification of the breakpoint containing region by LR PCR the PCR product was sequenced. The resulting sequence was compared with the human reference sequence available in NCBI Build 35 to identify the precise breakpoints. The surrounding sequence was then studied in further detail as discussed in Chapter 4.

3.3 Results; Refinement of chromosome translocation breakpoints using custom-made fosmid microarrays



A high resolution custom-made fosmid array was constructed to study the translocation breakpoints of the three patients' rearrangements. The array consisted of 2244 elements; 327 large insert clones (PACs and BACs) selected at 10Mb intervals across the human genome spotted in duplicate, 517 fosmids spotted in triplicate to cover genomic regions of interest for the rearrangements being investigated and 5 Drosophila clones spotted in triplicate as controls to allow for quantitation of background hybridisation. To give the best possible coverage for the area of interest, fosmid clones were selected at full redundancy from the 'Fosmid End Pairs' track on the UCSC website (http://genome.ucsc.edu/) according to NCBI build 35 of the human genome. The clones were DOP PCR amplified then re-amplified by an amino-linking PCR to attach an amino group to the 5' end of each product to allow for covalent bonding to the microarray slide. The fosmid microarrays were used in array painting and CGH hybridisation experiments as described in the methods section.

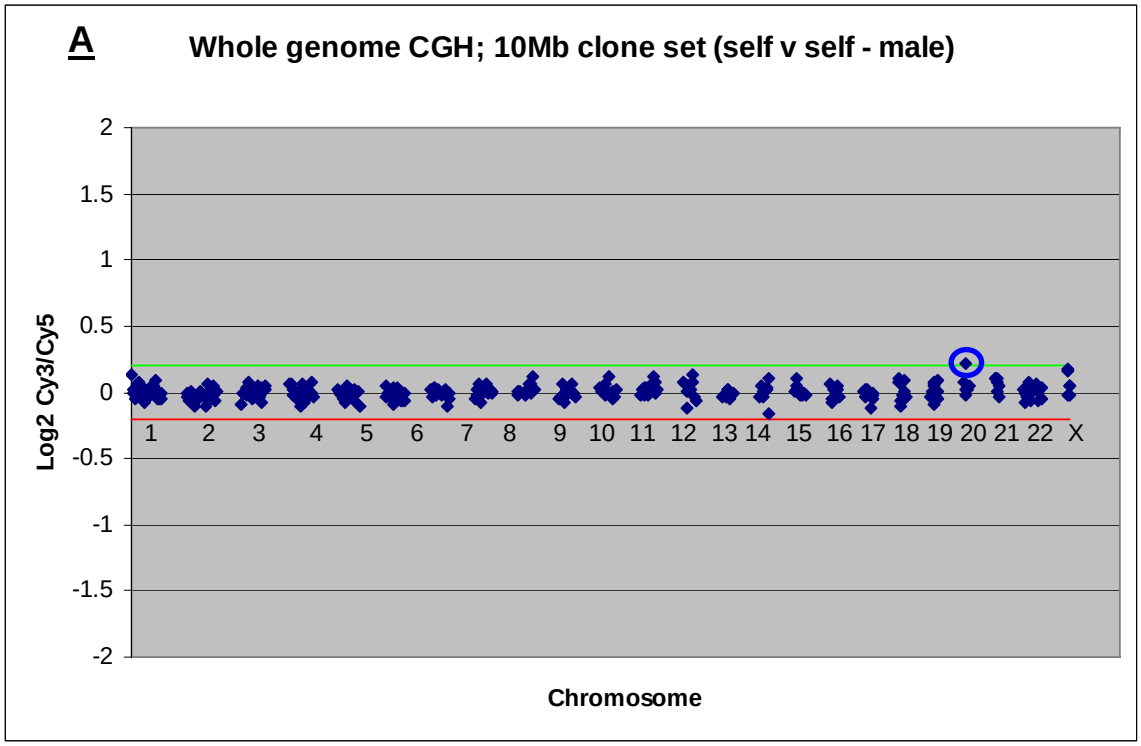
All fosmid clones were spotted in triplicate and the $\log_2$ ratio (Cy3/Cy5 intensities) of each spot within a trio was compared with the other two spots. If all 3 spots were within 2 standard deviations of the mean, the ratio was used for analysis. If the standard deviation of all 3 clones was found to be greater than 2, the standard deviation for all 3 combinations of 2 spots was calculated and if 2 clones within each trio were found to be within 2 standard deviations of the mean, they were used for the analysis, and the other spot discounted. Any clones where any 2 of the 3 spots fell outside 2 standard deviations of the mean were excluded from further analysis.

3.3.1 Testing the custom-made fosmid microarray

Prior to using the fosmid microarray for breakpoint mapping of patient rearrangements, the clones on the array were tested using a self versus self hybridisation and a test sample with known amplifications and deletions. Using a $\log_2$ scale, a clone showing a heterozygous deletion will report a ratio close to -1 and a clone showing a duplication will report a ratio close to 0.58. Clones which reported unexpected ratios in these experiments were excluded from further analyses. Thresholds were set by adding or subtracting 4 standard deviations of the autosomal clone $\log_2$ ratios to the mean. Clones which displayed ratios above or below these thresholds were deemed to be amplified or deleted respectively.

3.3.1.1 Self versus self hybridisation

The self versus self experiment was performed using a pool of 20 normal male DNAs. One BAC clone on chromosome 20 (RP1-64G16) was identified as showing a significantly high ratio. All fosmid clones (Figure 3.2) were seen to lie close to a $\log_2$ ratio of zero indicating that there was no difference in hybridisation between samples with the exception of G248P87165E3 on chromosome 5 and G248P81609G9 on chromosome 11. Because the samples hybridised to the microarray were the same, the discrepancy in hybridisation to these clones must have arisen from a dye bias between the Cy3 and Cy5 dCTP labelled samples. RP1-64G16, G248P87165E3 and G248P81609G9 were excluded from further analyses on the basis of these results.


A
Whole genome CGH; 10Mb clone set (self v self - male)
Log2 Cy3/Cy5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X
Chromosome

B

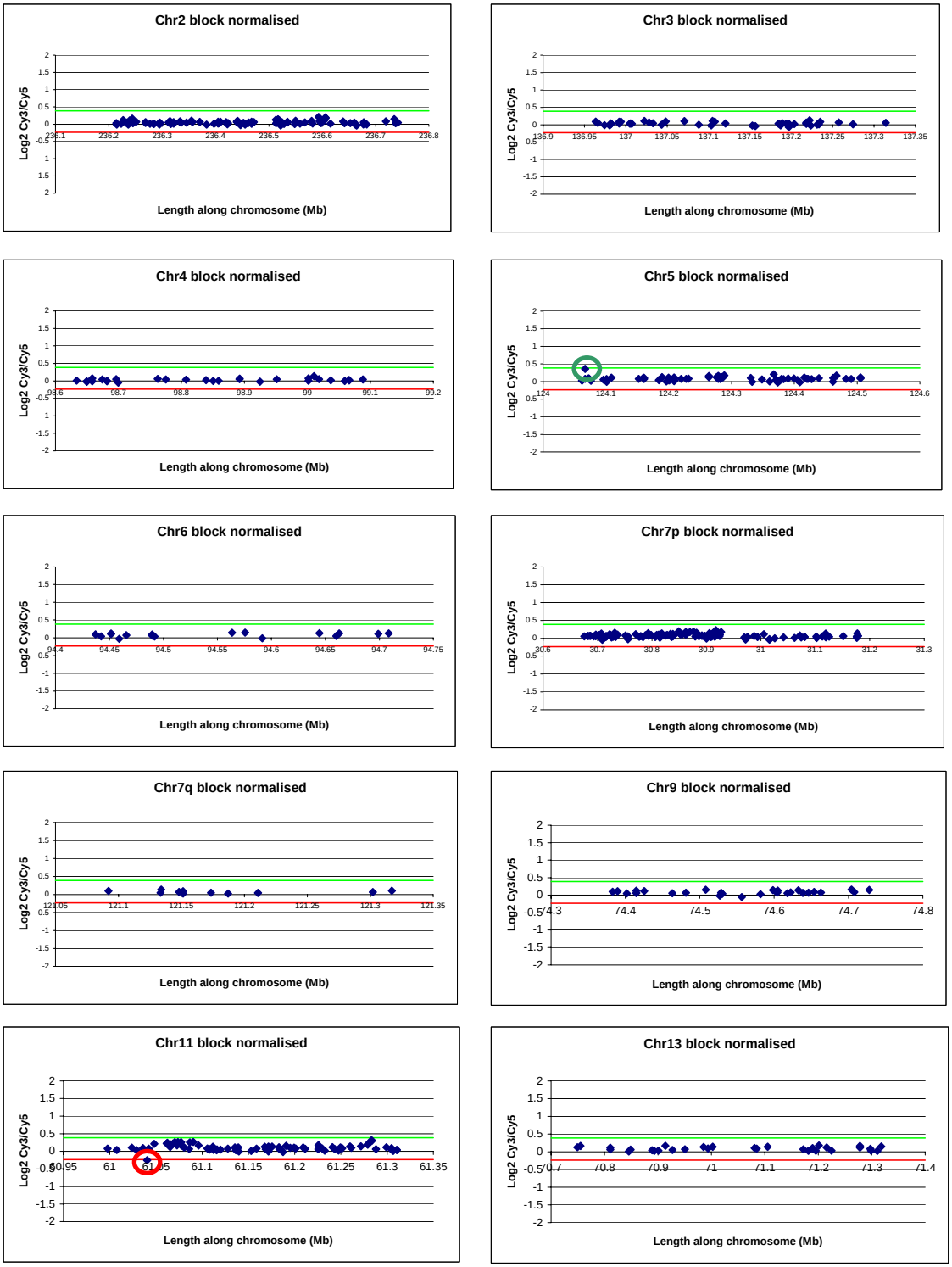


Figure 3.2 Self versus self CGH hybridisation onto custom-made fosmid microarray; A 10Mb clones, B fosmid clone results. RP1-64G14 G248P87165E3 G248P81609G9.

3.3.1.2 769p hybridisation

To investigate whether the clones on the microarray in general would respond correctly to a change in copy number, a primary renal cell adenocarcinoma cell line, 769p, well characterised with respect to copy number gains and losses was hybridised to the microarray.

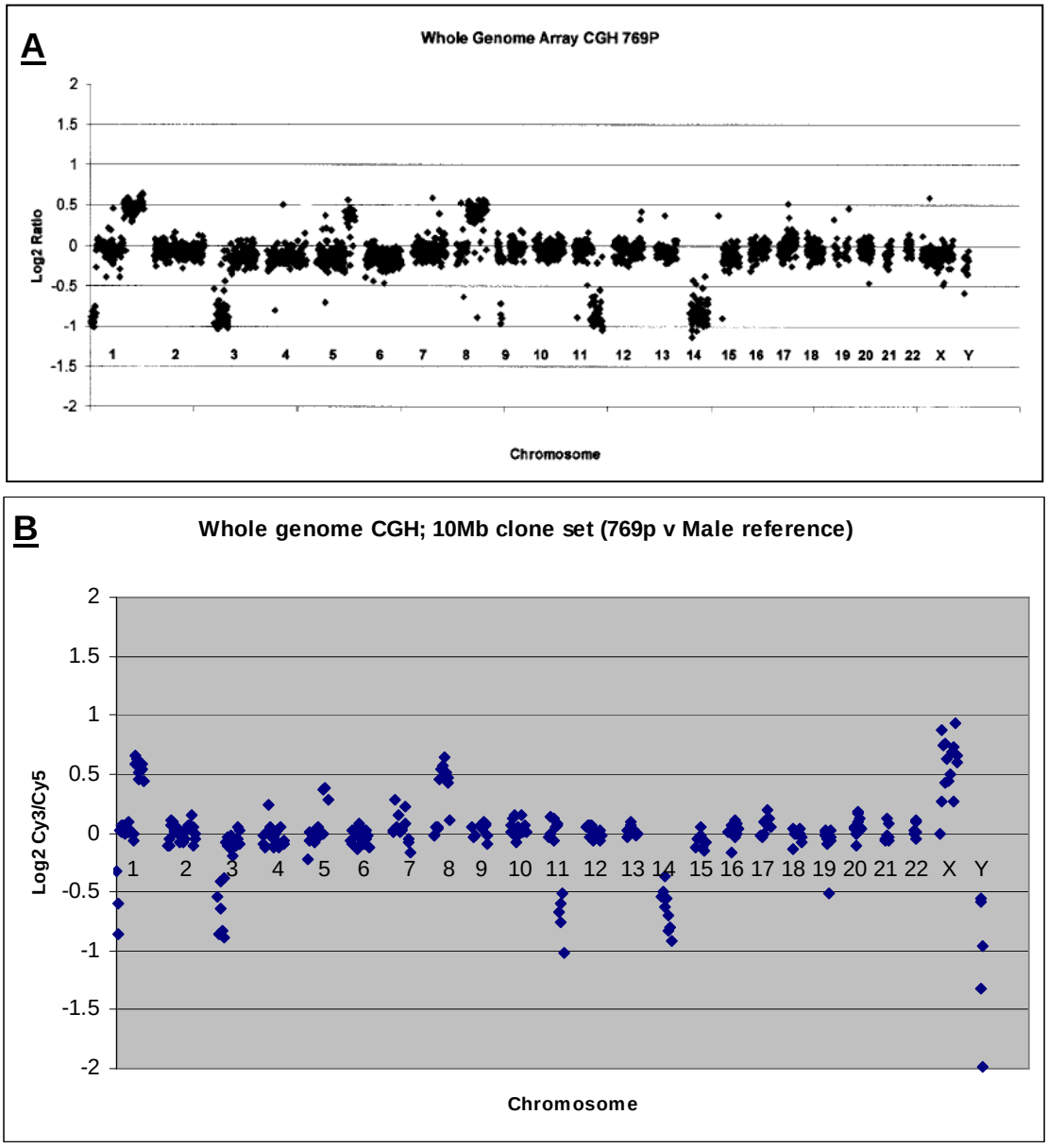


Figure 3.3 ***A Taken from Fiegler et al 2003 (Fiegler et al. 2003a). Array CGH profile of renal cell carcinoma cell line 769p against a female reference on a 1 Mb resolution whole genome microarray. B Results for large insert clones at 10Mb resolution in a 769p versus male reference hybridisation on custom-made fosmid microarray.***

The large insert clones at 10Mb resolution on the custom-made fosmid microarray reported regions of copy number gain (1q, 5q, 8q) and copy number loss (1p, 3p, 9p, 11q, 14) comparable to those reported by Fiegler *et al* on a 1Mb resolution microarray (Fiegler et al. 2003a) (Figure 3.3).

Whilst none of the regions covered by the fosmids on the array were in areas of amplification or deletion within the cell line (Figure 3.4) this 769p versus reference hybridisation was used to verify that none of the fosmids responded to regions of gain or loss within the cell line. There were 3 individual fosmid clones which reported a significant shift in ratio; G248P8541B6 which reported a gain on chromosome 7p and clones G248P87179H5 and G248P88359H5 which reported losses on chromosome 11. As these fosmid clones lay in regions of high redundancy of fosmid clones and as all other overlapping clones behaved as expected it was likely that these clones were mispicked or mismapped.

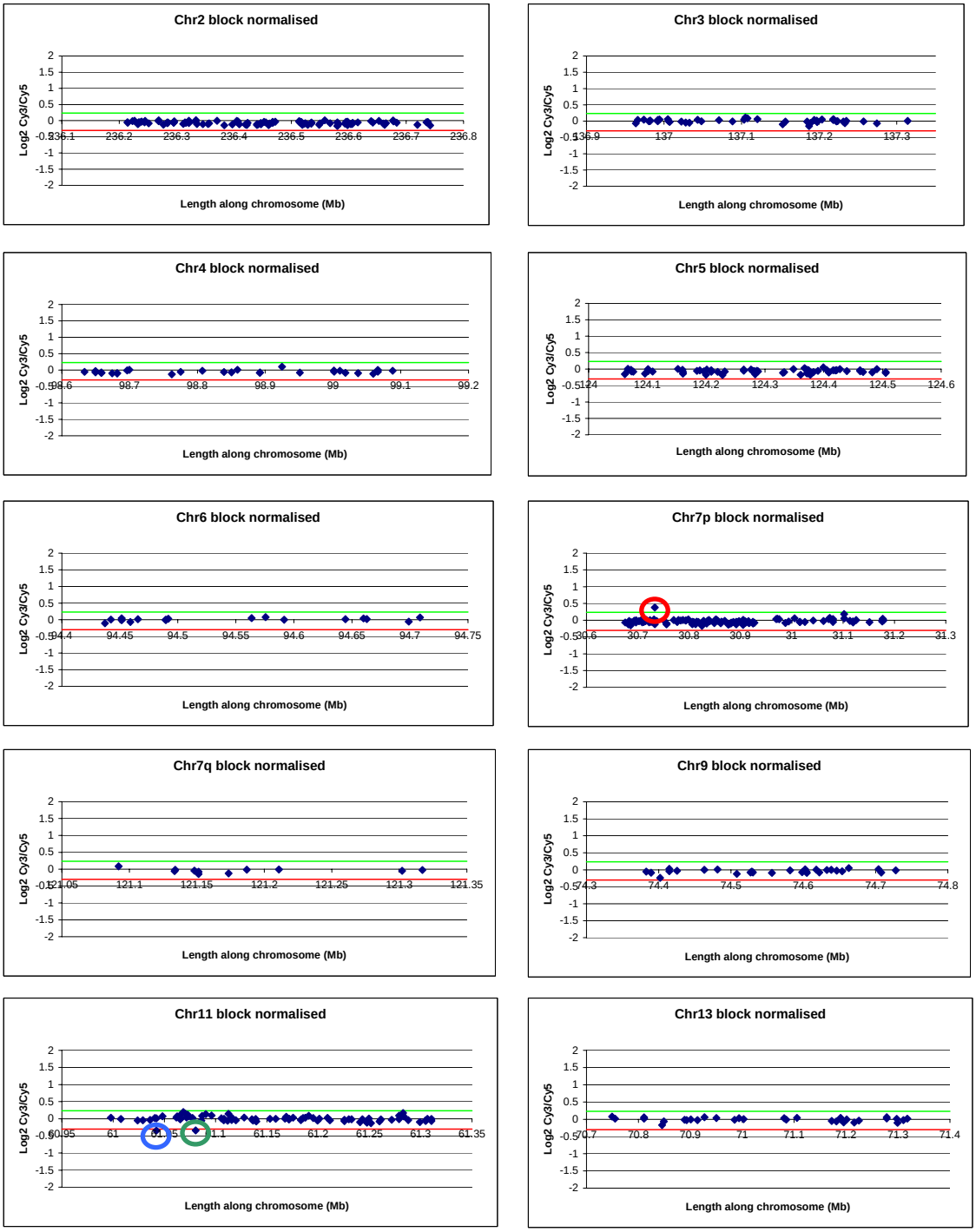
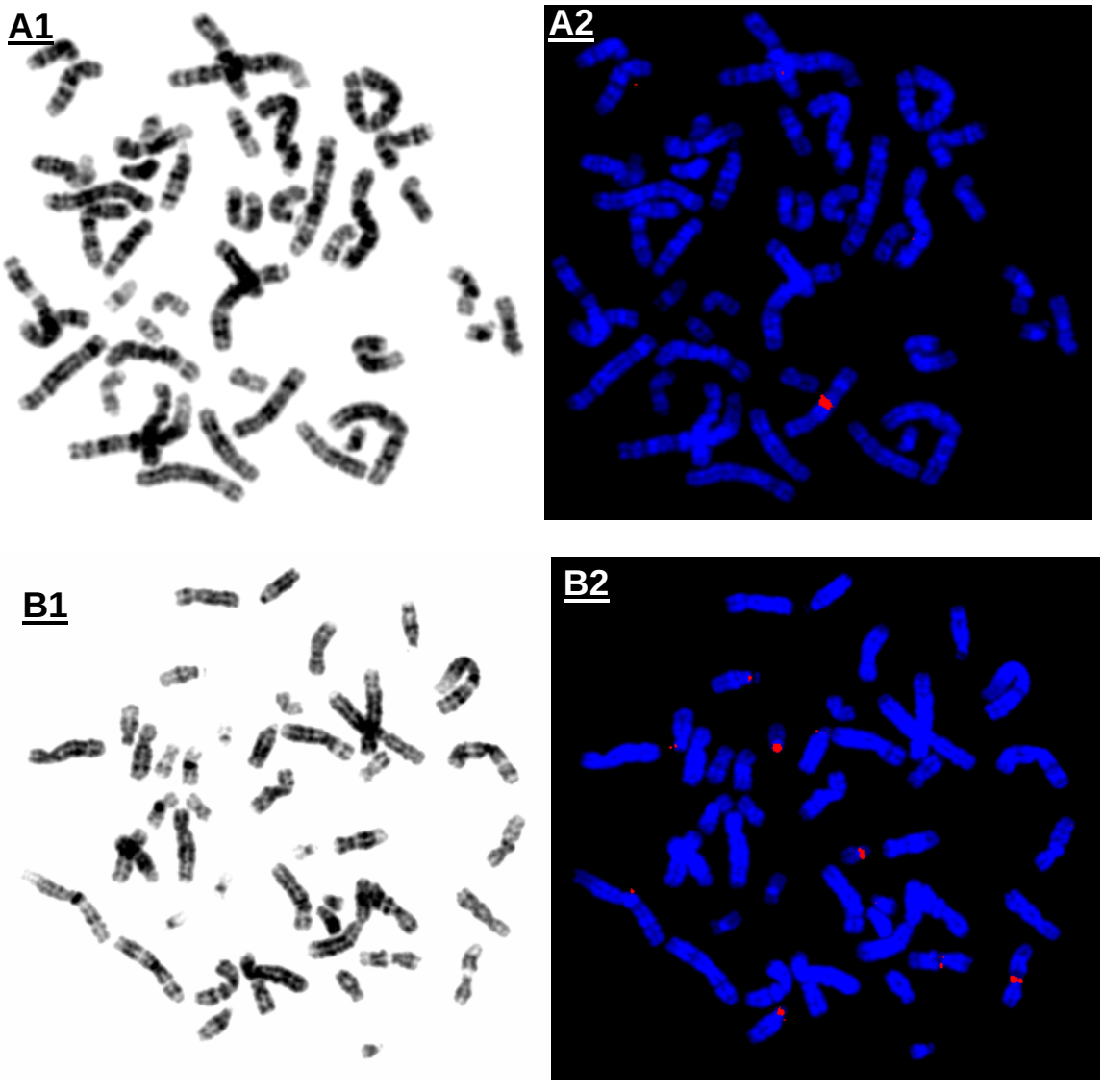


Figure 3.4 CGH results for renal cell carcinoma cell line on custom-made fosmid microarray for fosmid clones. G248P8541B6 G248P87179H5 G248P88359H5.

The three clones showing unexpected ratios were mapped by FISH to verify their chromosomal locations (Figure 3.5). G248P8541B6 hybridised to Xq13.1-Xq13.3

not 7p as expected. The elevated ratio seen by this clone after the CGH experiment was also seen for the other chromosome X clones spotted on the microarray. G248P87179H5 gave signals on the acrocentric chromosome centromeres (13, 14, 15, 21, 22). G248P88359H5 showed a signal on 11q13.1-11q13.4 as well as the centromere of chromosome 3 and the acrocentric chromosomes. The FISH data unequivocally explained the elevated ratio results seen after microarray hybridisation, and these clones were excluded from further analysis.



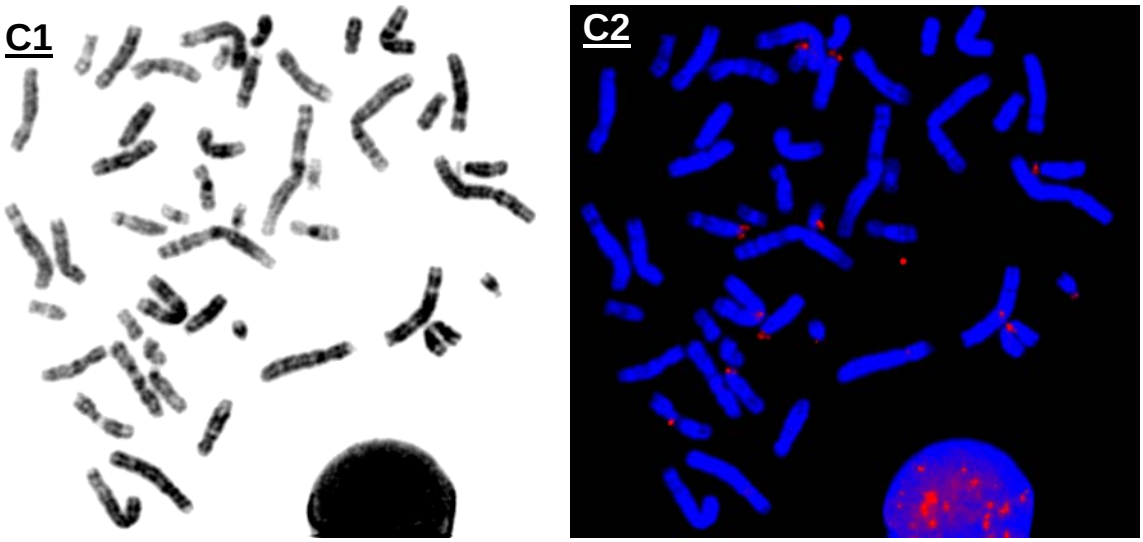


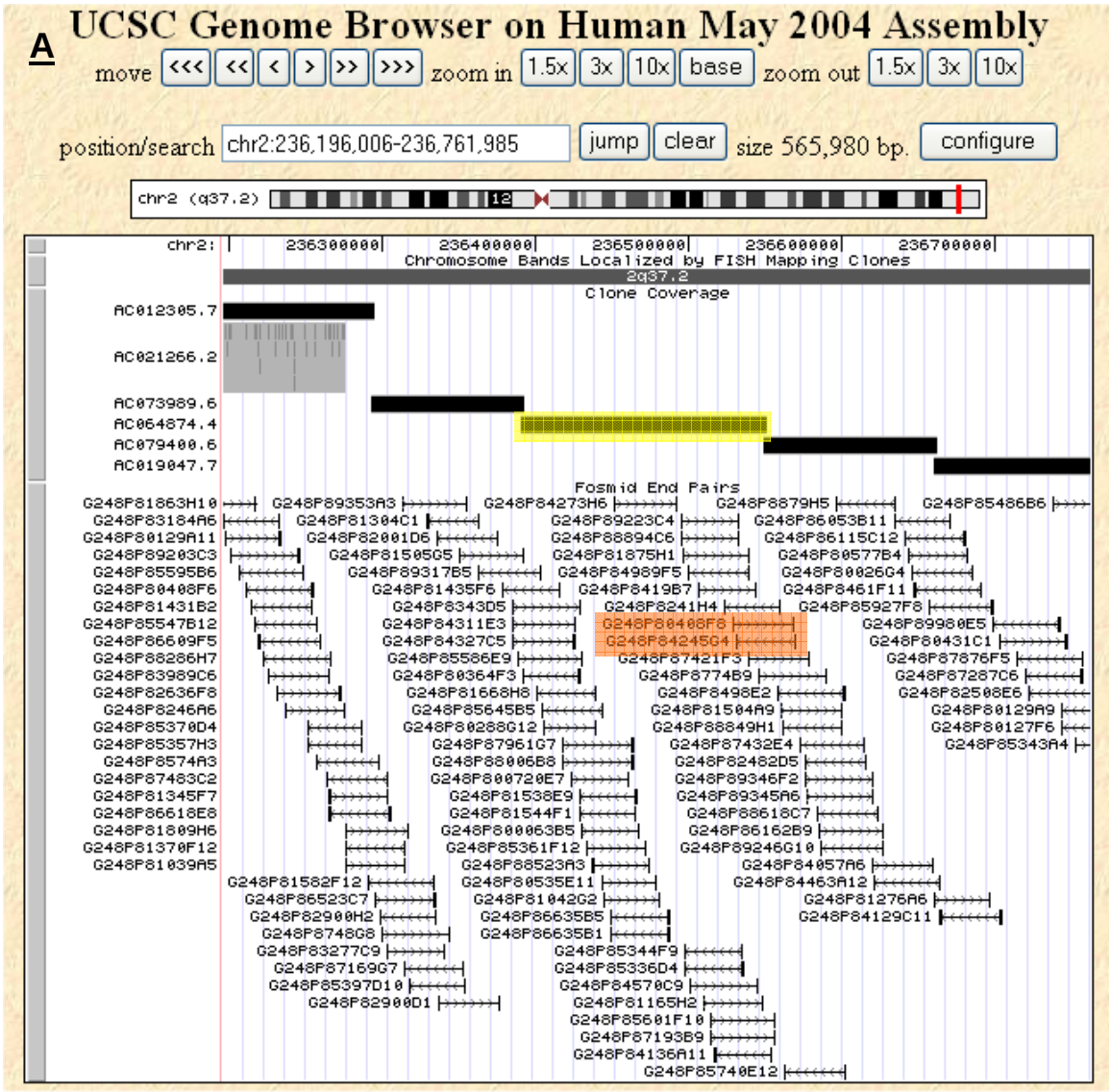
Figure 3.5 FISH results for clones showing unexpected ratios in a 769p versus reference array CGH hybridisation. 1 inverted DAPI banding of metaphase chromosomes, 2 metaphase FISH image. Results for A G248P8541B6 on Xq13.1-Xq13.3, B G248P87179H5 on the centromeres of acrocentric chromosomes and C G248P88359H5 on 11q13.1-11q13.4 and the centromeres of chromosomes 3, 13, 14, 15, 21 and 22.

3.3.2 Array painting analysis of patient derivative chromosomes on custom-made fosmid microarrays

Previous FISH analysis identified spanning BAC clones for all the three patient translocation breakpoints (Gribble et al. 2005). To further refine the approximately 150Kb regions defined by these spanning clones, derivative chromosomes were array painted onto the custom-made DNA fosmid microarray. This method was used to refine both breakpoints for patients t(2;7)(q37.3;p15.1) and t(7;13)(q31.3;q21.3), but only the chromosome 11 breakpoint for patient t(3;11)(q21;q12) as the chromosome 3 breakpoint had been refined to 2.2Kb prior to the start of this project.

3.3.2.1 Patient t(2;7)(q37.3;p15.1)

Chromosome 2 and 7 breakpoint spanning regions were well represented on the array with highly redundant and contiguous fosmid clone coverage (Figure 3.6).



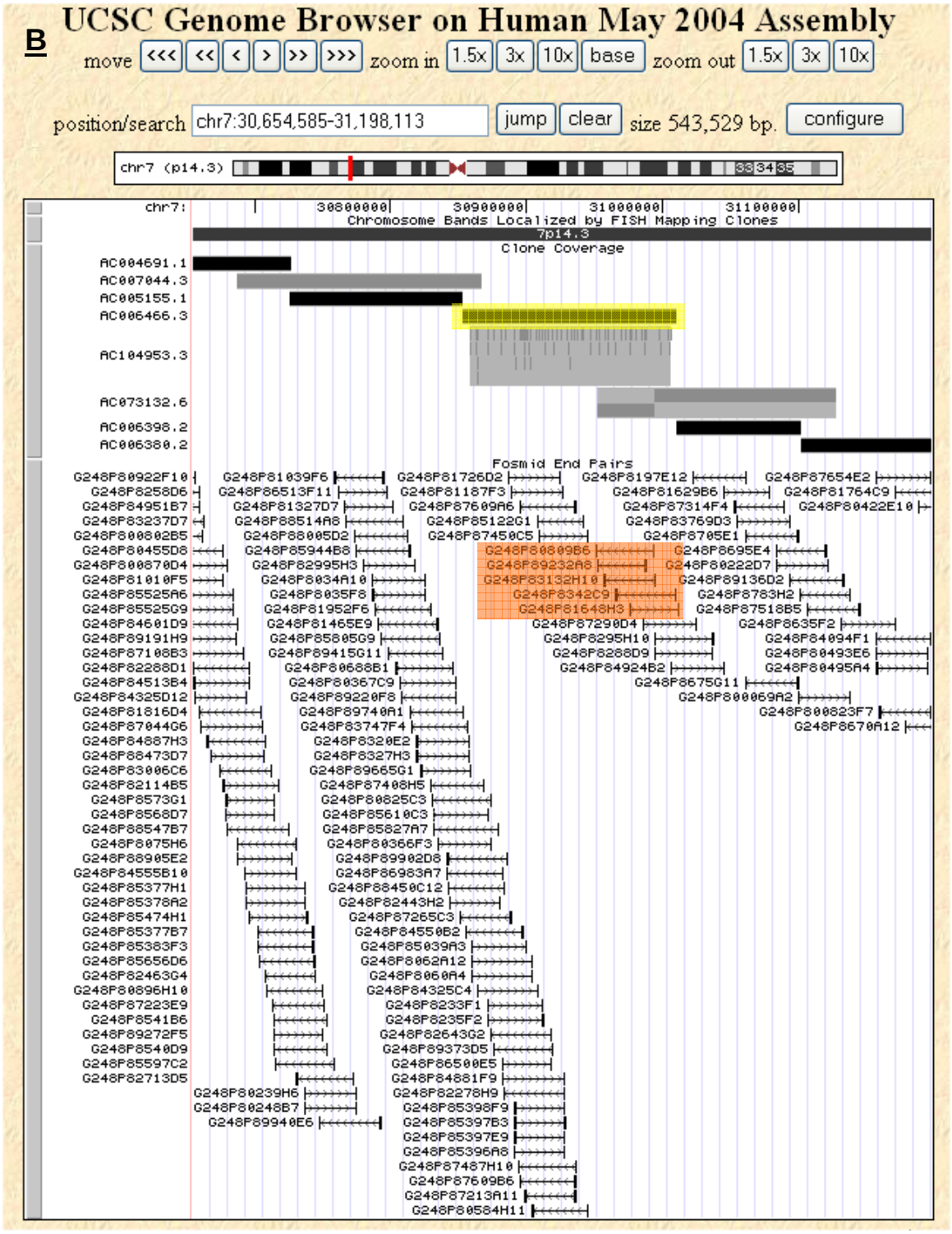


Figure 3.6 ***UCSC view showing fosmid coverage across A chromosome 2 and B chromosome 7 regions of interest. The spanning BAC for chromosome 2 was RP11-680O16, the chromosome 7 spanning BAC clone was CTA-471E18. No BAC end sequence was available for either spanning clone. Spanning BAC clones are highlighted in yellow. Spanning fosmid clones identified by the fosmid array are highlighted in orange.***

Array painting analysis showed a clear transition from high to low ratios for both chromosome 2 and 7 fosmid clones, indicating a simple translocation between the two chromosomes (Figure 3.7).

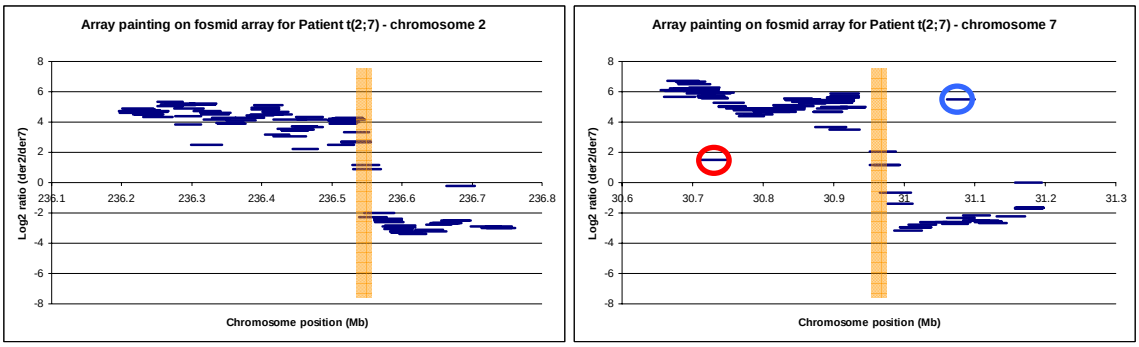


Figure 3.7 Array painting analysis of patient t(2;7)(q37.3;p15.1) using the custom-made fosmid microarray. G248P87223E9 G248P8675G11. The approximate breakpoint region is highlighted in orange.

Analysis of the chromosome 2 clones identified two spanning fosmids; G248P80408F8 at 236,528,698 to 236,567,965bp and G248P84245G4 at 236,530,153 to 236,569,814bp. This reduced the identified chromosome 2 breakpoint region from 150Kb to less than 38Kb. Array painting analysis of the chromosome 7 region identified 5 potential spanning clones; G248P89232A8, G248P80809B6, G248P83132H10, G248P8342C9 and G248P81648H3. Array painting has previously been shown to be a quantitative method (Fiegler et al. 2003b), therefore, the breakpoint containing region could be narrowed down to the 12Kb minimum overlap region from 30,975,849 to 30,987,680bp covered by all of these clones.

Figure 3.7B showed two fosmid clones in regions of good clone coverage with unexpected ratios; G248P87223E9 and G248P8675G11. Subsequent analysis by FISH to verify their chromosomal location showed that G248P87223E9 hybridised to 20q11.21-20q11.23 and G248P8675G11 to 2q12-2q14.2 (Figure 3.8) which is proximal to the chromosome 2 translocation breakpoint proving that these clones had been mis-picked during the production of the microarray.

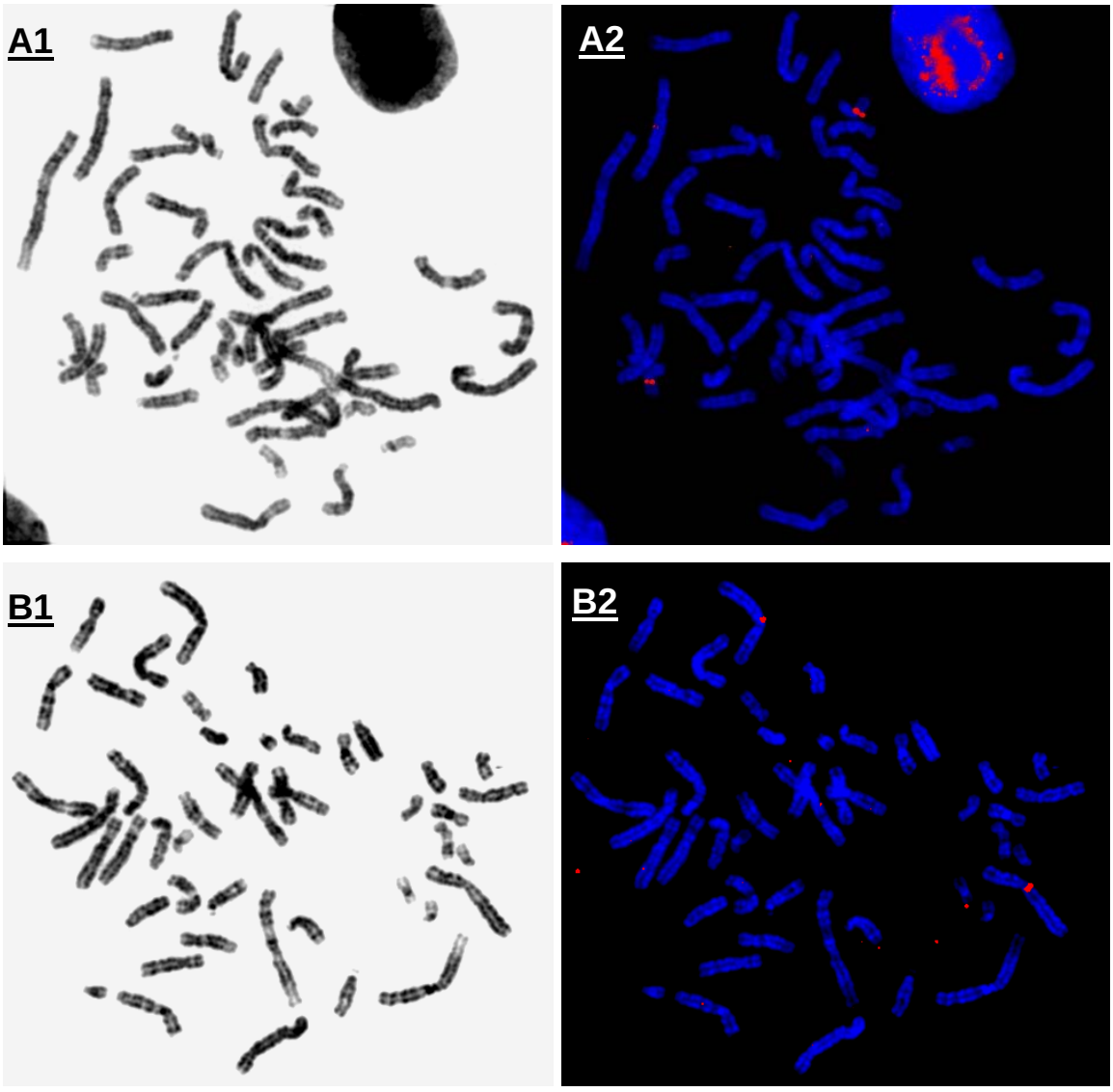


Figure 3.8 FISH results for clones showing unexpected ratios in the patient t(2;7)(q37.3;p15.1) array painting hybridisation; 1 DAPI banding of metaphase chromosomes, 2 metaphase FISH image. A G248P97223E9 at 20q11.21-20q11.23 and B G248P8675G11 at 2q12-2q14.2.

Utilising the $\log_2$ ratios of the clones spanning a chromosome breakpoint it was possible to predict the breakpoint location to within a few kilobases. Excel interpolation analysis of the array data (using the midpoint and $\log_2$ ratio for each spanning clone) predicted the chromosome 2 breakpoint to be at 236,556,071bp and the chromosome 7 breakpoint to be at 30,984,039bp (Figure 3.9).

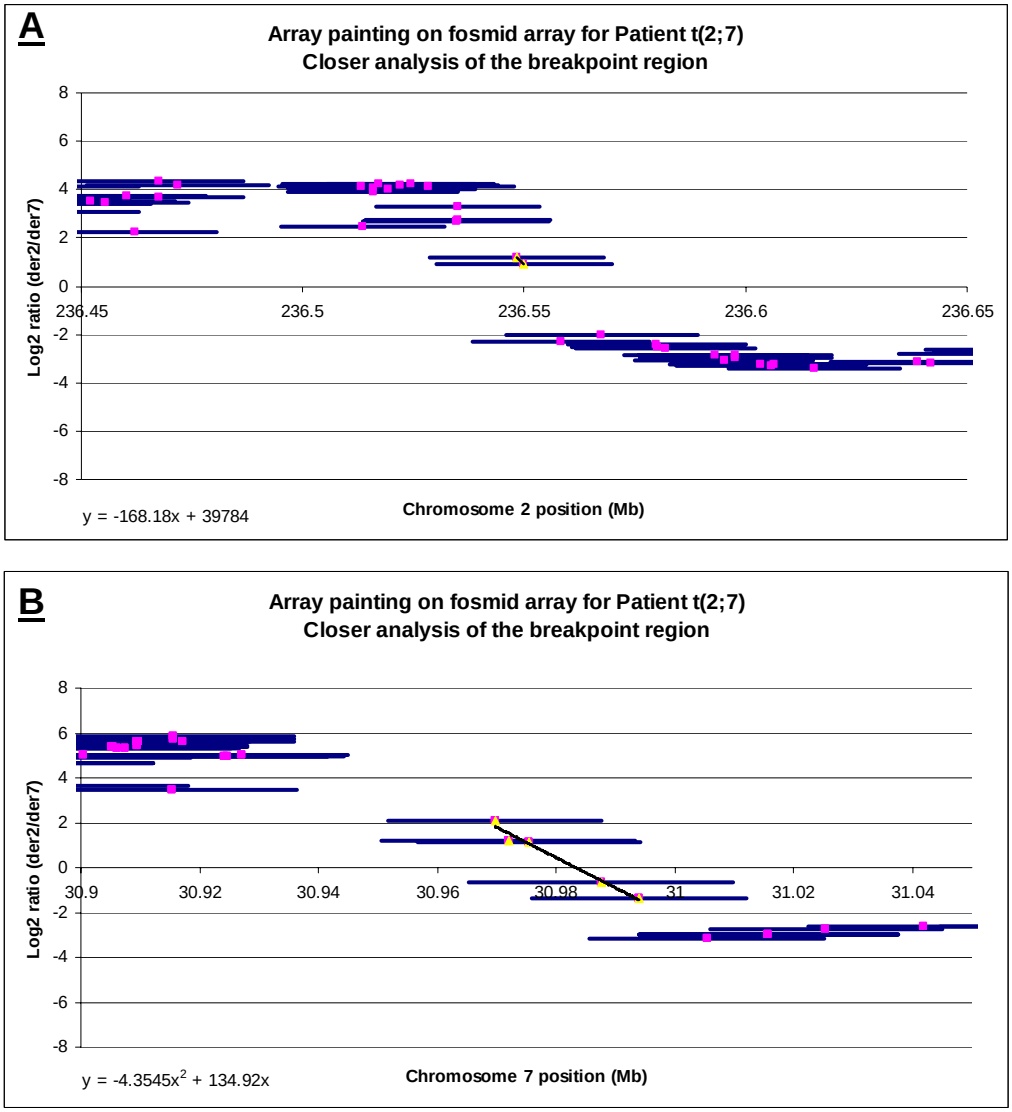


Figure 3.9 Analysis of the array data around the breakpoints for A chromosome 2 and B chromosome 7 for patient t(2;7)(q37.3;p15.1). The pink spots mark the midpoint of each clone.

3.3.2.2 Patient t(3;11)(q21;q12)

For patient t(3;11)(q21;q12) only chromosome 11 clones were included on the array as the chromosome 3 breakpoint had been refined to 2.2Kb previously by STS PCR analysis.

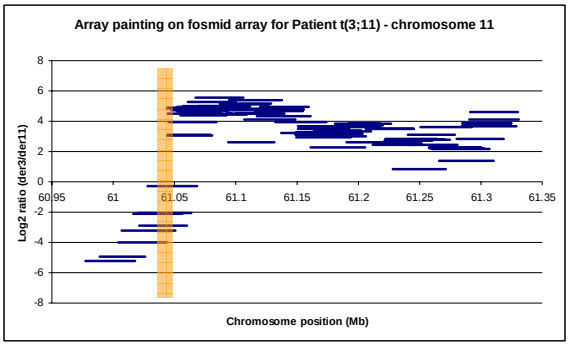


Figure 3.10 Chromosome 11 array painting results for patient t(3;11)(q21;q12) on the custom-made fosmid microarray. The breakpoint containing region is highlighted in orange.

Array painting analysis mapped the chromosome 11 breakpoint to a 23Kb region from 61,027,847 to 61,050,897bp contained within the overlapping clones which showed intermediate ratios on the array; G248P88450F7, G248P80771G8, G248P81609G9, G248P8917A11, G248P87074D8 and G248P82315G10 as highlighted in Figure 3.10 and Figure 3.11.

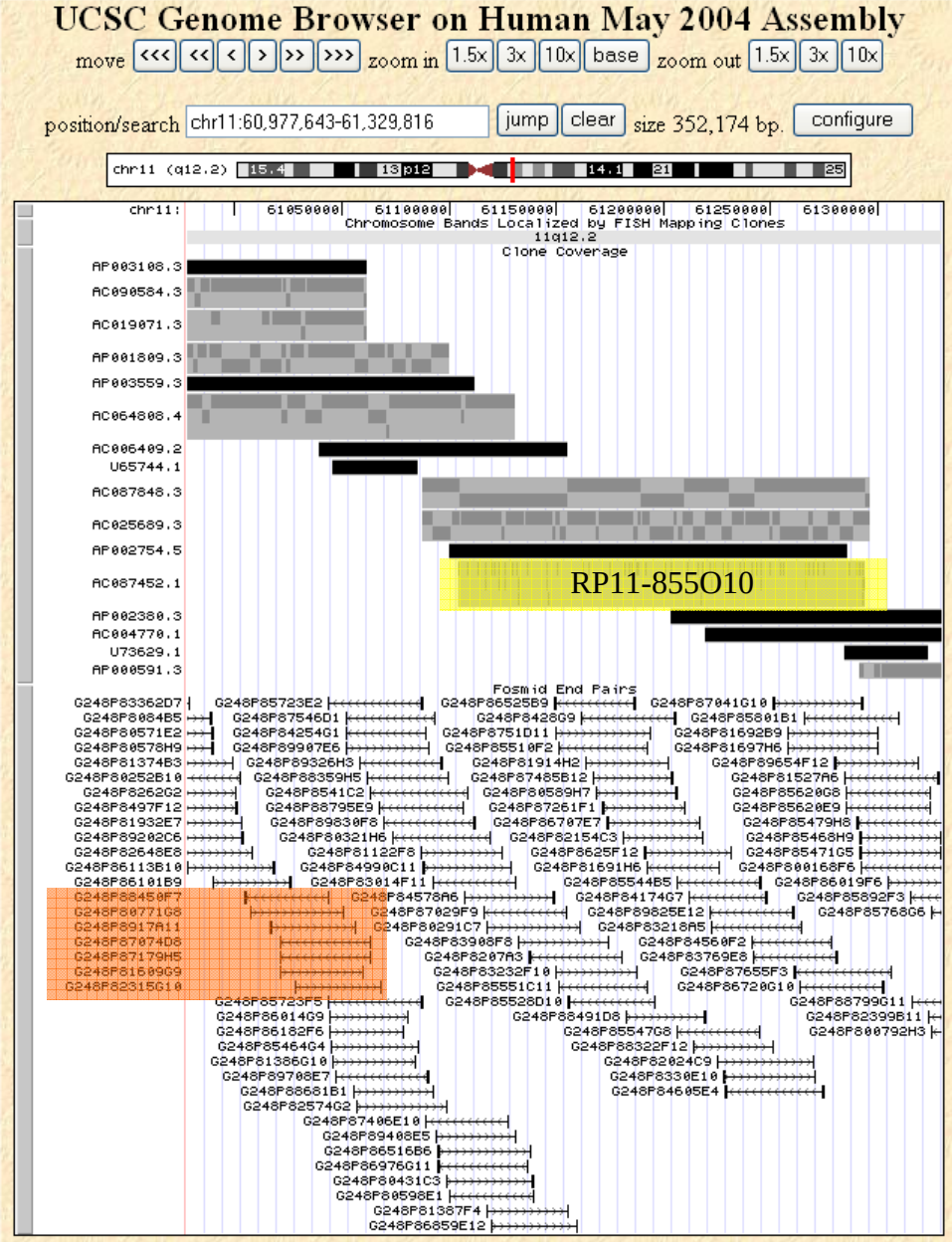


Figure 3.11 UCSC page showing fosmid coverage across the chromosome 11 breakpoint spanning region for patient t(3;11)(q21;q12). The accessioned sequence for the spanning BAC RP11-855O10 is highlighted in yellow. No BAC end sequence was available for this clone. The spanning fosmids are highlighted in orange.

The apparent discrepancy between the spanning BAC clone and the spanning fosmid clones is due to the lack of end sequence data for RP11-855O10. Only the accessioned sequence is represented in Figure 3.11. The full insert length is likely to be much longer.

3.3.2.3 Patient t(7;13)(q31.3;q21.3)

Full fosmid clone coverage was not available for either of the breakpoint regions in patient t(7;13)(q31.3;q21.3). For the genomic regions identified by the spanning BACs, there was a gap of 49Kb in the chromosome 7 fosmid map from 121,232,180 to 121,281,674bp and a gap of 38Kb in the chromosome 13 map from 71,022,384 to 71,060,273bp (Figure 3.13).

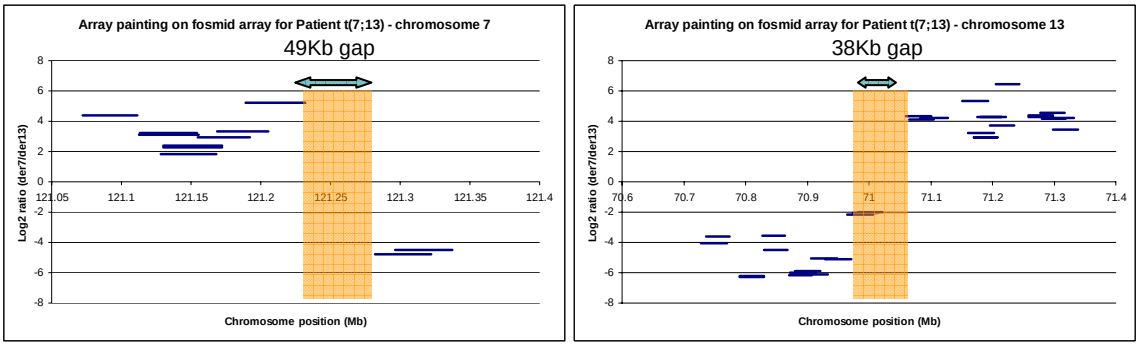
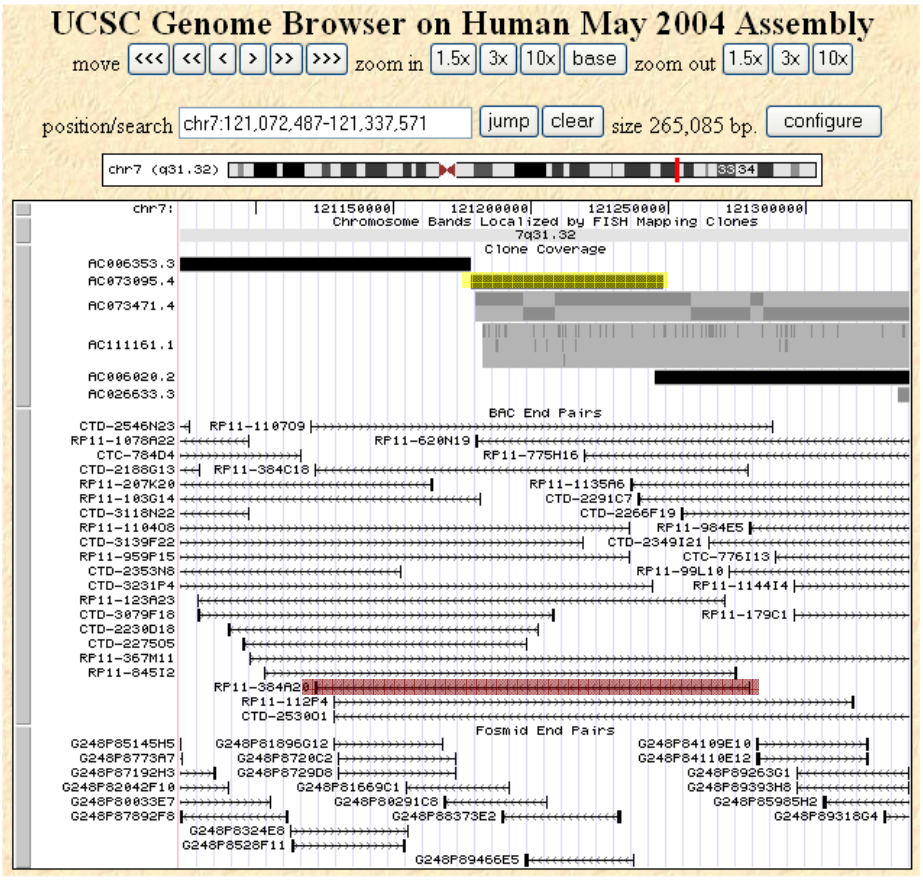


Figure 3.12 Array painting results for patient t(7;13)(q31.3;q21.3) on the custom-made fosmid microarray. Orange arrows depict the approximate breakpoint regions identified.

Array painting (Figure 3.12) refined the chromosome 7 breakpoint to between clones G248P88373E2 and G248P84109E10 corresponding to the 38Kb gap in the fosmid coverage. The chromosome 13 breakpoint appeared to lie towards the distal end of the clones showing an intermediate ratio of approximately -2; G248P88693H1, G248P83744B9 and G248P85941D11. These fosmids lie next to a 38Kb gap from 71,022,389 to 71,060,273bp. Although there were gaps in the fosmid coverage for this region, the spanning BAC clone had been fully sequenced so sequence was available for STS PCR mapping.

A



B

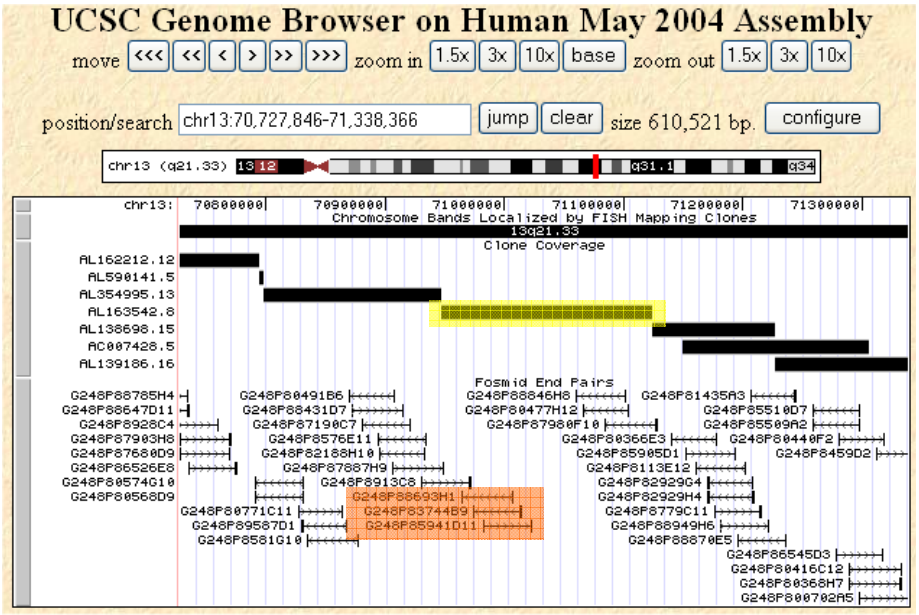
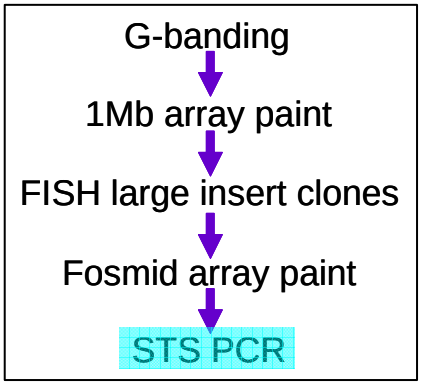


Figure 3.13 ***UCSC download for A chromosome 7 and B chromosome 13 regions of interest for patient t(7;13)(q31.3;q21.3). Spanning BAC clones are highlighted in yellow, and spanning fosmids from the array paint experiment are highlighted in orange. A Spanning BAC RP11-384A20 has been end sequenced and is highlighted in red. B No BAC end sequence was available for RP11-360I23.***

3.4 Further refinement of chromosome translocation breakpoints by STS PCR, PCR microarrays and oligonucleotide microarrays

3.4.1 Refinement of breakpoints by STS PCR



Previous array painting experiments delineated the translocation breakpoints to genomic regions varying from 12-49Kb. Breakpoints were further refined by STS PCR analysis to enable the direct amplification of junction fragments by LR PCR. Genomic regions of interest known to contain the chromosome breakpoints were extended both proximally and distally by a further 3Kb and the sequence exported from NCBI Build 35 for STS PCR primer design (see methods for primer parameters and Appendix A3 for primer sequence).

3.4.1.1 Patient t(2;7)(q37.3;p15.1)

STS PCR primers spaced at 10Kb intervals were designed to cover the chromosome 2 and 7 spanning fosmids. The first round of STS PCR refined the chromosome 2 breakpoint to a 10Kb region from 236,557,221 to 236,567,273bp between primer pair 4 which gave a weak band on derivative 2 and primer pair 5 which gave a strong band on derivative 7 (Figure 3.14).

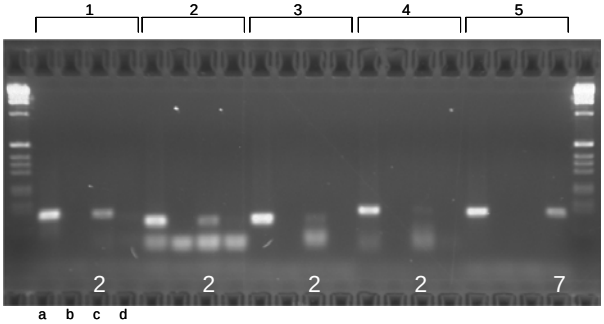


Figure 3.14 Chromosome 2 STS PCR for patient t(2;7)(q37.3;p15.1) at 10Kb resolution resolved the breakpoint to between primers 4 and 5. Templates used; a) whole genomic DNA from a reference sample as a positive control, b) water as a negative control, c) DOP amplified flow-sorted derivative 2, d) DOP amplified flow-sorted derivative 7.

In a subsequent round of PCR using primers designed at 1Kb resolution within this region, only 2 primers gave informative results; both on the derivative 7 (Figure 3.15).

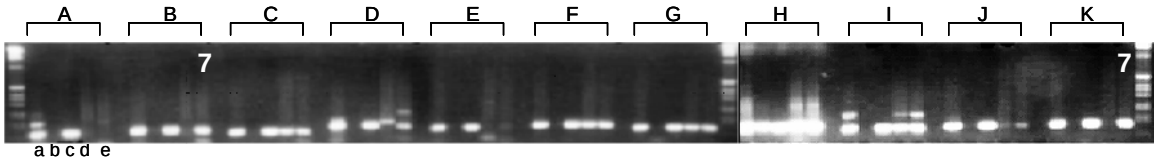


Figure 3.15 Chromosome 2 STS PCR results for patient t(2;7)(q37.3;p15.1) at 1Kb resolution. All informative primers gave products on the derivative 7. Templates used; a) whole genomic DNA from a reference sample as a positive control, b) water as a negative control, c) chromosome 2 spanning BAC clone DNA, d) DOP amplified flow-sorted derivative 2, e) DOP amplified flow-sorted derivative 7.

A further set of primers were designed every 1Kb extending proximal to primer pair 4 to primer pair 3. These primer pairs (L to U) showed a transition from positive PCR products obtained on the derivative 2 (M) to the derivative 7 (Q) (Figure 3.16) which indicated that the breakpoint was between primer pairs M and Q (236,548,584 to 236,553,794bp). Primer pairs N, O and P failed to amplify a product on either derivative chromosome template DNA.

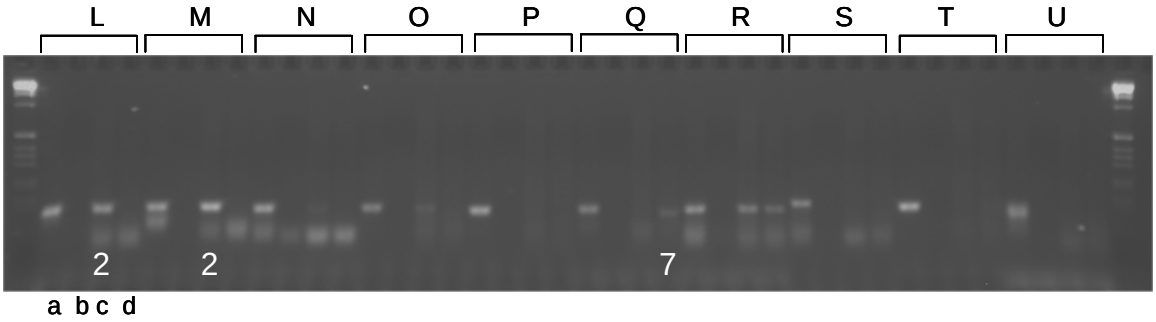


Figure 3.16 Chromosome 2 STS PCR results at 1Kb resolution for patient t(2;7)(q37.3;p15.1) refined the breakpoint region to between primers M and Q. Templates used; a) whole genomic DNA from a reference sample as a positive control, b) water as a negative control, c) DOP amplified flow-sorted derivative 2, d) DOP amplified flow-sorted derivative 7 material.

This data suggested that the weak primer pair 4 result obtained during the 10Kb resolution STS PCR was misleading, and that the breakpoint actually lay between primer pairs 3 and 5. This second round of PCR at 1Kb resolution narrowed the breakpoint to a 5,210bp region from 236,548,584 to 236,553,794bp.

For the chromosome 7 breakpoint, an initial round of STS PCR using primers designed at a 10Kb resolution showed the breakpoint was within a 19,294bp region from 30,979,917 to 30,999,211bp between primers 2 and 4 (Figure 3.17). This region is double the theoretical region that should be resolved using primers at 10Kb resolution as the primer pair in between (primer 3) failed to give a clean PCR result.

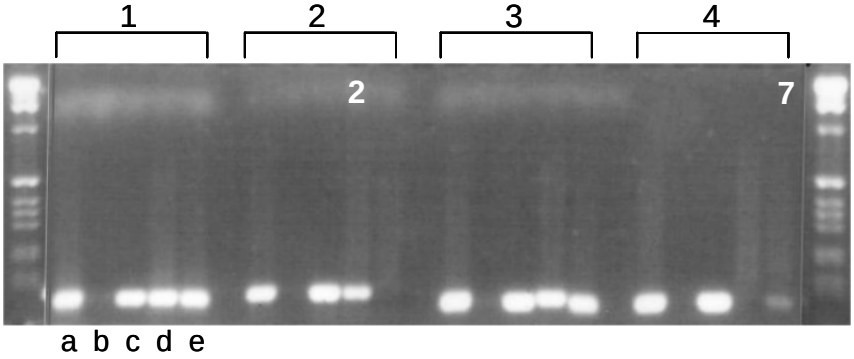


Figure 3.17 Chromosome 7 STS PCR results for patient t(2;7)(q37.3;p15.1) using primers spaced approximately 10Kb apart run with 1Kb marker. Templates used; a) whole genomic DNA from a reference sample as a positive control, b) water as a negative control, c) Chromosome 7 spanning BAC clone, d) DOP amplified flow-sorted derivative 2 and e) DOP amplified flow-sorted derivative 7.

A second round of PCR using primers spaced at approximately 1Kb intervals (Figure 3.18) only managed to refine the breakpoint region to 17,251bp from 30,981,960 to 30,999,211bp between primer B and primer 4 from the initial round of PCR. Half of the primer pairs (D,E,H,I,J,M,O,Q,R) designed across this ~20Kb region produced products on both the derivative 2 and derivative 7 template DNA. Analysis of the 20Kb region of chromosome 7 used to design primers revealed large regions of homology with other regions of chromosome 7 and

regions along chromosome 2 which may explain these double positive results (see Discussion chapter for further analysis of this region).

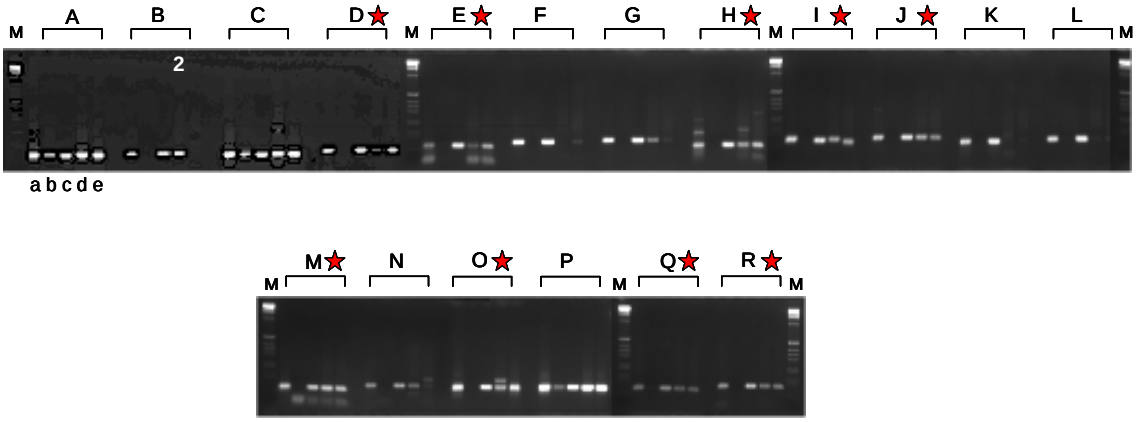


Figure 3.18 Chromosome 7 STS PCR results for patient t(2;7)(q37.3;p15.1) using primers spaced approximately 1Kb apart between primers 2 and 4 from Figure 3.17 run with 1Kb marker. Templates used; a) whole genomic DNA from a reference sample as a positive control, b) water as a negative control, c) Chromosome 7 spanning BAC clone, d) DOP amplified flow-sorted derivative 2 and e) DOP amplified flow-sorted derivative 7.

Alternative strategies of array painting on a PCR product array (3.4.2) and an oligonucleotide array (3.4.3) were investigated to refine the breakpoint region to a level sufficient for LR PCR.

3.4.1.2 Patient t(3;11)(q21;q12)

The chromosome 3 translocation breakpoint had been previously investigated prior to the start of this project. Briefly; STS PCR mapping across the spanning BAC clone sequence at approximately 30Kb resolution mapped the translocation breakpoint to a 40Kb region from 130,728,241bp to 130,767,994bp on chromosome 3. A subsequent round of PCR using primers spaced approximately every 2Kb across this region refined the breakpoint to 2.2Kb from 130,754,507bp to 130,756,748bp.

For chromosome 11, STS PCR mapping using primers spaced at approximately 10Kb intervals mapped the breakpoint to a 20,422bp region from 61,020,792 to 61,041,214bp between primers 1 and 3 (Figure 3.19).

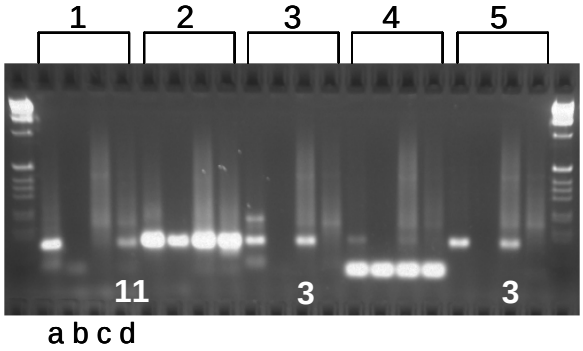


Figure 3.19 Chromosome 11 STS PCR results for patient t(3;11)(q21;q12) at 10Kb resolution resolved the breakpoint to between primers 1 and 3. Templates used; a) whole genomic DNA from reference individual as positive control, b) water as negative control, c) DOP amplified flow sorted derivative 3 material, d) DOP amplified flow sorted derivative 11 material.

A subsequent round of PCR mapping using primers at approximately 2Kb intervals refined the breakpoint region to 7,285bp from 61,026,849 to 61,034,134bp on chromosome 11 between primers C and E (Figure 3.20).

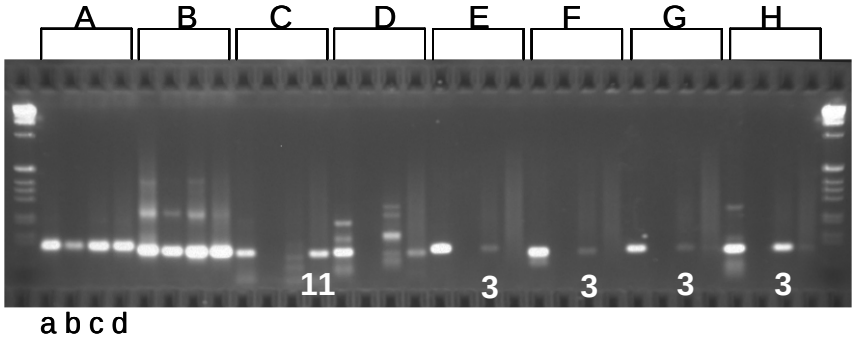


Figure 3.20 Chromosome 11 STS PCR results for patient t(3;11)(q21;q12) at 1Kb resolution refined the breakpoint region to between primers C and E. Templates used; a) whole genomic DNA from reference individual as positive control, b) water as negative control, c) DOP amplified flow sorted derivative 3 material, d) DOP amplified flow sorted derivative 11 material.

3.4.1.3 Patient t(7;13)(q31.3;q21.3)

An initial round of STS PCR at 10Kb resolution mapped the chromosome 7 breakpoint to a 19,851bp region from 121,238,076 to 121,257,927bp between primer 1 which gave a strong band on derivative 7 and primer 3 which gave a weaker band on derivative 13 (Figure 3.21).

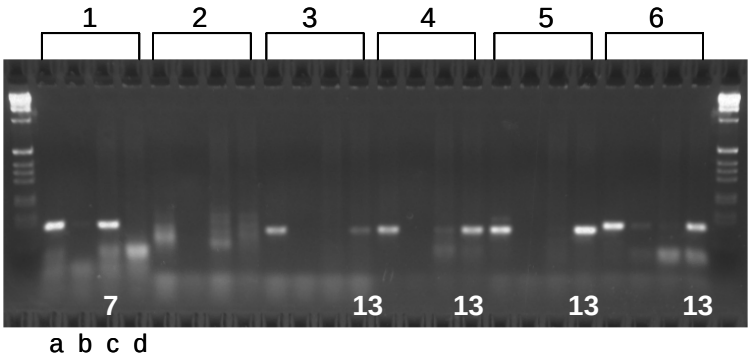


Figure 3.21 Chromosome 7 STS PCR results for patient t(7;13)(q31.3;q21.3) at 10Kb resolution resolved the breakpoint to between primers 1 and 3. Templates used; a) whole genomic DNA from reference individual as positive control, b) water as negative control, c) DOP amplified flow-sorted derivative 7 material, d) DOP amplified flow sorted derivative 13 material.

A subsequent round of PCR refined the breakpoint region to 3,345bp from 121,242,659 to 121,246,004bp (Figure 3.22).

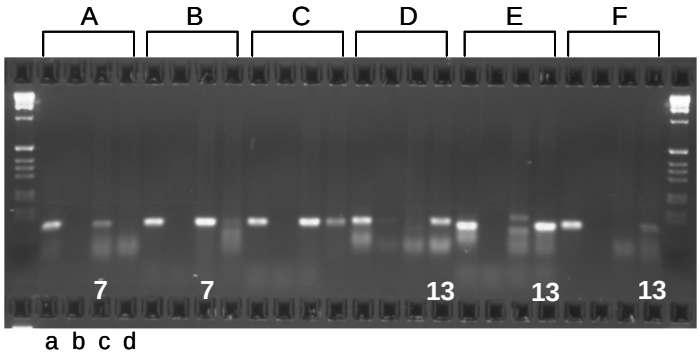


Figure 3.22 Chromosome 7 STS PCR results for patient t(7;13)(q31.3;q21.3) at 1Kb resolution refined the breakpoint region to between primers B and D. Templates used; a) whole genomic DNA from reference individual as positive control, b) water as negative control, c) DOP amplified flow sorted derivative 7 material, d) DOP amplified flow sorted derivative 13 material.

For the chromosome 13 breakpoint, the initial round of STS PCR mapped the breakpoint to a 7,199bp interval between primer pair 2 at 71,016,957bp and primer pair 3 at 71,024,156bp (Figure 3.23).

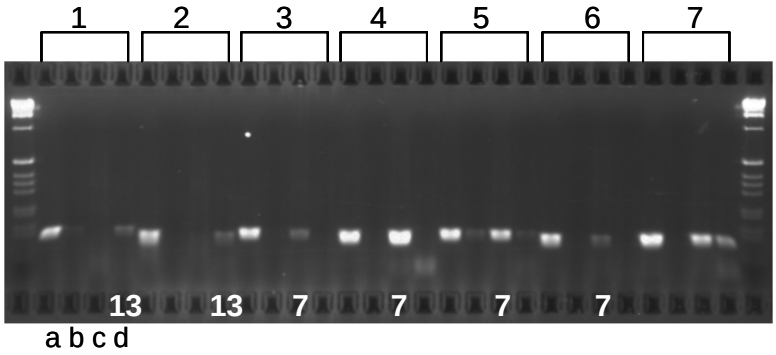


Figure 3.23 Chromosome 13 STS PCR results for patient t(7;13)(q31.3;q21.3) at 10Kb resolution resolved the breakpoint to between primers 2 and 3. Templates used; a) whole genomic DNA from reference individual as positive control, b) water as negative control, c) DOP amplified flow sorted derivative 7 material, d) DOP amplified flow sorted derivative 13 material.

A subsequent round of PCR mapping refined the breakpoint to a 334bp region between primer pairs F and G at 71,023,629 and 71,023,963bp respectively (Figure 3.24).

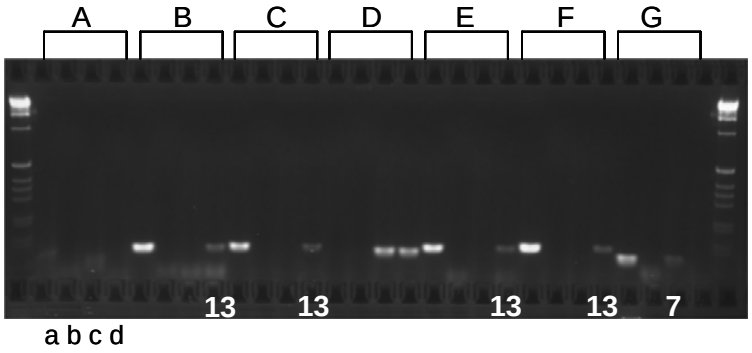


Figure 3.24 Chromosome 13 STS PCR results for patient t(7;13)(q31.3;q21.3). a Initial PCR resolved the breakpoint to between primers 2 and 3. b Subsequent mapping refined the breakpoint region to between primers F and G. Templates used; a) whole genomic DNA from reference individual as positive control, b) water as negative control, c) DOP amplified flow sorted derivative 7 material, d) DOP amplified flow sorted derivative 13 material.

3.4.1.4 Summary of STS PCR data

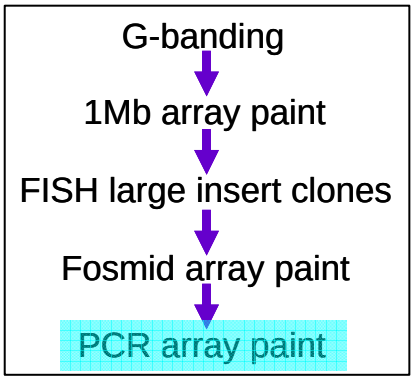
Table 3.2 summarises the mapping of breakpoints by STS PCR. LR PCR can routinely amplify products of up to 10Kb, so with the exception of patient t(2;7)(q37.3;p15.1) all patients had been mapped by STS PCR to a resolution sufficient for junction fragment amplification.

Patient	Chromosome	Fosmid microarray	STS PCR
t(2;7)(q37.3;p15.1)	Chr 2	38Kb	5,210bp
	Chr 7	12Kb	17,251bp
t(3;11)(q21;q12)	Chr 3	n/a	2,241bp
	Chr 11	23Kb	7,285bp
t(7;13)(q31.3;q21.3)	Chr 7	49Kb	3,345bp
	Chr 13	35Kb	334bp

Table 3.2 Summary of breakpoint regions after array painting onto the fosmid microarray and STS PCR.

In virtually all the translocations studied, the breakpoints were refined by STS PCR. However, for patient t(2;7)(q37.3;p15.1) the chromosome 7 breakpoint was refined to 12Kb by fosmid array analysis but only to 17Kb by STS PCR mapping. All primer pairs in this 17Kb region failed to amplify a specific product on either derivative chromosome. To attempt to further refine this breakpoint, higher resolution microarrays were utilised.

3.4.2 Refinement of breakpoints by custom-made PCR product microarrays



STS PCR analysis failed to refine the chromosome 7 breakpoint in patient t(2;7)(q37.3;p15.1) to a resolution sufficient for amplification of the translocation junction by LR PCR, indicating the need for an alternative strategy. PCR primer pairs were designed to create 1Kb products tiling the 17Kb chromosome 7 breakpoint region as defined by STS PCR analysis (Figure 3.25). These primers were used to amplify products to create a custom PCR product array. Each forward primer was

synthesised with an amino group attached to the 5' end to allow for covalent bonding of the product to the microarray slide. This array was then array painted using derivative chromosomes to map the breakpoint further.

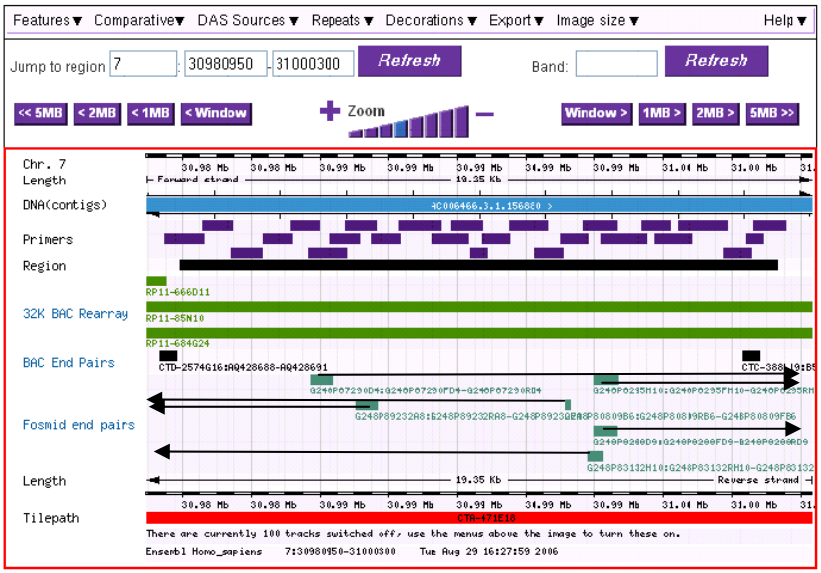


Figure 3.25 Ensembl view showing alignment of PCR products (tiling the 17Kb chromosome 7 region defined by STS PCR analysis) to the human reference sequence. The black arrows on the fosmid end pairs depict the direction of the remainder of the cloned sequence for each fosmid.

Array painting onto the fosmid array (discussed in 3.3.2.1) showed that the fosmid clones depicted in Figure 3.25, G248P89232A8, G248P80809B6 and G248P83132H10 spanned the breakpoint, but that G248P87290D4, G248P8295H10 and G248P8288D9 were distal to the breakpoint.

Array painting onto the PCR product array (Figure 3.26) showed a transition from high to low ratios indicating a shift from one derivative chromosome to the other between a PCR product at 30,982,601 to 30,983,464bp and another at 30,985,159 to 30,985,963bp, refining the breakpoint to 1,695bp. One product (30,983,446 to 30,984,330bp) with an intermediate ratio was observed indicating that this product spanned the breakpoint.

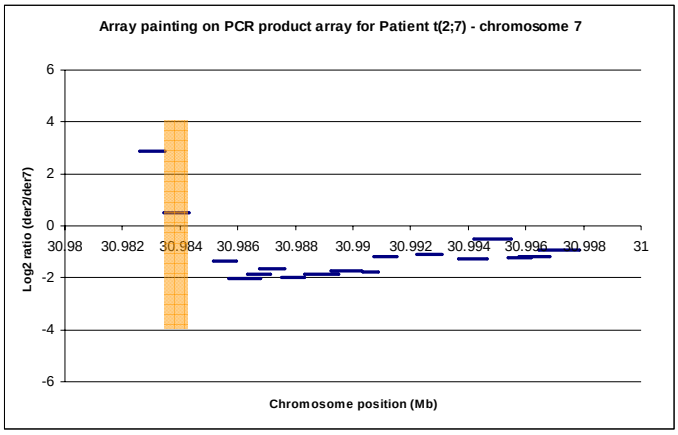
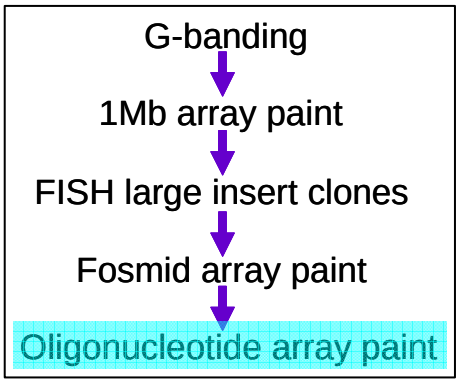


Figure 3.26 Array painting analysis for patient t(2;7)(q37.3;p15.1) on the PCR product array. The breakpoint region is highlighted by the orange region.

3.4.3 Refinement of breakpoints by custom-made oligonucleotide microarrays



A third strategy for refining translocation breakpoints by array painting on oligonucleotide arrays was also investigated for patient t(2;7)(q37.3;p15.1). An ultra-high resolution microarray (NimbleGen Systems, Inc) was constructed using unique, isothermal oligonucleotide probes ranging from 45 to 77bp. The probes were selected from repeat masked sequence spanning the selected regions with 1bp spacing where possible to provide the highest attainable resolution. The chromosome 2 region investigated by the array was from 236,450,000 to 236,650,000bp and the chromosome 7 region from 30,950,000 to 31,300,000bp (Gribble et al. 2006).

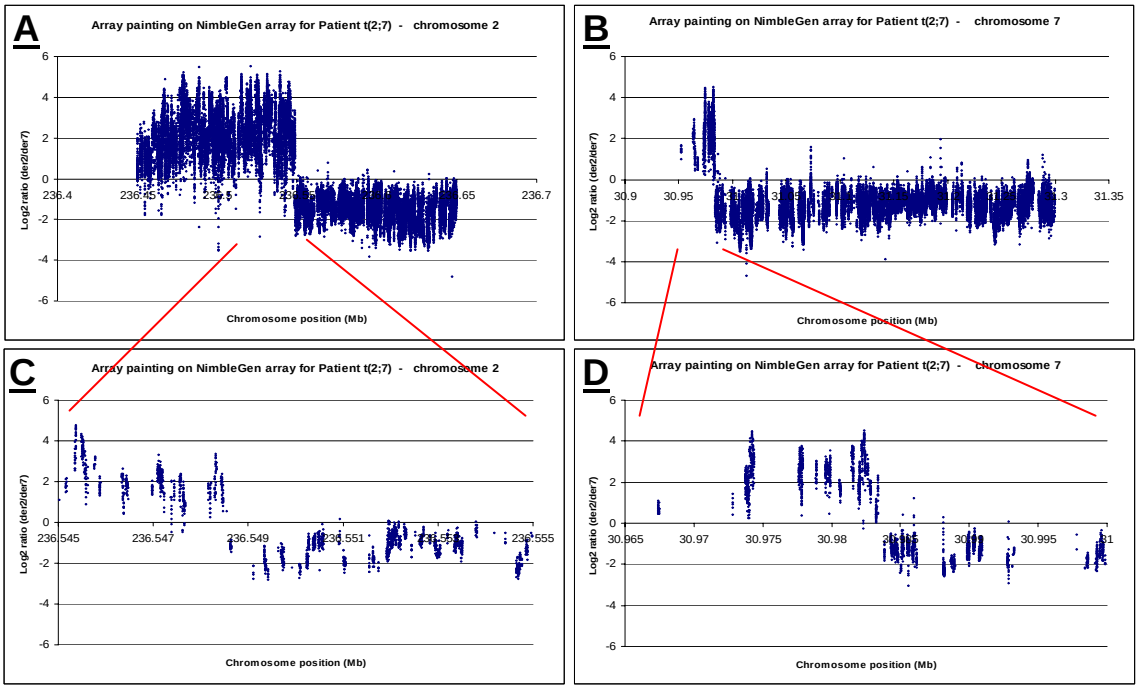
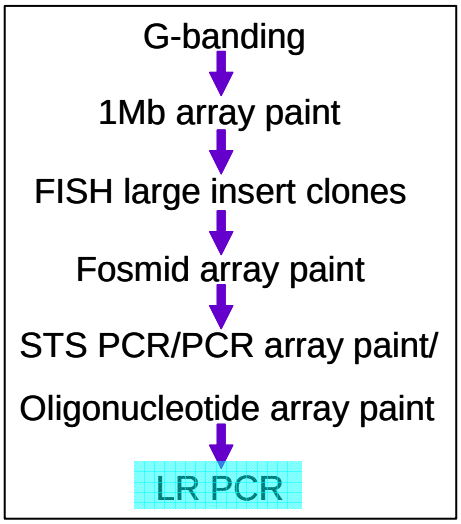


Figure 3.27 Array painting results for patient t(2;7)(q37.3;p15.1) on the NimbleGen oligonucleotide array. A and B show the results for chromosomes 2 and 7. C and D show the breakpoint regions at increased magnitude for chromosomes 2 and 7 respectively.

The ultra-high resolution array painting results (Figure 3.27) showed that the chromosome 2 breakpoint lies between the oligonucleotides at 236,548,539 and 236,548,680bp; a region of 141bp and the chromosome 7 breakpoint lies between the oligonucleotides at 30,983,401 and 30,983,890bp a region of 489bp. This refinement was so precise primers could be designed to amplify the junction fragments directly by PCR.

3.5 Chromosome translocation breakpoint amplification by LR PCR



In all cases, once the breakpoint regions had been refined sufficiently, LR PCR was used to amplify junction fragments.

Patient	Derivative	Primers		Fragment size
t(2;7)	der2	3572_2(2)_1f	ATATACACCATAACAGAAATGACACAGAAG	1.8Kb
		3572_7(2)_2f	GTCAGTCTAAGCATTGAGGTAAAAGACTC	
	der7	3572_7(1)_1r	CCTGCAGATAACACTTAGCTAGAAATAGG	2.0Kb
		3572_2(4)_2r	TAGAAACTGTGCAACTAAAAAGTTTGATAA	
t(3;11)	der3	3606_3(5)_1f	TTTCAAAGGAGTTTAGTTTAAGGATGCTAC	0.9Kb
		3606_11(5)_1r	TCTTACTACCTAGGAGTGTCTGAAGATGAG	
	der11	3606_seq1	ACAGTAACACTGTTTCGAGTTACTACACTG	0.8Kb
		3606_seq2	ATACGTAGCATTAAGCTCATTTCTCAGAC	
t(7;13)	der7	3615_7_2f	AATATAATTCAGATCAAGTGCACTAACTTC	0.7Kb
		3615_13_2r	TAAATCAATAGGGTGACTATAGTTTACAGC	
	der13	3615_13_3f	TTTACAACTAGGTGCAAGAGATTTACTAAC	3.5Kb
		3615_7_2r	GTATATGTAAATTCAGCTACAGAAACAGGT	

Table 3.3 Primers used for amplification of junction fragments for patients t(2;7)(q37.3;p15.1), t(3;11)(q21;q12) and t(7;13)(q31.3;q21.3).

Junction fragments were amplified successfully for all derivative chromosomes (Figure 3.28). Each primer pair (Table 3.3) was tested on genomic DNA from a normal reference individual to ensure that the amplified product was unique to the patient.

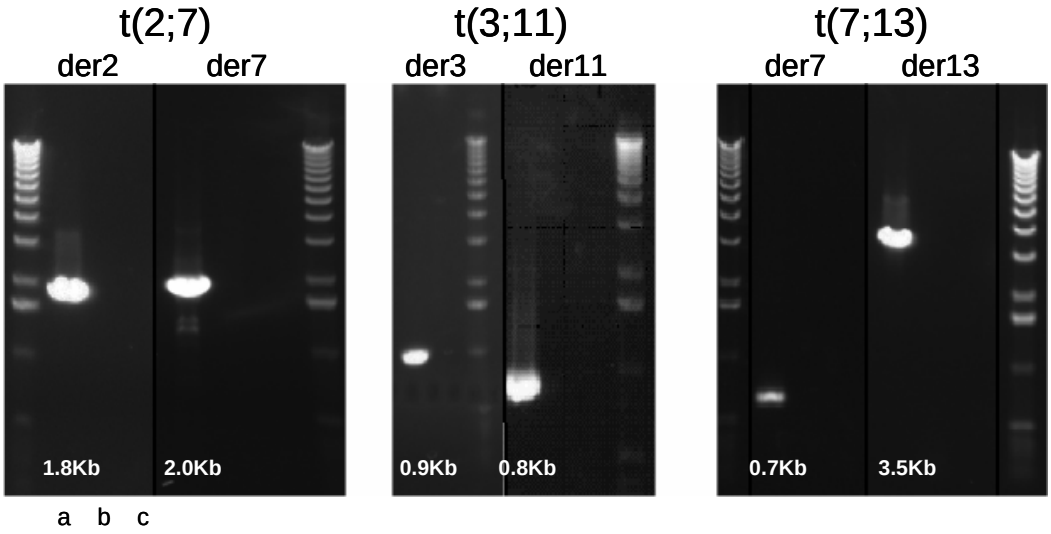
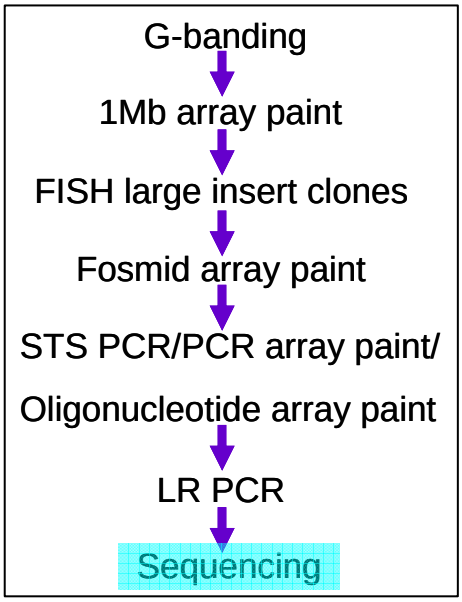


Figure 3.28 Agarose gel analysis of junction fragments for all 3 patients run against 1Kb marker. Templates used; a) patient genomic DNA, b) reference genomic DNA, c) water as negative control.

3.6 Sequence of junction fragments



The same PCR primers used to generate junction fragments were used to sequence the products. Approximately 400-500bp of sequence was obtained from both the forward and reverse primers. A subsequent round of primer design at the end of the sequence read generated the next sequence read, "walking" across the product to generate the sequence necessary. The sequence reads were blasted back against the sequence from NCBI Build 35 to align the derivative sequence to the reference sequence for exact identification of the translocation breakpoints (Figure 3.29).

```
t(2;7)(q37.3;p15.1)
2r/c;  aaatgaaaatcccccaagatcacccaggaagcagccctgggtataagcctcacactcccaacat
chr2;  actgtctgcccatgttgggagtgtgaggcttatacccagggctgcttcctgggtgatcttgggg
der2;  actgtctgcccatgttgggagtgtgaggcttataTTAAGGCTTTAAATATCTGAATGTAGCCAT
der7;  cagcccaggaagcagtttaatctattaatctttgTTAATAGATTAAACAAAAACTGAGCGTAAA
chr7;  GGTACCTCATGGCTACATTCAGATATTTAAAGCCTTAATAGATTAAACAAAAACTGAGCGTAAA
7r/c;  ACCCTGAGTTTACGCTCAGTTTTTGTTTAATCTATTAAGGCTTTAAATATCTGAATGTAGCCAT

t(3;11)(q21;q12)
chr3   acaccctgatcctgagttcactcctcggcccgcccatccaagcgccccgtgcggctggcgtt
der3;  acaccctgatcctgagttcactcctcggcccGGGCTGGATGGGCAGGTAGGGGGCGGGCTCCGG
der11; CGGGGCGGGGAATCTCTCGGCTTGTGCTTGCcccatccaagcgccccgtgcggctggcgttctc
chr11; CGGGGCGGGGAATCTCTCGGCTTGTGCTTGCTCCGCGGTGGGCTGGATGGGCAGGTAGGGGGCG

t(7;13)(q31.3;q21.3)
chr7;  tagtgattcggccttgcatgctacgcctgtatttcccagtgtcgatgtgtcatttgaatccatc
der7;  tagtgattcggccttgcatgctacgcctgGCTTTGTATGAAAATGGTCCATAAGATTGGATGCT
der13; GTGATACATAATGATTTTCTTTCAAAAGGGCTTTttcccagtgtcgatgtgtcatttgaatcca
chr13; GTGATACATAATGATTTTCTTTCAAAAGGGCTTTGTATGAAAATGGTCCATAAGATTGGATGCT
```

Figure 3.29 Sequence obtained across the junction regions for all three patients discussed in this chapter. Highlighted bases are; deleted bases (red), duplicated bases (green), inserted bases (yellow), bases of ambiguous origin which could have come from either donor chromosome (blue).

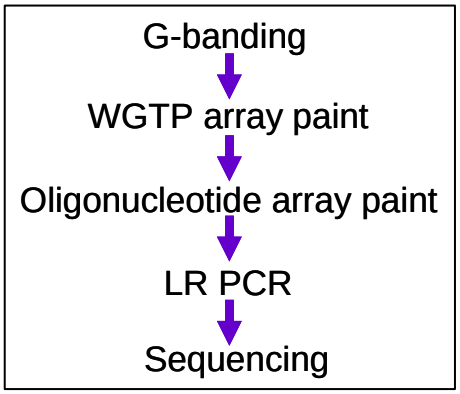
The breakpoint positions for each patient are detailed in Table 3.4. Each breakpoint was accompanied by varying numbers of deleted and/or duplicated bases. For patients t(3;11)(q21;q12) and t(7;13)(q31.3;q21.3) there were homologous bases at the breakpoints present on both donor chromosomes so the breakpoint to the single basepair level could not be determined; patient t(3;11)(q21;q12) had a 2bp homology (GC) and patient t(7;13)(q31.3;q21.3) a single basepair (T) homology.

Patient	Chromosome	Breakpoint position	Additional complexity at the breakpoint	
t(2;7)	2	236,548,579	236,548,580-236,548,586 deleted	19bp insertion on der7
	7	30,983,603	30,983,604-30,983,607 duplicated	
t(3;11)	3	130,755,505	130,755,506 deleted	
			130,755,507-130,755,508 either deleted or on der11	
	11	61,032,619	61,032,620-61,032,621 either deleted or on der11	
			61,032,622-61,032,629 deleted	
t(7;13)	7	121,245,446	121,245,447-121,245,448 deleted	
			121,245,449 either deleted or on der13	
	13	71,023,877	71,023,878-71,023,881 duplicated	
			71,023,822 either deleted or on der13	

Table 3.4 Translocation positions from NCBI Build 35 and additional sequence complexity at the breakpoints for all 3 patients.

Analysis of the sequence obtained from the two junction fragments and normal reference sequence did not reveal any homology at the breakpoint between the donor chromosomes in any of the three patients. Further investigation of the surrounding sequence, gene analysis and possible mechanisms are discussed in detail in Chapter 4.

3.7 Most rapid method of translocation breakpoint mapping



Advances in array technology over the course of this study have enabled the production of a whole genome tile path (WGTP) array consisting of large insert clones which tile the whole genome, resulting in a resolution of approximately 150Kb. A single hybridisation on the Sanger WGTP array can map both breakpoints of a reciprocal translocation within large insert clones and replaces a

more conventional approach of a 1Mb array paint and subsequent FISH hybridisations (Gribble et al. 2005). Once a spanning large insert clone has been identified by array painting using the WGTP array, the genomic region can be investigated by an oligonucleotide array paint analysis (NimbleGen Systems, Inc.). Using this custom-made ultra-high resolution oligonucleotide array as described in 3.4.3 will normally identify the breakpoints at a resolution sufficient for the direct amplification of junction fragments by PCR.

To demonstrate this approach, patient t(2;7)(q37.3;p15.1) was array painted onto the Sanger WGTP array to identify the spanning BAC clones (Figure 3.30). The chromosome 2 spanning clone was identified as RP11-401E12 and the spanning chromosome 7 clone identified as RP11-259J14.

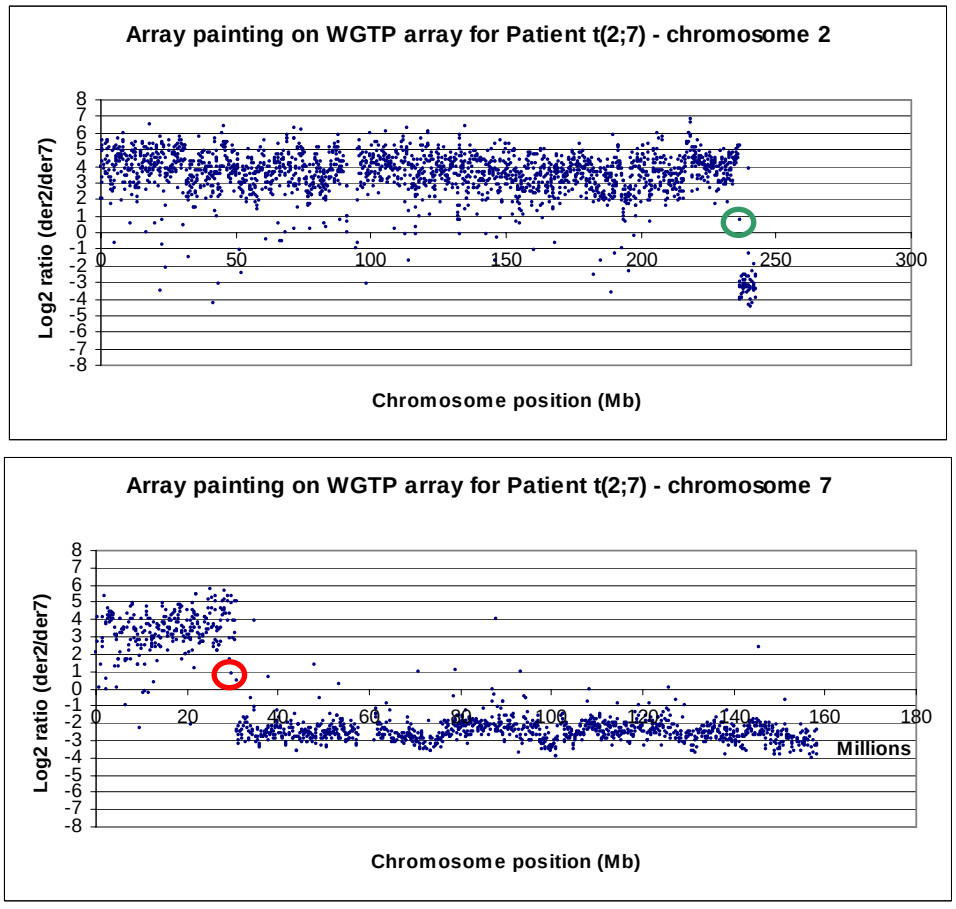


Figure 3.30 Array painting results for patient t(2;7)(q37.3;p15.1) on the whole genome tilepath array. RP11-401E12 and RP11-259J14.

The spanning clones identified by the WGTP array paint were not identical to the clones previously identified by FISH as the clones used for FISH experiments were not selected for generation of the WGTP array (Table 3.5). However, the clones identified by both approaches contain similar and overlapping genomic regions. All clones selected for the Sanger WGTP array were verified by end sequencing.

	Clones found by FISH	Clone found by WGTP array
Chr 2	RP11-680O16	RP11-401E12
	236,390,125 to 236,562,868bp	236,389,125 to 236,551,779bp
Chr 7	CTA-471E18	RP11-259J14
	30,853,334 to 31,010,213bp	30,899,687 to 31,060,782bp

Table 3.5 Comparison of spanning clones for patient t(2;7)(q37.3;p15.1) found by FISH and WGTP array painting.

A degree of variation was seen amongst the clones after hybridisation to derivative chromosomes. This is shown by the outliers in Figure 3.30. This could have arisen because of the repetitive nature of the human genome or from the amplification step used to increase the amount of probe prior to hybridisation to the microarray. It is estimated that approximately 5.4%-50% of the human genome is repeated (Cheung et al. 2001; Lander et al. 2001). Regions of homology within the genome can alter ratios as the probe cross-hybridises to multiple clones on the microarray. Also seen in the data was a wave phenomenon. Amplification of the flow-sorted derivative chromosomes may introduce a level of bias if certain regions of the template DNA are amplified preferentially, resulting in an unequal amount of DNA being generated across the whole template and so hybridising to the microarray causing the wave pattern.

Array painting onto the WGTP array followed by array painting on a custom-made oligonucleotide array is currently the most rapid and efficient approach to breakpoint mapping.

3.8 Investigation of further rearrangement around translocation breakpoints using fosmid microarrays

Previous reports have shown that translocations are sometimes accompanied by additional complexity at or around the breakpoints. A combination of three studies (Kumar et al. 1998; Astbury et al. 2004; Patsalis et al. 2004) using FISH and PCR reported that 8 out of 30 apparently balanced reciprocal translocations are more highly rearranged than identified by G-banding. An initial CGH screen on a 1Mb resolution microarray showed a further imbalance in patient t(2;7)(q37.3;p15.1) on chromosome 3 (Gribble et al. 2005). To investigate any additional complexity close to the breakpoints in each of the 3 patients array CGH was performed on the custom-made fosmid array (Figure 3.31).

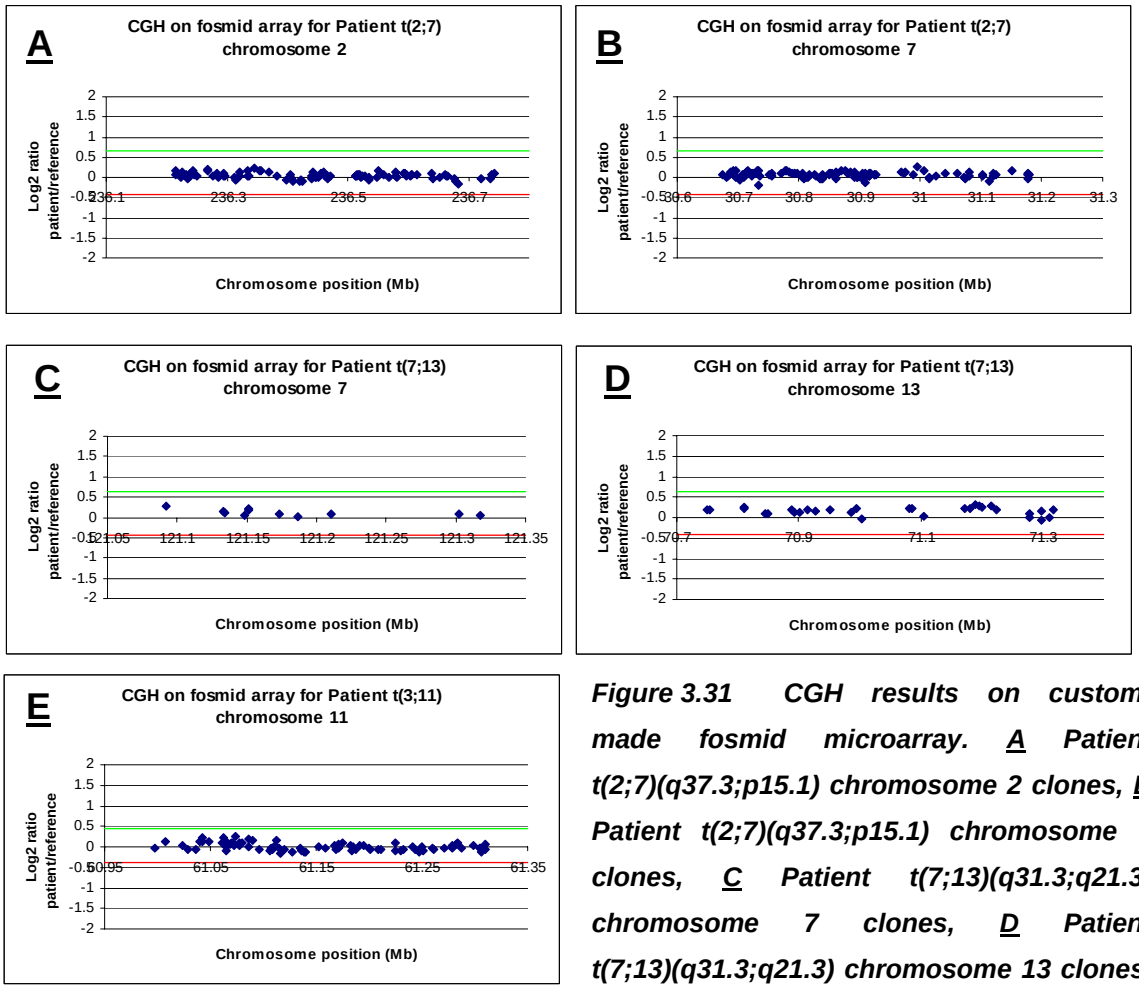


Figure 3.31 CGH results on custom-made fosmid microarray. A Patient t(2;7)(q37.3;p15.1) chromosome 2 clones, B Patient t(2;7)(q37.3;p15.1) chromosome 7 clones, C Patient t(7;13)(q31.3;q21.3) chromosome 7 clones, D Patient t(7;13)(q31.3;q21.3) chromosome 13 clones, E Patient t(3;11)(q21;q12) chromosome 11 clones.

None of the patients showed any additional complexity around the breakpoints at the resolution of the fosmid array.

3.9 Investigation of additional imbalance in patient t(2;7)(q37.3;p15.1)

Whilst no additional complexity was found close to the translocation breakpoints, the initial 1Mb array CGH screen identified a duplication of material at 3p26.3 in patient t(2;7)(q37.3;p15.1) which was confirmed by genotyping to be present in the father and the proband (Gribble et al. 2005). The frequency of interstitial duplications is believed to be approximately 1 in 4000 (Shaffer and Lupski 2000). A possible mechanism for these duplications (and corresponding deletions) is through non-allelic homologous recombination by virtue of homology of the sequence along the affected chromosome (Figure 3.32).

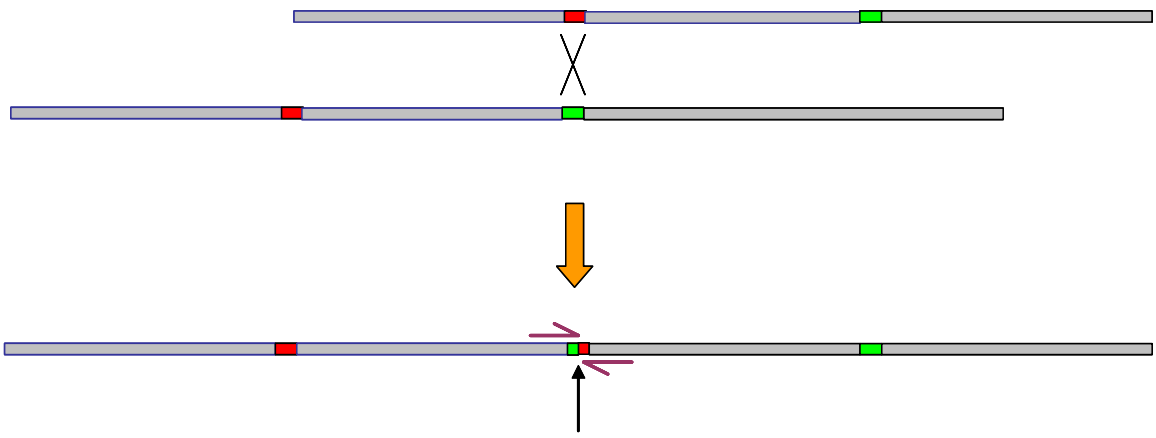


Figure 3.32 Possible mechanism for the 3dup(p26.3)(p26.3) in patient t(2;7)(q37.3;p15.1). The black arrow indicates the duplication junction. The purple arrows show the orientation of primers necessary to amplify the proposed duplication junction.

The two sister chromatids align close to each other during meiosis to undergo homologous recombination. If the chromatids are misaligned, then unequal crossing over can occur resulting in the duplication seen in the patient.

The 3dup(p26.3)(p26.3) identified by the 1Mb array CGH screen was mapped further using a custom-made fosmid microarray and LR PCR.

3.9.1 Delineation of chromosome 3 duplication breakpoints in patient t(2;7)(q37.3;p15.1) by microarray analysis

A high resolution fosmid array was constructed using 497 fosmids at full redundancy selected from NCBI Build 35 to refine the duplication breakpoints initially identified by the 1Mb array CGH screen (Gribble et al. 2005).

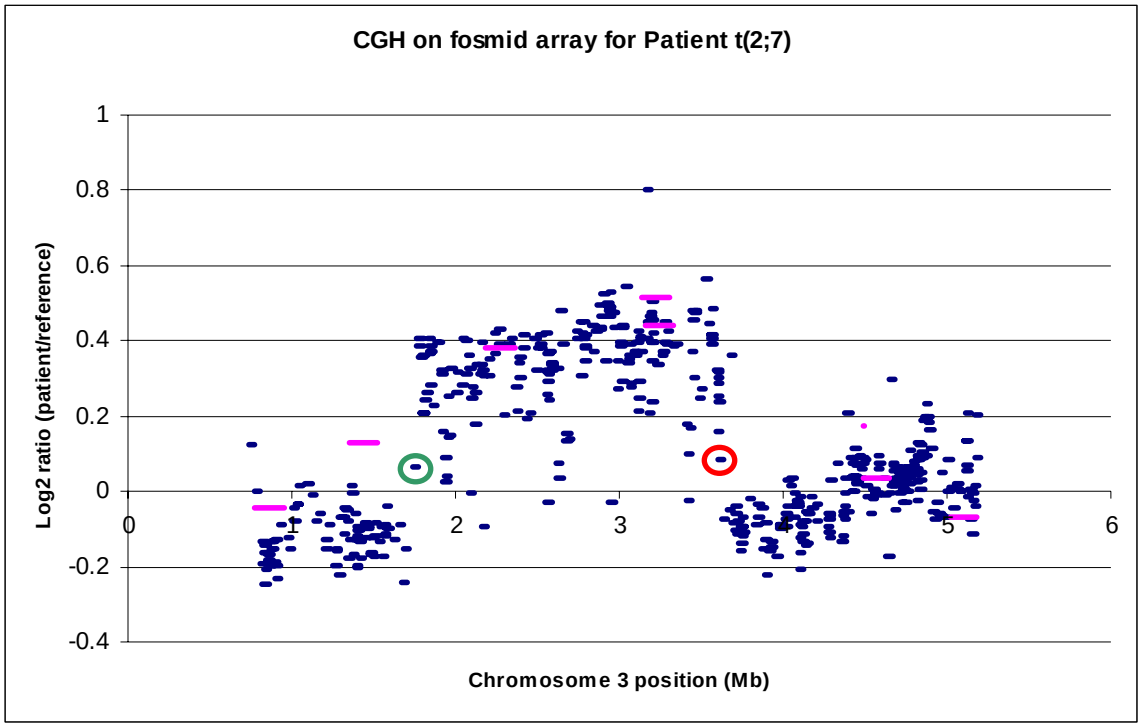


Figure 3.33 CGH results for patient t(2;7)(q37.3;p15.1) on custom-made fosmid microarray. Fosmid clones are in blue, 1Mb clones from original CGH screen (Gribble et al. 2005) in pink. G248P8110H1 G248P89401F1.

Fosmid array CGH was used to identify and refine the duplication breakpoints. CGH using patient DNA and a reference DNA on this microarray identified the distal breakpoint in clone G248P8110H1 and the proximal breakpoint in G248P89401F1 (Figure 3.33). As part of the original investigation into this amplification, genotyping showed that a duplication was also present in the phenotypically normal father at the same location (Gribble et al. 2005). To confirm that the duplication was the same in the patient as it was in the father a

CGH experiment competitively hybridising the patient's DNA against the father's DNA was performed (Figure 3.34).

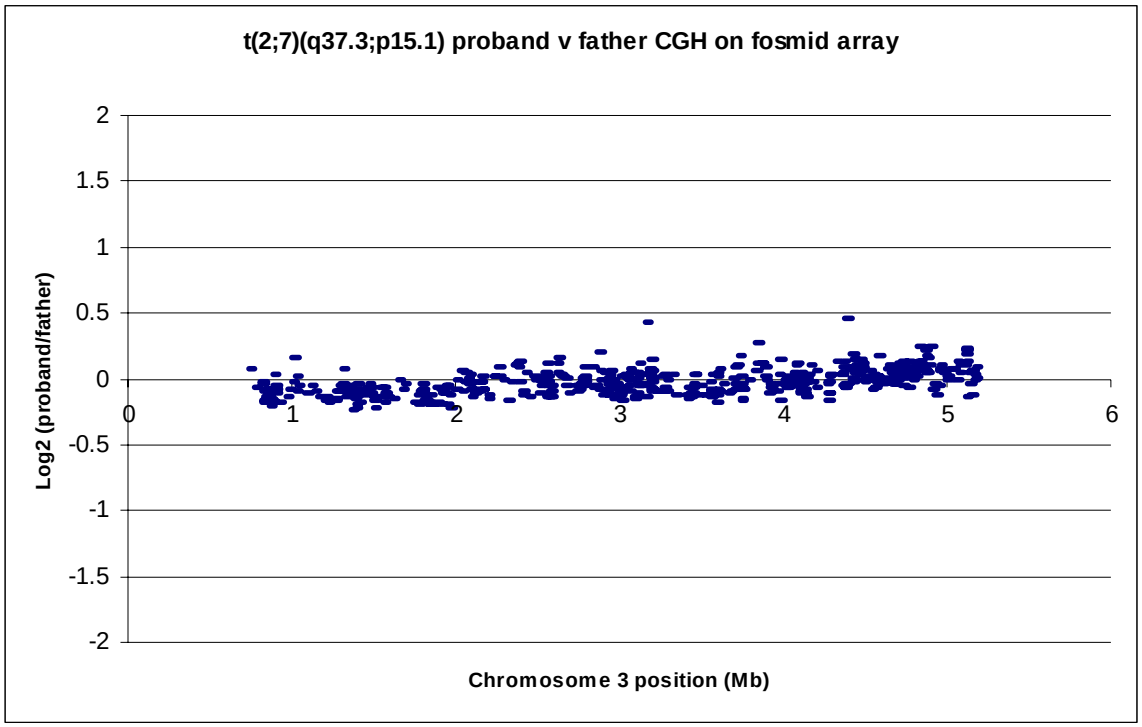


Figure 3.34 CGH results for patient t(2;7)(q37.3;p15.1) versus the patient's father on custom-made fosmid microarray.

No shift in ratios was observed from the CGH experiment for the clones spotted on the microarray, indicating that there was no difference in copy number between the father and the proband verifying that the same duplication was present in both DNA samples.

To further refine the breakpoints an oligonucleotide array was designed for the distal region 1,600,000 to 1,900,000bp and the proximal region 3,500,000 to 3,800,000bp on chromosome 3.

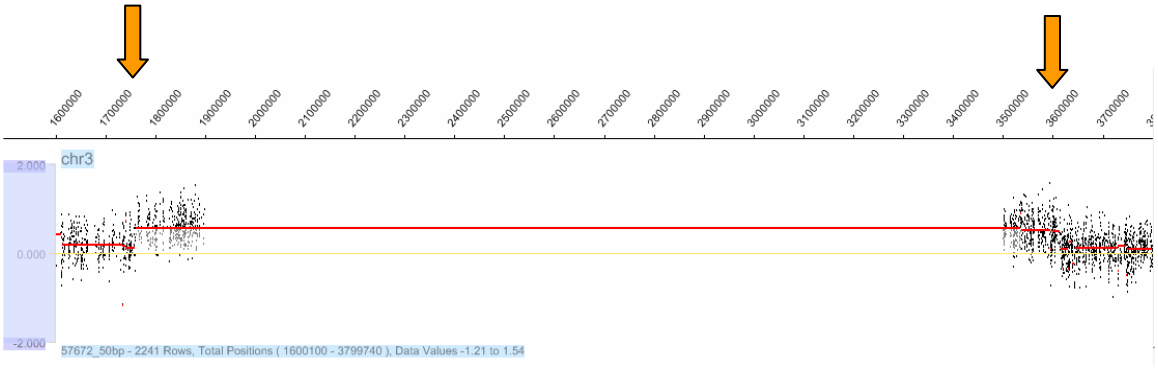


Figure 3.35 CGH for patient t(2;7)(q37.3;p15.1) onto a custom-made oligonucleotide array to refine the chromosome 3 duplication. The red lines show the averaged data over 50bp segments. Breakpoint regions are highlighted by the orange arrows.

Array CGH on the custom-made oligonucleotide array (Figure 3.35) refined the distal breakpoint to within 4,947bp from 1,759,196 to 1,764,153bp and the proximal breakpoint to within 1,105bp from 3,613,988 to 3,615,093bp.

3.9.2 Amplification and sequence of chromosome 3 duplication junction in patient t(2;7)(q37.3;p15.1)

LR PCR with primers designed to the breakpoint regions refined by the oligonucleotide array amplified products using patient t(2;7)(q37.3;p15.1) DNA as template. The same LR PCR primer pairs used on the father's DNA also produced a product of the same size as the proband. No product was obtained using the same primer pairs on the mother's DNA (Figure 3.36). The product obtained using the proband's DNA was sequenced to identify the duplication breakpoints.

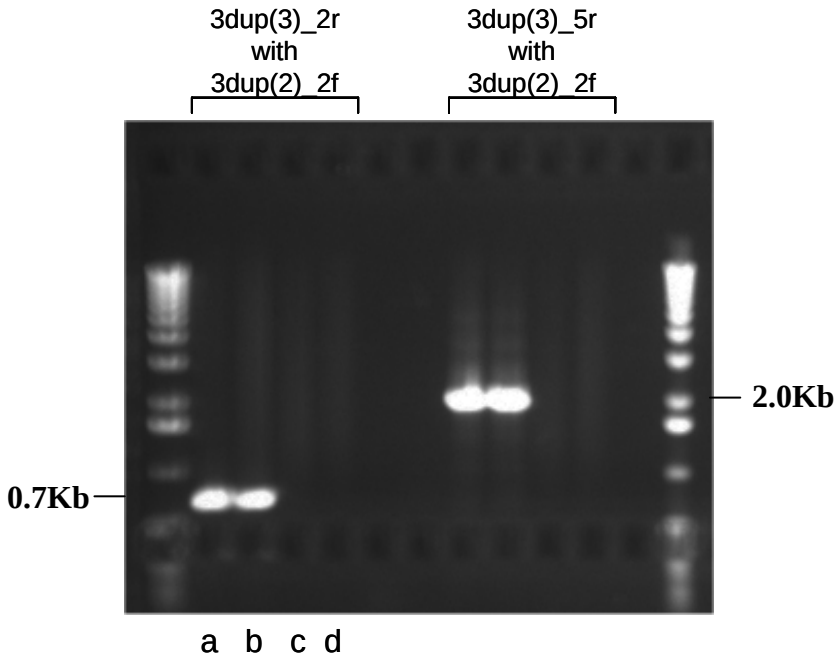


Figure 3.36 Agarose gel analysis of LR PCR results for amplification of chromosome 3 duplication junction in patient t(2;7)(q37.3;p15.1) run with 1Kb marker. Templates used; a) Patient genomic DNA, b) genomic DNA from father, c) genomic DNA from mother, d) water for negative control.

The breakpoint junction fragments were sequenced and the sequence obtained was aligned using ClustalW (http://www.ebi.ac.uk/clustalw/index.html?) and sequence homologies highlighted using the Genedoc software (http://www.psc.edu/biomed/genedoc/). The precise breakpoints within the sequence could not be found as both the proximal and distal breakpoints fall within Alu elements with 97% homology at approximately 1.7 and 3.6Mb along chromosome 3 (Figure 3.37). The distal breakpoint was within a 260bp region from 1,756,993 to 1,757,252bp and the proximal breakpoint was within a 258bp region from 3,614,132 to 3,614,389bp.

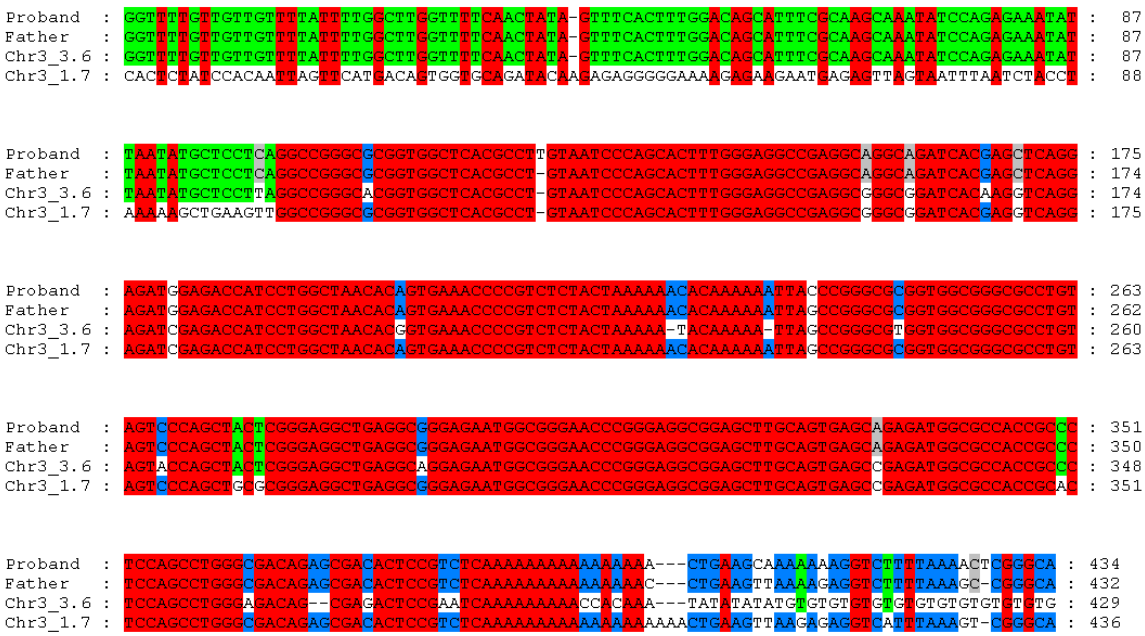


Figure 3.37 Sequence alignment of chromosome 3 duplication junction for patient t(2;7)(q37.3;p15.1), the patient's father, Alu elements at 3.6 and 1.7Mb on chromosome 3 at the rearrangement breakpoints. Shading; patient and father sequence match both Alu sequences (red), patient and father sequence match the Alu sequence at 3.6Mb on chromosome 3 (green), patient and father sequence match the Alu sequence at 1.7Mb on chromosome 3 (blue).

This sequence supports the hypothesis that the duplication arises from unequal crossing over during homologous recombination between sister chromatids mediated by an Alu repeat.

3.10 Conclusions

This chapter describes multiple techniques to refine chromosome breakpoints; Figure 3.38 shows the resolution of each of these techniques.

Simultaneous CGH and array paint hybridisations onto the same microarrays can identify and map regions of copy number loss or gain and chromosome translocation breakpoints.

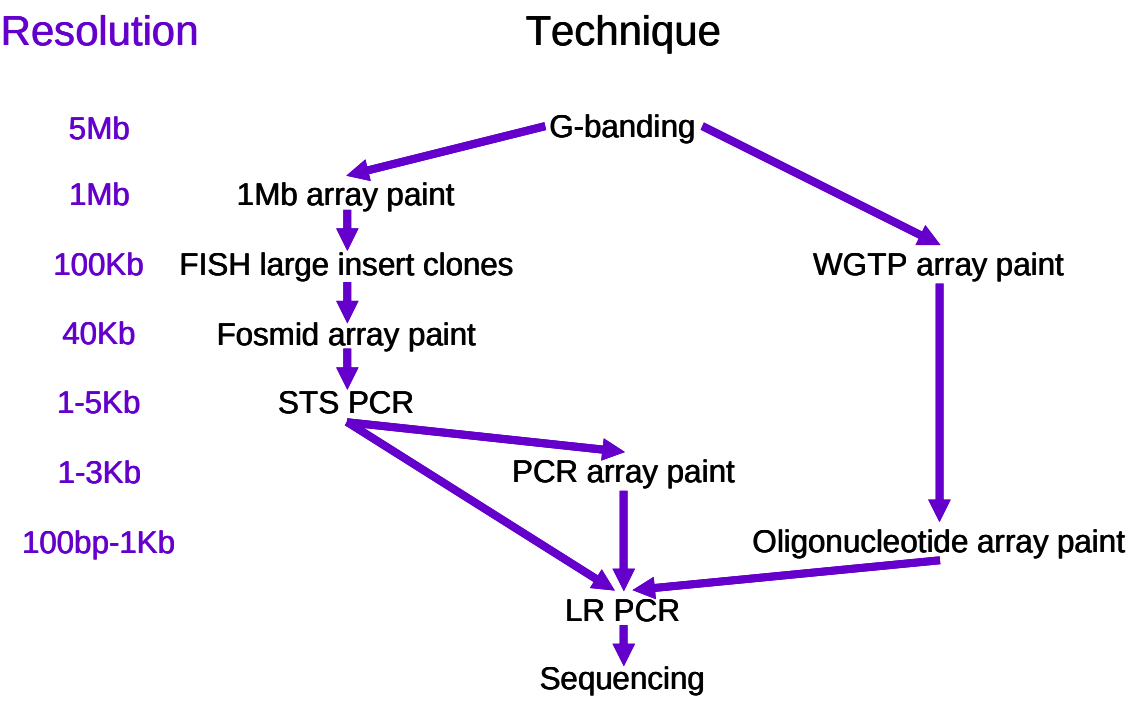


Figure 3.38 Schematic showing techniques used in this chapter to map rearrangement breakpoints.

Once the rearrangement breakpoints were mapped and sequenced the genomic regions spanning and flanking the breakpoints were submitted for bioinformatic analysis to look at the genomic architecture around the breakpoints and gene disruption. This analysis, along with possible mechanisms for the rearrangements are discussed in Chapter 4.