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ORIGINAL PAPER

# Integrated physical and transcript map of 5q31.3-qter

M Kostrzewa[1], BW Krings[1], MJ Dixon[2], K Eppelt[1], A Köhler[1], DL Grady[3], D Steinberger[1], ND Fairweather[4], RK Moyzis[3], AP Monaco[4] and U Müller[1]

[1]*Institut für Humangenetik, Justus-Liebig-Universität Giessen, Germany*
[2]*School of Biological Sciences, University of Manchester, UK*
[3]*Department of Biological Chemistry, University of California-Irvine, USA*
[4]*Wellcome Trust Centre for Human Genetics, Oxford, UK*

**We have constructed a physical and transcript map of 5q31.3-qter. The contig comprises 173 yeast artificial chromosomes (YACs) to which 159 sequence tagged sites (STSs), 47 expressed sequence tags (ESTs), and 32 genes were assigned. Previously published partial YAC contigs of the region have been refined and integrated. Given that the region contains 25 Mbp of DNA the average spacing of markers is approximately 100 kb.**

**Keywords: chromosome 5; physical and transcript map; YAC contig**

## Introduction

Genome-wide mapping of human DNA has made significant progress.[1–3] Of human chromosome 5, about half of the distal long arm (5q31.3-qter) has been cloned in yeast artificial chromosomes (YACs). Two YAC contigs of 3-5 Mbp cover 5q31.3-q33.[4,5] This region contains the genes *TCOF1* and *DTDST* that are mutated in Treacher Collins syndrome[6,7] and DTD (diastrophic dysplasia), respectively.[8] 5q31-q33 is also the critical region of gene loss in the 5q-syndrome, a myelodysplastic disorder.[9] More distally, about 1.5 Mbp of 5q33 have been cloned including a cluster of the $GABA_A$ receptor subunits $\alpha_1$, $\alpha_6$, $\beta_2$, and $\gamma_2$.[10] In addition, there are two YAC contigs spanning 7–10 Mbp of 5q34-q35.[11,12] This region encompasses the disease gene of craniosynostosis Boston type which was identified as the homeotic gene *MSX2*.[13,14]

More than 50 genes [OMIM, http://www3.ncbi.nlm-.nih.gov:80/omim] and more than 130 expressed sequence tags (ESTs) have been assigned to distal 5q (Whitehead Institute/MIT Centre for Genome Research, http://www-genome.wi.mit.edu).[15] Most of these transcripts were localised by either linkage analysis, fluorescence *in situ* hybridisation (FISH), or radiation hybrid mapping (RH). Only relatively few transcripts were fine-mapped and assigned map positions on the various YAC contigs of 5q31.3-q35. Many markers assigned to 5q31.3-qter, however, appear to fall in those regions that have not been cloned.

Detailed physical mapping of all markers assigned to a given region of the genome is necessary in order to facilitate its systematic analysis including sequencing. Therefore, we set out to construct a complete physical map of 5q31.3-qter in YACs, by integrating known maps, sequence tagged sites (STSs), ESTs, and genes. We were able to construct an integrated physical and transcript map of the 25 Mbp 5q31.3-qter region.

## Materials and Methods

YAC pools were screened by PCR according to the instructions of CEPH (Centre d'Etude Polymorphisme Humain, Paris, France), ICRF (Imperial Cancer Research Fund, London, Great Britain), and ICI (Zeneca, Macclesfield, Great Britain).

Correspondence: Ulrich Müller, Institut für Humangenetik, Justus-Liebig-Universität, Schlangenzahl 14, D35392 Giessen, Germany. Tel: +49 641 99 41600; Fax: +49 641 99 41609; Email: ulrich.mueller@humangenetik.med.uni-giessen.de
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Liquid yeast/YAC DNA was prepared by SDS/proteinase K lysis of cells, phenol/chloroform extraction and ethanol precipitation. Yeast/YAC DNA embedded in agarose blocks was prepared according to standard protocols.[16] Pulsed-field gel electrophoresis (PFGE), blotting, and hybridisation of YACs with total human DNA was performed as described previously.[12] YAC sizes were determined by comparison with chromosomes of *Saccharomyces cerevisiae* strain YPH49.

End fragments of YAC 20AB9 were isolated as described elsewhere.[5] All other YAC end fragments were isolated by inverted PCR as previously described.[12,17] PCR products were blunt end cloned in the Eco RV site of pBluescript II SK + (Stratagene) and sequenced with T7 polymerase (Pharmacia). Alternatively, PCR products were excised from agarose gels, isolated using the Prep-A-Gene system (BioRad), and sequenced directly using the Thermosequenase fluorescent labelled primer sequencing kit (Amersham) and IRD labelled primers 5'-CGGTAGCCAAGTTGGTTTAAGG-3' and 5'-GGTCCTTCCAAGATGGTTCAGA-3' (left arm) or 5'-CGCCCGATCTCAAGATTACG-3' and 5'-CCATTCACTTCCCAGACTTGC-3' (right arm). Sequencing products were separated and analysed on a LI-COR automated sequencing machine. All newly designed primer pairs for chromosome 5-specific STSs were tested for chromosome 5 specificity by PCR with a human/hamster somatic cell hybrid containing chromosome 5 as the only human chromosome (GM10114).

Previously published primers were used for the amplification of the following genes: *FGF1* (fibroblast growth factor),[18] *HSST* (heparan sulfate-N-deacetylase/N-sulfotransferase),[6] *TCOF1* (gene mutated in Treacher Collins syndrome),[6] *GM2A* (GM2 ganglioside activator protein),[19,20] *MFAP3* (microfibrillar-associated protein 3),[21] *SGD* (sacroglycan delta),[22] *IL12B* (interleukin 12B),[23] *GABRA6* (gamma-aminobutyric acid A receptor, alpha 6),[24] *LCP2* (lymphocyte cytosolic protein 2),[25] *F12* (coagulation factor XII), *NPT2* (sodium phosphate cotransporter), and *FLT4* (fms-related tyrosine kinase 4).[12]

For fluorescence *in situ* hybridisation (FISH) one microgram of whole yeast DNA of YACs was nick translated or amplified by inter-Alu PCR using primers $Cl_1$ and $Cl_2$[26] and labelled by random priming. Biotin-dUTP or digoxigenin-dUTP labelled probes were precipitated after addition of 50 μg cot1 DNA and fish sperm DNA (Boehringer Mannheim). Hybridisation to metaphase chromosomes was performed at 37°C. Probes were preannealed for 45 min at 37°C and hybridized. Slides were washed for 15 min at 43°C in 50% formamide/2 × SSC once, and twice for 8 min at 60°C in 0.1 × SSC. Signals were detected using the appropriate antibodies. Pictures were taken on Kodak Extrachrome 200 photographic film.

# Results

## *Construction of YAC contig of 5q31.3-qter*

YACs comprising the known contigs at 5q31.3-q33,[4,5] at 5q33,[10] and at 5q34-q35.1[11,12] were obtained from CEPH (Centre d'Etude du Polymorphisme Humain). Screening with anonymous markers[27,28] and genes assigned to 5q31.1-qter allowed the extension of these maps. While several specific primer pairs of genes were taken from the literature, primer pairs for *CDX1* (caudal type homeobox transcription factor 1) *DHLAG* (CD 74 antigen), *PDGFRB* (platelet-derived growth factor receptor beta), *GLRA1* (glycine receptor alpha 1), *GRIA1* (glutamate receptor, ionotropic, AMPA1), *ITK* (T-cell-specific tyrosine kinase1), *ADRA1B* (adrenergic receptor, alpha 1B), *HMMR* (hyaluron-mediated motility receptor), and *HK3* (hexokinase 3) were newly designed. The primer sequences are given in Table 1.[29–37] A total of 36 STSs, including 7 YAC arms, and 9 genes were mapped to these contigs. The exact map positions of these markers had not been known before. Genes assigned to the three contigs included *FGF1, DHLAG, PDGFRB, CDX1, HSST, TCOF1, GM2A, ADRA1B*, and *LCP2*. During this analysis the CEPH mega YAC library, the ICRF (Imperial Cancer Research Fund), and ICI (Imperial Chemical Industries) libraries were screened and 29 additional YACs integrated into these contigs (Figure 1). In addition, we were able to map several of the YACs described by Chumakov *et al*[1] to these contigs. All YACs were checked for chimerism by fluorescence *in situ* hybridization and sizes were determined by pulsed-field gel electrophoresis. Both the state of chimerism of the YACs and their sizes are listed in Figure 1.

We then started to close the gaps between the contigs by screening the CEPH library with STS primers previously assigned to distal 5q.[27,28] Forty five STSs, including those defining 9 YAC arms, and 10 genes (*GLRA1, GRIA1, MFAP3, SGD, ITK, IL12B, HMMR, F12, NPT2* and *FLT4*) were assigned to these regions. This screening facilitated incomplete closure of the gaps and necessitated the construction of YAC endpieces to bridge the gaps, and to refine the map. YACs used for isolation of endpieces and relevant primer pairs are listed in Table 1. Using these new markers, most gaps in the contig could be closed. D5S numbers of these newly isolated STSs are given in Table 1 and their exact location is shown in Figure 1. The only region not completely covered by YACs is 5q35.2-qter. Although we screened YAC libraries with various markers from this region, we only isolated a few overlapping, highly chimeric YACs from which endpieces could not be isolated. This is similar to the experiences at other telomeric regions, including 5p.[38] The region not covered by YACs is probably smaller than 3 Mbp, which is the estimated DNA content of a high resolution band.

### *Construction of transcript map of 5q31.3-qter*

We used two sources of published ESTs from terminal 5q and assigned them to the map. The panel of Del Mastro[15] allowed assignment of 8 (out of 78 published) ESTs to the contig. An additional 39 ESTs (out of 44 tested) from the Whitehead Institute were assigned map positions. Mapping of the Whitehead ESTs was

**Table 1** Newly developed markers in 5q31.3-qter

| *Marker* | *D number* | *Accession number* | *Primer sequence* | *Product size bp* | *Ann. temp. °C* | *Sequence source* |
|---|---|---|---|---|---|---|
| 20AB9R | D5S2924 | | TGCTTTACGAATCTGGGTGC<br>TACTCCCTACAAATGGAAA | 198 | 55 | This study |
| 20AB9L | D5S2923 | | ACTACATCTACCCACATGAAT<br>TCATGAACTCACAATCATT | 130 | 55 | This study |
| 119H2L | D5S2908 | Y13737 | TAAATTTTCCTTCCCCTCTC<br>CACTGGAACAGAGAATGGTG | 164 | 60 | This study |
| 936H1L | D5S2909 | Y13748 | CTAAAGGTAAGACAAAGGAAAGA<br>TTTCACTGGGTACAACATTCTT | 190 | 60 | This study |
| 936H1R | D5S2910 | Y13749 | TTGCAGTCATTTACTTCTACTCA<br>CAGTCTCACCATTTCTATGTCAG | 165 | 60 | This study |
| 809B9L | D5S2911 | Y13741 | AGAAGGCTAGAGGGCACTG<br>TCAGAGTAAAGCTCCTCATGC | 194 | 60 | This study |
| 930D10L | D5S2912 | Y13747 | GTCTCTTTTTACAGTTTTTG<br>GACACATTTAGATTGCAAG | 128 | 52 | This study |
| 822E10L | D5S2913 | Y13742 | GCAGGCATCTAAGATATAAC<br>AGCCAAGCTATTACATCTAC | 139 | 52 | This study |
| 822E10R | D5S2914 | Y13743 | GGCATTAACCCATATTTATC<br>TAAATCTTGTAAAAACCTGTCA | 171 | 56 | This study |
| 693F4L | D5S2915 | Y13738 | AGGCCTAGTGGAAGAGTGTTG<br>TGGGGTTATTATTAGTCTCCTTG | 165 | 63 | This study |
| 944E2L | D5S2916 | Y13750 | ACATGATTTGATACACTGAGAAGA<br>CATCCAGTTATCACGTCTCCT | 215 | 60 | This study |
| 693F4R | D5S2917 | Y13739 | AGAACTAGACATTTACAATAACATC<br>ACAATCATTAGTTTCATGTTTG | 111 | 57 | This study |
| 737H11L | D5S2921 | Y13740 | CCCAGTGAAGCCCTCTAAG<br>AATAGAGAAGACAAACCAGAAGC | 141 | 61 | This study |
| 889G5L | D5S2918 | Y13746 | ATTGGCATTTTCCCTACAG<br>TAGACTAGAGAATTTCAAGAGGG | 88 | 60 | This study |
| 856E9R | D5S2919 | Y13745 | ATCATTTTGAGAGATGTATC<br>CCATCTAGCATTCTACTTC | 143 | 50 | This study |
| 856E9L | D5S2920 | Y13744 | TAGCAACTGAGGAACAAACG<br>AGTGTCCCGTCATTTTTCC | 170 | 61 | This study |
| *CDX1* | | | GAGCGCAAAGTGAACAAGAAG<br>TGAGACTCGGACCAGACCTC | 265 | 64 | Ref. 29 |
| *HLADG* | | | GACCAAGTGCCAGGAAGAGG<br>GGGGAAGACACACCAGCAGT | 136 | 63 | Ref. 30 |
| *PDGFRB* | | | TGGAACAGTTGCCGGATTC<br>AAGGGGACAGCTGATAAGGG | 182 | 56 | Ref. 31 |
| *GLYRA1A* | | | AGAGGGCCAAGAAGATCGAC<br>GCTATTCCCACGTTCCCCT | 171 | 62 | Ref. 32 |
| *GRIA* | | | GAGAGAATGGTCGGGTGGTC<br>TTTTTGCACTGAAGGGTTTG | 178 | 64 | Ref. 33 |
| *ITK* | | | CCTCATTCCATAGAGCATTAGA<br>TAGAGGAAGGGCAGAGACAT | 194 | 60 | Ref. 34 |
| *ADRA1B* | | | CCCTTCTTCATCGCTCTACC<br>AATCTCCGAACACGCACAG | 146 | 63 | Ref. 35 |
| *RHAMM* | | | CAATACAAACTGTTACCGAGC<br>CACATATTTAAGGATTGGCAG | 178 | 60 | Ref. 36 |
| *HK3* | | | CGCAGTTGACTCGTGTCTGA<br>GAGGGGTCACACATGGAAT | 141 | 58 | Ref. 37 |

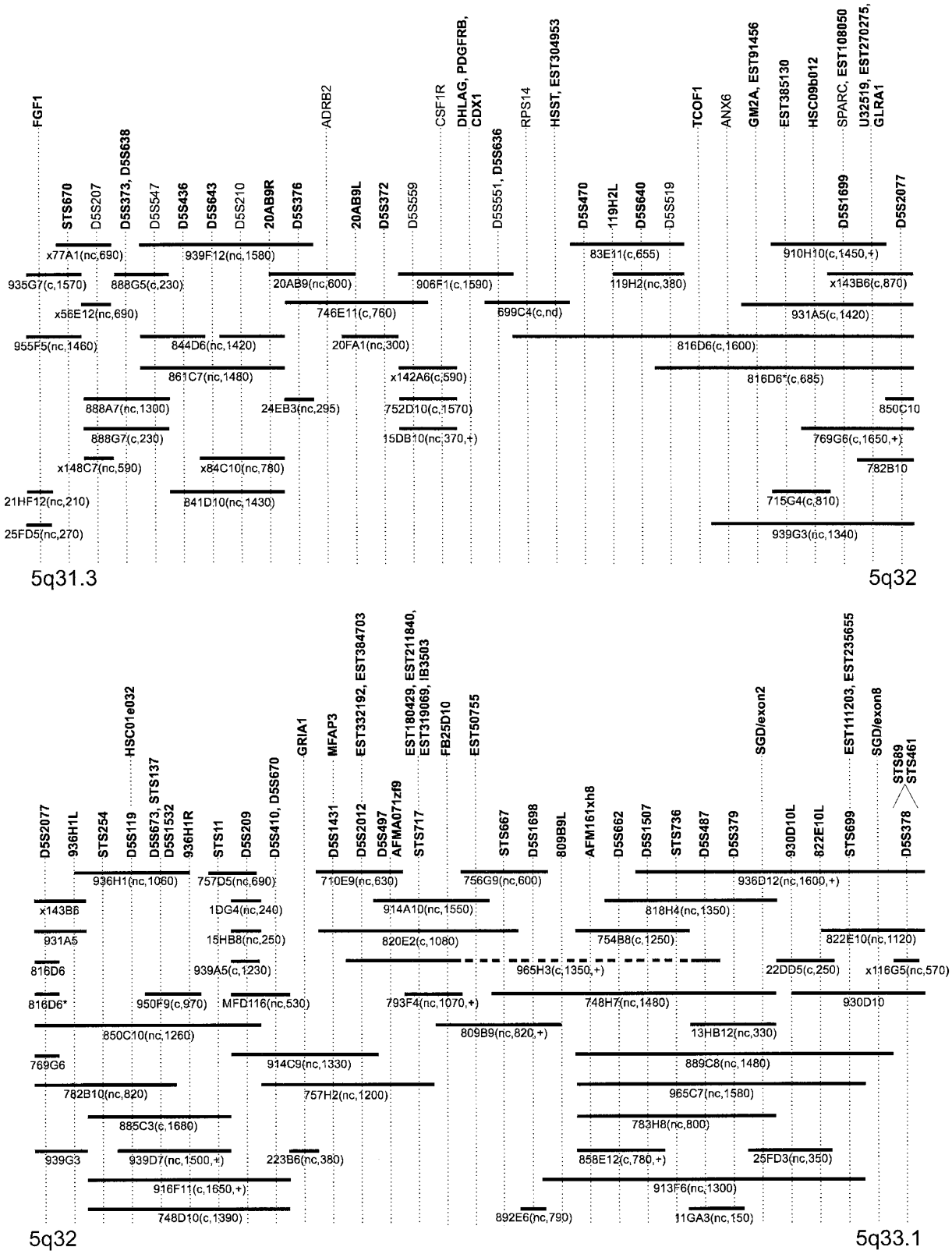


**Figure 1** *YAC contig of 5q31.3-qter. Below each YAC its name, status of chimerism (nc = nonchimeric; c = chimeric), and size in kb is given; + behind the size of the YAC indicates the largest of several YACs within a clone. Anonymous markers are shown in the first lane above the map, genes and ESTs are indicated in the second lane. Bold type indicates markers integrated in this contig as part of this investigation. The remaining markers have previously been assigned to partial YAC contigs of the region.*[4,5,10–12] *Dotted lines indicate deletions within YACs.*

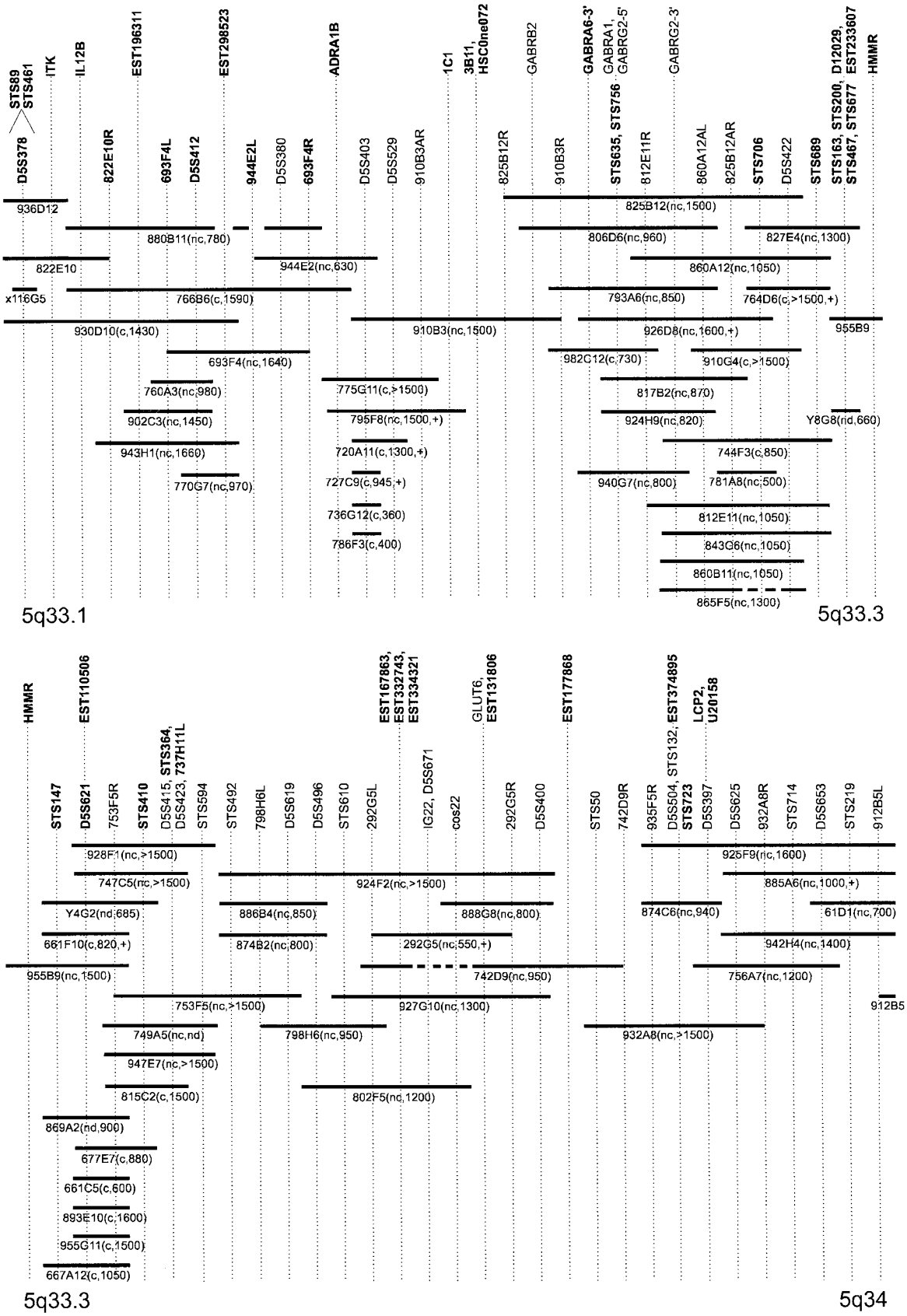


**Figure 1** *Continued*

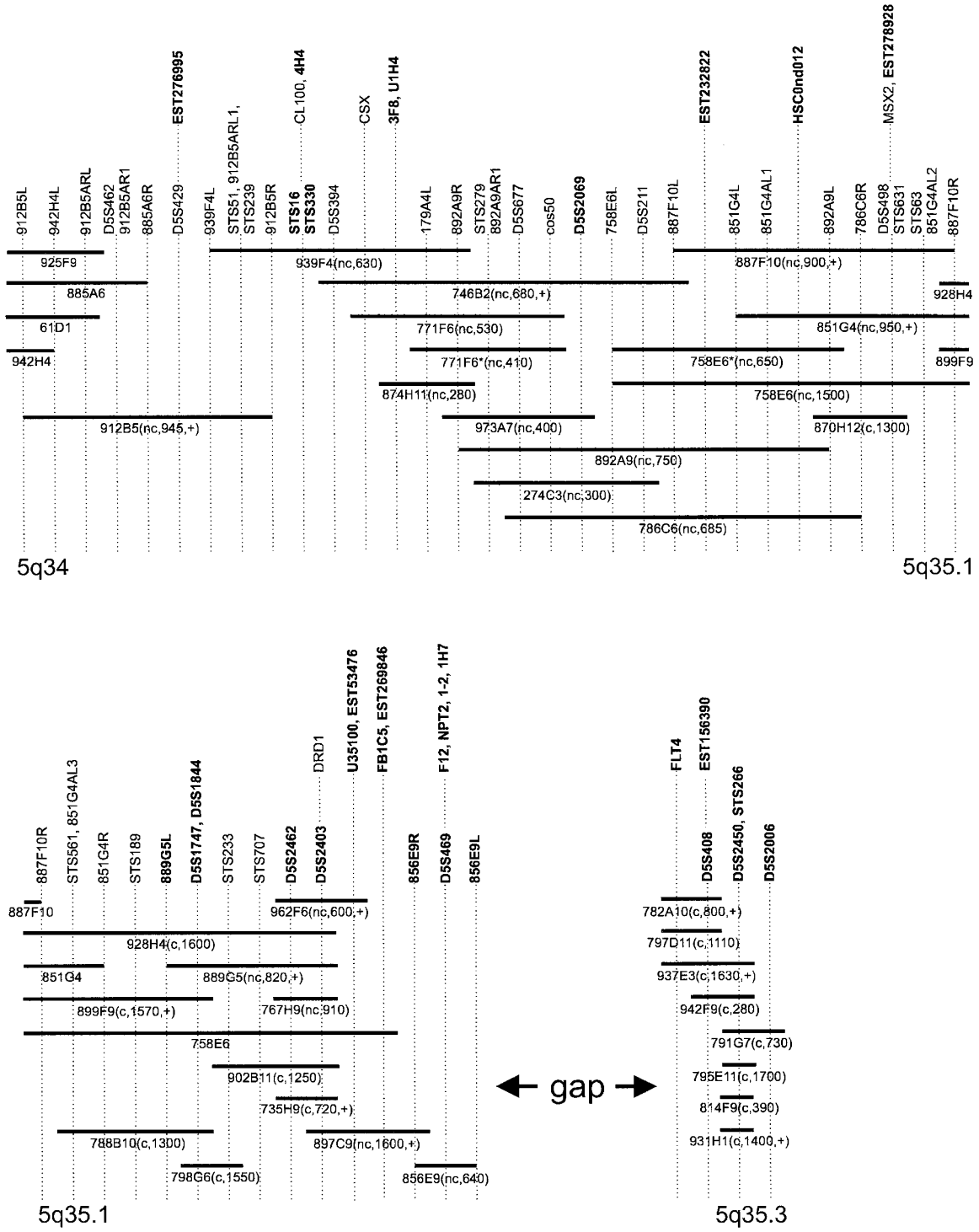


**Figure 1** *Continued*

further facilitated by the previous assignment of 9 of these ESTs to YACs, i.e. HSC09b012, EST108050, HSC01e032, IB3503, FB25D10, EST111203, HSC0ne072, EST110506, and HSC0nd012 (Figure 1).

Several genes, including *LOX* (lysyl oxidase),[39] *GRL* (glucocorticoid receptor),[23] *PDEA* (phosphodiesterase alpha),[40] *HK3*,[37] *NPM1* (nucleophosmin 1),[41] *CANX* (calnexin),[42] and *FGFR4* (fibroblast growth factor

receptor 4)[23] had previously been assigned to 5q31.3-qter by FISH, RH, or PFGE. However, we could not assign them to our contig.

## Discussion

We have constructed a physical and transcript map of the majority of 5q31.3-qter. Genes and ESTs previously assigned to the distal long arm of 5q were fine-mapped on this contig. Of approximately 50 genes localized to distal 5q [OMIM, http://www3.ncbi.nlm.nih.gov:80/omim], 32 could be assigned map positions on the contig. Similarly, a high percentage of ESTs assigned to the region by the Whitehead Institute/MIT Centre for Genome Research (http://www-genome.wi.mit.edu) could be mapped on this contig. In contrast, only a few of these ESTs could be assigned to the contig that had been mapped by radiation hybrids to distal 5q.[15] This might be due to their being located in the very distal region of 5qter which we could not clone and/or to more proximal locations in the long arm of chromosome 5. In addition, we were not able to assign genes *LOX*, *GRL*, *PDEA*, *HK3*, *NPM1*, *CANX*, and *FGFR4*, previously mapped to 5q31-qter, to this contig.[23,37,39–42] This might be due to the fact that *LOX* (location given as 5q23.3-5q31.2),[39] *GRL* (5q22-5q35),[23] and *PDEA* (5q31.2-5q34)[40] lie proximal to our contig and *HK3* (5q35.2),[37] *NPM1* (5q35),[41] *CANX* (5q35),[42] and *FGFR4* (5q22-5q35)[23] are located distal to or within the gap of our contig.

BLAST homology searches identified the two ESTs EST304953 and U20185 (Whitehead Institute/MIT Centre for Genome Research) as portions of known genes. EST304953 is homologous to *HSST*, and U20185 makes up part of *LCP2*. No additional homologies between ESTs and genes were found despite identical map positions of some. Two ESTs might lie within introns of *SGD*, since they were localised to an interval between the 5' and 3' end of *SGD*. It will be interesting to learn whether these ESTs make up parts of a gene or genes transcribed in the opposite direction to *SGD* or are intronic transcripts of the same strand.

There is a difference between the order of *FGF1* and *GRL* in this and a previous contig.[4] Whilst Li *et al*[4] localised *GRL* within 200 kb of D5S207, we did not find it on four D5S207 positive non-chimeric YACs ranging in size from 590 kb to 1300 kb. *GRL* could also not be assigned to the proximally adjacent non-chimeric YACs 955F5, 21HF12, 25FD5 of 210 kb to 1460 kb. These YACs contain *FGF1*, a gene not assigned to the contig of Li *et al*.[4] The data suggest that GRL is located proximal to *FGF1*. The gene *TCOF1* which is mutated in Treacher Collins syndrome turned out to be located in a more distal region than previously assumed. While the Treacher Collins Syndrome Collaborative Group assigned this gene to an interval flanked by *CDX1* and *DHLAG*,[6] the present investigation assigns it to an area $>380$ kb distal to the original interval. It is flanked by *HSST* and *ANX6*. This discrepancy might be explained by chimeric YACs or cosmids in this or the previous study.

The present investigation allowed refinement of the order of $GABA_A$ receptor subunit genes previously mapped to 5q33.[10] Genes coding for subunits *GABRB2* (gamma-aminobutyric acid A receptor, beta 2), *GABRA1* (gamma-aminobutyric acid A receptor, alpha 1), *GABRA6*, and *GABRG2* (gamma-aminobutyric acid A receptor, gamma 2) had been placed within an interval of less than one Mb. Of these, subunit genes *GABRA1* and *GABRA6* could not be separated. It has now turned out that primers used for amplification of *GABRA6*[43] are in fact part of subunit gene *GABRG2* (M Bailey, personal communication). We have therefore used another primer pair derived from the 3' non-translated region of subunit gene *GABRA6*[24] and were able to localise *GABRA6* to a region flanked by *GABRB2* and *GABRA1*. Furthermore, it is now clear (Figure 1) that the *GABRG2* gene is transcribed in a proximal to distal direction in 5q33.

The contig described covers between 23 Mbp and 26 Mbp of DNA. Given the assignment of a total of 240 markers to this contig, they are spaced at intervals of approximately 100 kb on average.

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