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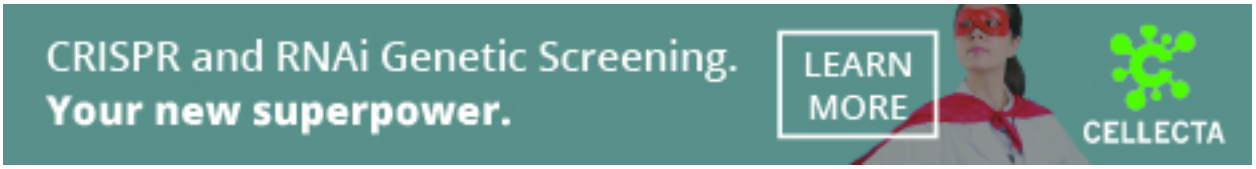

RESEARCH

# Mapping the RP10 Locus for Autosomal Dominant Retinitis Pigmentosa on 7q: Refined Genetic Positioning and Localization within a Well-defined YAC Contig

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Retinitis pigmentosa is a genetically heterogeneous disease that has autosomal dominant, autosomal recessive and X-linked forms. Autosomal dominant retinitis pigmentosa (adRP) has thus far been associated with eight distinct loci, including the rhodopsin and peripherin/RDS genes as well as unidentified genes on chromosomes 7p, 7q, 8q, 17p, 17q, and 19q. The RP10 locus for adRP on chromosome 7q was first mapped in a Spanish family; later, an unrelated American family was identified that also showed linkage to 7q. By combining the linkage results from both families, we are able to assign the disease gene to a 5-cM interval on 7q. Based on extensive physical mapping of this region, the genetic interval is now fully contained within a ~5-Mb segment on a well-defined YAC contig. These studies significantly reduce the size of the RP10 critical region, exclude a number of possible candidate genes, and provide the necessary cloned DNA for the positional cloning of the RP10 gene.

Retinitis pigmentosa (RP) is the name given to a group of degenerative retinal disorders that primarily affect the photoreceptors and retinal pigment epithelium (Heckenlively 1988). A characteristic feature of RP is the bone spicule-like pattern of pigment deposited on the retina, although this deposition is thought to be a consequence and not a cause of the disease. With over 1.5 million people affected worldwide, RP is the most common form of hereditary retinal dysfunction.

Clinical characteristics of RP include night blindness and loss of peripheral vision in early adulthood. This is followed by progressive constriction of the mid- and central visual fields and often culminates in legal or complete blindness. In addition to pigment deposition, progression of the disease is accompanied by retinal atrophy and vascular attenuation. The disease is marked by great variability in expressivity and penetrance, even within families.

The clinical heterogeneity of RP can be explained, in part, by the genetic heterogeneity. The disease occurs in autosomal dominant (adRP), autosomal recessive (arRP), X-linked (xlRP), and syndromic forms (Humphries et al. 1993). This heterogeneity is compounded further, as, to date, there are eight known loci for adRP, five for arRP, and four for XlRP (Daiger et al. 1995). Jordan et al. (1993) reported the localization of one adRP locus, RP10, to chromosome 7q in a Spanish family with a maximum lod (log odds) score of 7.5 at 0% recombination with the microsatellite marker D7S480. A second, independent (American) family linked to 7q was later reported by McGuire et al. (1995); this family had a maximum lod score of 5.3 at 0% recombination with the more distal marker D7S514. These two localizations placed the disease locus within an 11-cM region on 7q (genetic distances provided

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by J. Weissenbach, pers. comm.). A third adRP family also mapping to 7q was reported recently (Millán et al. 1995).

Here we combine the linkage data from the Spanish and American families in an effort to map the RP10 locus more precisely. Using a combination of two-point linkage testing and haplotype analysis, we have established that the genetic markers D7S686 and D7S530 flank the RP10 gene and are separated by 5 cM. These two markers have been mapped to a ~5-Mb interval on a large, well-characterized yeast artificial chromosome (YAC) contig. Knowledge of the refined location of the RP10 locus in conjunction with complete YAC-based cloned coverage should facilitate the isolation of the responsible gene.

## RESULTS AND DISCUSSION

### Family Descriptions

Pedigrees for the two families analyzed in this study are shown in Figure 1. Family UTAD045 is American, and family FA84 is of Spanish origin. Both families have early-onset disease and exhibit characteristic features of RP, including night blindness, extinguished electroretinogram (ERG) responses, and bone spicule-like pigmentary deposits. Neither family has defects in color vision. Different 7q haplotypes are segregating with the disease in the two families, thus excluding descent of RP10 from a common ancestor.

In addition to these two families, a third family linked to RP10 was reported recently by Millán et al. (1995). This small family is also of Spanish descent. Millán et al. (1995) suggest that the two Spanish families are unrelated based on genotypes at the D7S480 locus. The disease is later onset with slower progression in the second Spanish family than in the first. Haplotyping within the RP10 critical region will be necessary to confirm that these are unrelated families.

### Linkage and Haplotype Analysis

Table 1 shows the maximum individual and combined two-point lod scores for 18 informative microsatellite markers from this region of chromosome 7q (Weissenbach 1992; Gyapay 1994). These markers have been placed in the

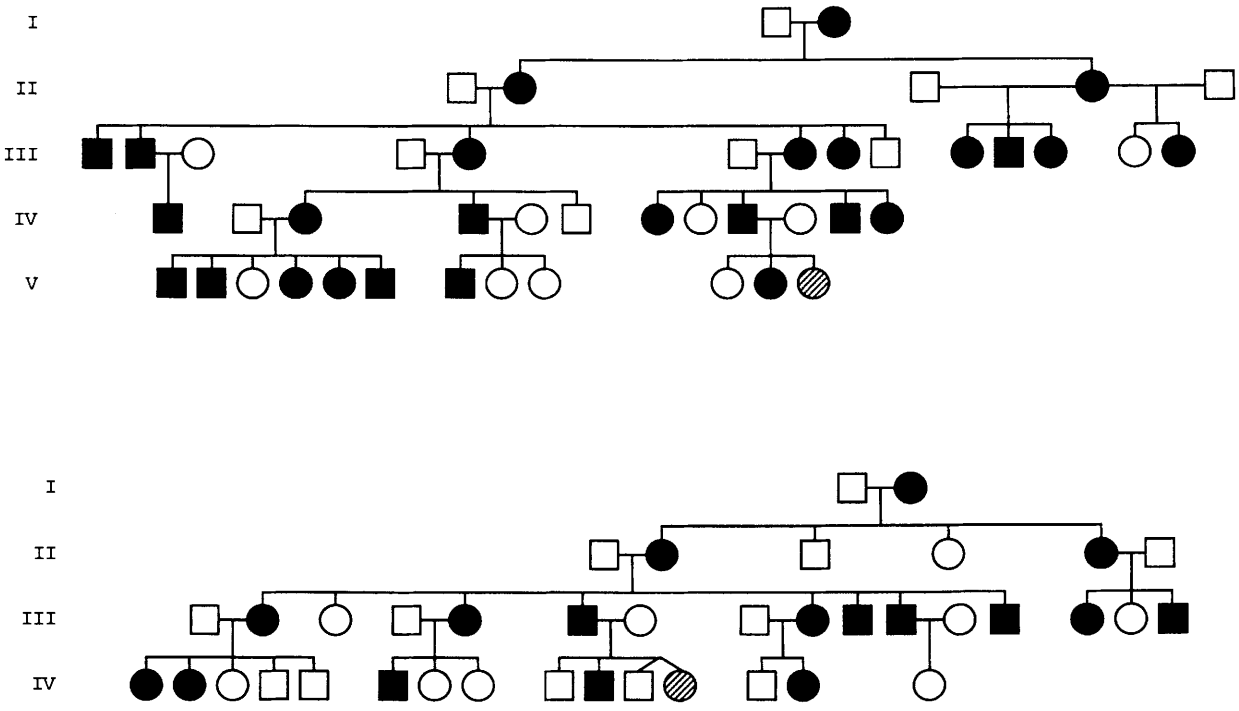


**Figure 1** Pedigrees of the two RP10 families studied. Family UTAD045 (*top*) is an American family with early-onset RP. Family FA84 (*bottom*) is a Spanish family also exhibiting early-onset RP. Both family pedigrees display classical rod–cone degeneration with an autosomal dominant pattern of inheritance. Unaffected individuals are shown as open symbols; affected individuals as solid symbols, and at-risk, "probably" affected individuals as hatched symbols.

**Table 1. Maximum Two-point lod Scores of the Individual Families and of Both Families Combined**

| | UTAD045 | | FA84 | | Combined | |
|---|---|---|---|---|---|---|
| | $Z_{max}$ | Θ | $Z_{max}$ | Θ | $Z_{max}$ | Θ |
| D7S486 | 2.99 | 0.10 | 3.61 | 0.10 | 6.60 | 0.10 |
| D7S522 | 2.19 | 0.10 | 2.04 | 0.00 | 3.98 | 0.10 |
| D7S633 | 3.03 | 0.05 | 1.67 | 0.00 | 4.65 | 0.05 |
| D7S677 | 3.72 | 0.05 | 0.08 | 0.40 | 2.72 | 0.20 |
| CFTR | 5.20 | 0.05 | 1.13 | 0.20 | 5.99 | 0.10 |
| D7S480 | 5.21 | 0.00 | 5.83 | 0.05 | 10.74 | 0.01 |
| D7S650 | 4.66 | 0.00 | 5.79 | 0.05 | 10.17 | 0.01 |
| D7S685 | 2.68 | 0.00 | 3.60 | 0.05 | 6.06 | 0.05 |
| D7S490 | 1.12 | 0.00 | 4.69 | 0.05 | 5.69 | 0.05 |
| D7S487 | 2.59 | 0.00 | 4.48 | 0.00 | 7.07 | 0.00 |
| D7S648 | 4.37 | 0.00 | 5.21 | 0.05 | 9.28 | 0.01 |
| D7S686 | 2.86 | 0.00 | 5.04 | 0.05 | 7.53 | 0.05 |
| D7S680 | 1.80 | 0.00 | 6.20 | 0.00 | 8.00 | 0.00 |
| D7S514 | 5.95 | 0.00 | 7.13 | 0.00 | 13.08 | 0.00 |
| D7S635 | 5.95 | 0.00 | 6.25 | 0.00 | 12.21 | 0.00 |
| D7S504 | 0.90 | 0.00 | 4.19 | 0.00 | 5.09 | 0.00 |
| D7S461 | 6.50 | 0.00 | 3.57 | 0.00 | 10.07 | 0.00 |
| D7S530 | 1.57 | 0.10 | 4.29 | 0.01 | 5.56 | 0.05 |

Microsatellite markers are listed in the most likely physical order (centromeric to telomeric). lod scores are calculated using the MLINK LINKAGE program.

best order based on physical mapping (see below). The highest combined lod score occurs with the marker D7S514 ($Z_{max}$ = 13.1) at 0% recombination. The combined lod scores are consistent with the values obtained with each family separately. The two-point lod scores reflect affected individuals in both families who are recombinant from D7S486 through CFTR [a microsatellite marker in intron 17 of the cystic fibrosis transmembrane regulator (CFTR) gene]. These results exclude the region proximal (centromeric) to CFTR in both families. Additional affected members from UTAD045 are recombinant for the distal marker D7S530. Thus, two-point linkage analysis places the RP10 locus between CFTR and D7S530.

Linkage analysis allowed placement of RP10 within an 11-cM region on chromosome 7q (J. Weissenbach, pers. comm.). The two-point lod scores, however, do not take into account genotypes at several loci considered simultaneously. By constructing haplotypes in the families, we are able to limit the disease region to 5 cM. Figure 2 shows the reconstructed haplotypes for family FA-84. Individual III-19 is an affected 37-year-old man with RP. Haplotype construction shows that he carries his mother's normal chromosome through marker D7S686, which is located 6 cM distal to CFTR. A recombination event occurred between D7S686 and D7S680, as III-37 carries the affected chromosome distal to D7S686. Thus, reconstruction of haplotypes effectively delimits the RP10 region to the 5-cM region between D7S686 and D7S530.

Fine-structure linkage analysis has not been completed for the second Spanish family reported by Millán et al. (1995). Preliminary data, however, may serve to further narrow the RP10 critical region. One definitely unaffected 44-year-old man is recombinant for the most distal RP10 markers, thereby placing the disease gene between D7S686 (proximal) and D7S514 (distal), reducing the RP10 region to 1 cM.

## Physical Mapping

As part of a global effort to assemble a highly integrated physical map of human chromosome 7 (Green and Green 1991; Green et al. 1994, 1995), we recently reported the construction of a

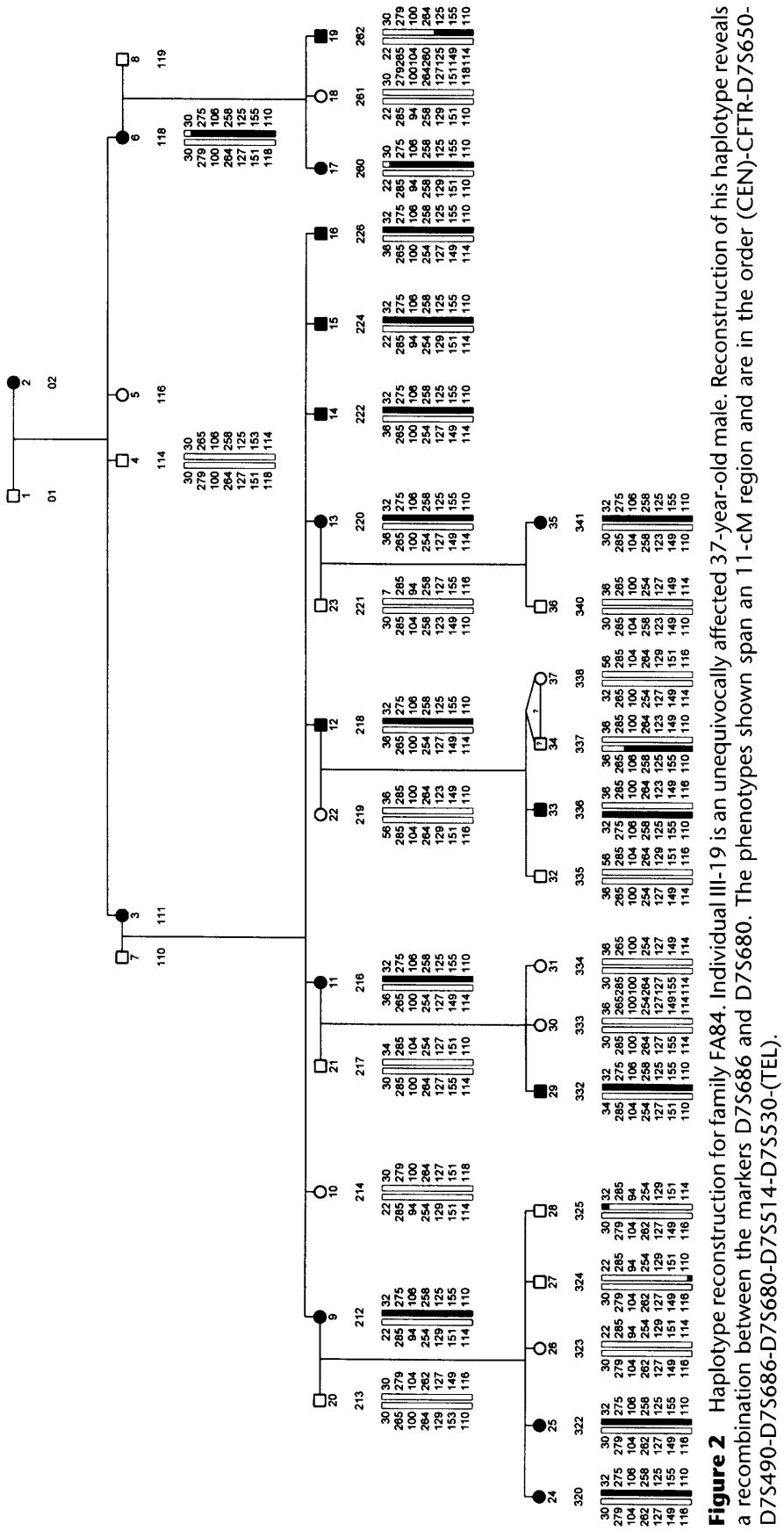

**Figure 2** Haplotype reconstruction for family FA84. Individual III-19 is an unequivocally affected 37-year-old male. Reconstruction of his haplotype reveals a recombination between the markers D7S686 and D7S680. The phenotypes shown span an 11-cM region and are in the order (CEN)-CFTR-D7S650-D7S490-D7S686-D7S680-D7S514-D7S530-(TEL).

YAC-based sequence-tagged site (STS)-content map of the region of 7q31.3 containing the human obese (OB) gene (Green et al. 1995b). This contig contained a significant portion of the genomic segment residing between D7S686 and D7S530 but did not include either of these genetic markers. Subsequent bidirectional expansion of the contig, which included the addition of 107 YACs and 53 STSs, yielded the map depicted in Figure 3 and established significantly greater contiguous and redundant clone coverage. Of particular relevance to RP10, the entire 5-cM critical region, as defined by the flanking markers D7S686 (sWSS1126) and D7S530 (sWSS1166), is fully contained within the YAC contig.

A total of 72 STSs have been assigned to the YAC contig shown in Figure 3 (Green et al. 1995b) and Table 2, with 19 corresponding to short tandem repeat-based genetic markers, 1 corresponding to a restriction fragment length polymorphism (RFLP), 31 derived from YACs (in particular, 27 from YAC insert ends), 12 derived from random chromosome 7 sequences, and 9 corresponding to EST or gene sequences. This contig contains a total of 150 YACs and provides a unique order for 67 of the 72 STSs (based on the STS content of the YACs). Of note, there is redundant YAC-based connectivity (i.e., two or more YACs connecting a pair of adjacent STSs) for all but five adjacent pairs of STSs in the contig, lending strong support for the relative STS order indicated in Figure 3. All 12 YACs analyzed by fluorescence in situ hybridization (FISH) were found to map to chromosome 7q31–32.

Although the contig shown in Figure 3 was deduced by SEGMAP without consideration of YAC sizes (thereby displaying STSs equidistant from one another), a similar analysis of the data by SEGMAP that accounted for YAC sizes indicates that the region covered by the contig is just over 9 Mb in size (data not shown). The interval between the key genetic markers D7S686 (sWSS1126) and D7S530 (sWSS1166) is estimated to be just under 5 Mb. Taken together, the genetic and physical mapping studies have thus delimited the RP10 critical region to a 5-cM, ~5-Mb interval of human chromosome 7q31–32 that is contained within a highly redundant set of overlapping YAC clones. The physical interval between D7S686 (sWSS1126) and D7S514 (sWSS1174)—the smaller RP10 critical region suggested by the Millán et al. study (1995)—is ~1.7 Mb in size.

## Candidate Genes

The localization of the RP10 critical region within the contig shown in Figure 3 suggests several possible candidate genes. For example, genes encoding acetylcholinesterase (ACHE), CFTR, and G-nucleotide-binding protein 2 (GNB2) have been mapped to YAC contigs other than the one shown in Figure 3 and are therefore excluded as the RP10 gene (E.D. Green, unpubl.). In addition, two retinal-specific genes have been mapped to chromosome 7q: one encoding a transducin subunit (GNGT1) and the second encoding blue cone pigment (BCP).

Transducin is a protein complex involved in signal coupling in the visual transduction pathway; thus, with the gene for the inhibitory γ subunit of transducin located on chromosome 7q (Fagan 1995), it became a viable candidate for RP10. Genetic analysis of our RP10 families with the microsatellite marker COL1A2, which maps physically within 1–2 Mb of the transducin γ gene, failed to reveal linkage between the RP10 locus and the transducin γ gene. Subsequently, the transducin γ subunit gene was mapped to a YAC contig from 7q21–q22, centromeric to the RP10 critical region.

The second known retinal-specific gene, BCP, maps within the larger (i.e., 5-cM) RP10 critical region shown in Figure 3 (see sWSS1325, Table 2). The BCP gene product is one of the three cone opsins responsible for color vision in bright light. In addition, BCP has 70% similarity to rhodopsin (Nathans 1986), the rod opsin that mediates vision in dim light and that is defective in 30% of adRP cases (Daiger et al. 1995). For these reasons, BCP could be considered a likely candidate gene for RP10. Physiologically, however, mutations in BCP are unlikely to cause RP10. There are an estimated $3 \times 10^6$ cones located in the center of the retina and $1 \times 10^7$ rods at the periphery. Both families have rod–cone degeneration with night blindness and loss of peripheral visual fields that is typical of RP. This suggests that the defect is in the rod photoreceptors rather than the cones. In addition, the families have normal color vision, indicating the presence of a functional blue cone opsin. Mutations have been encountered in the BCP gene that are analogous to known, severe mutations in rhodopsin; in all cases, these mutations cause tritanopia (blue color blindness) and not RP (J Nathans, pers. comm.). Beyond these physiological arguments, molecular data strengthen the

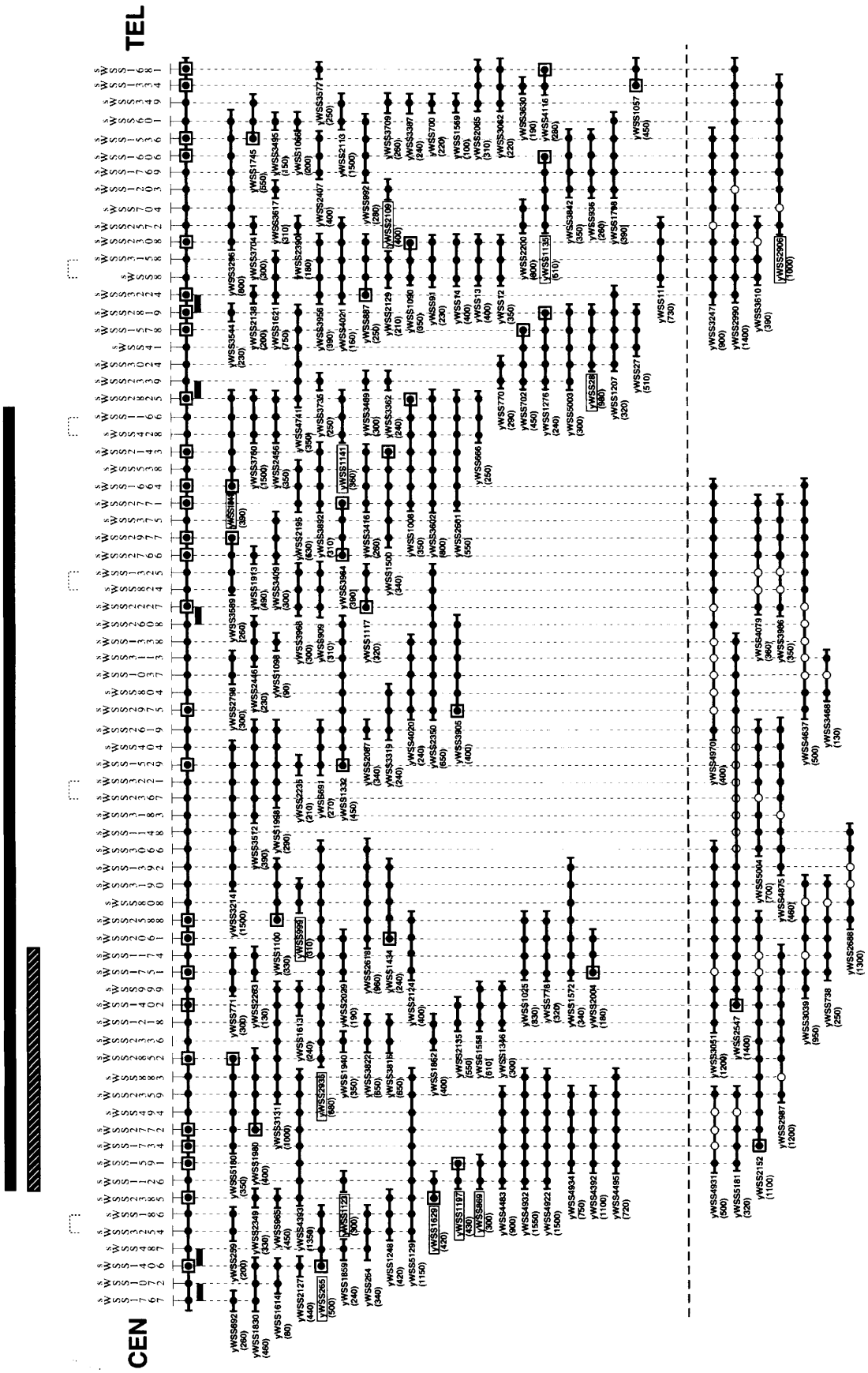


**Figure 3** *(See facing page for legend.)*

case against BCP as the RP10 gene. The entire coding regions of two affected individuals from each family were sequenced and no variants were found (data not shown). Finally, the linkage data with the family reported by Millán et al. (1995) would place the BCP gene telomeric to the smaller (i.e., 1-cM) RP10 critical region. For these reasons, the BCP gene does not appear to be a strong candidate for RP10.

## SUMMARY

By combining linkage mapping and haplotype analysis with two large families, we have narrowed the critical region containing the RP10 gene from 11 to 5 cM on chromosome 7q. Based on physical mapping studies reported here, this region is contained within a ~5-Mb segment on a large, highly redundant YAC contig. With the additional genetic mapping of a third family by Millán et al. (1995), the RP10 critical region may be as small as a 1-cM, ~1.7-Mb physical interval. Together these studies significantly reduce the RP10 critical region and provide the necessary clone coverage that should facilitate the identification and evaluation of additional retinal-specific candidate genes. This information and associated reagents should hasten the cloning of the RP10 gene and promote the search for effective treatments of this degenerative disorder.

## METHODS

### Families and DNA

All individuals in the kindred family FA-84 were examined by an ophthalmologist at the Fundacion Jiminez Diaz in Madrid, Spain (Jordan et al. 1993), which included both direct and indirect ophthalmoscopy. Younger members of the family not showing any symptoms of RP upon first examination were reassessed by an ophthalmologist 1 year later. The family pedigree and diagnostic information for family UTAD045 were obtained with the help of the Foundation Fighting Blindness using the Foundation's patient registry (McGuire et al. 1995). Clinical records were reviewed by an ophthalmologist at the Hermann Eye Center (Houston, TX). Human studies were reviewed and approved by the Committee for the Protection of Human Subjects, University of Texas, Houston. Informed consent was given by all participants. Two 10-cc vacutainers of whole blood were collected from a total of 36 affected and 32 unaffected individuals. Genomic DNA was extracted using the PureGene kit (Gentra).

### Detection of Microsatellite Polymorphisms

The forward primer of each microsatellite marker (Map Pairs, Research Genetics) was end-labeled with [$\gamma$-$^{32}$P]ATP

**Figure 3** YAC contig containing the RP10 critical region. The YAC-based STS–content map of the region of chromosome 7 containing the RP10 critical region is depicted, as deduced by SEGMAP/v. 3.44 (Green and Green 1991; C.L. Magness and P. Green, unpubl.). The 72 STSs (see Green et al. 1995b and Table 2) mapped to the YAC clones are listed along the *top*. Of these STSs, 67 are uniquely ordered based on the STS content of the YACs, with those STSs not uniquely ordered grouped by horizontal brackets above the STS names. Bidirectional expansion of the YAC contig reported by Green et al. (1995b) resulted in several minor changes in the precise order of some of the STSs. In all but five cases (indicated by solid bars immediately below and between the names of the STSs), each adjacent pair of STSs was connected by more than a single YAC clone. Also indicated are the positions of the centromere (CEN) and 7q telomere (TEL) relative to the STSs. Each of the 150 YAC clones is depicted by a horizontal bar, with its name given to the left and estimated YAC sizes (in kb, measured by PFGE) in parenthesis. The presence of an STS in a YAC is indicated (●) at the appropriate position. When an STS corresponds to the insert end of a YAC, a square is placed around the corresponding circle, both along the top (near the STS name) and at the end of the YAC from which it was derived. For the 20 YACs at the *bottom* (below the horizontal broken line), 1 or more STS expected to be present (based on the established STS order) was not detected (as assessed by testing the individual YACs with the corresponding STS-specific PCR assay at least twice). These are depicted (○) at the appropriate positions. A subset of the YACs were isolated from a human–hamster hybrid cell line-derived library (Green et al. 1995a), with their original names as indicated. The remaining YACs were isolated from total human genomic libraries, and their original library locations are provided in Table 3. Boxes are placed around the names of the 23 YACs that were found by FISH analysis to map to 7q31–q32 (Green et al. 1994, 1995b; and E.D. Green, unpubl.). The contig is displayed in its "uncomputed" form, where YAC sizes are not used to estimate clone overlaps or STS spacing, and all of the STSs are therefore spaced in an equidistant fashion. In the computed form, where YAC sizes and STS content are used to estimate the relative distance separating each pair of adjacent STSs as well as the extent of clone overlaps, the total YAC contig appears to span just over 9 Mb. The larger (i.e., 5-cM) RP10 critical region is indicated by the solid bar at the top of the contig map; the smaller (i.e., 1-cM) critical region is indicated by the hatched bar (see text for additional details).

**Table 2. STSs in the YAC Contig Containing the RP10 Critical Region**

| STS Name | Alias | Locus | Source | PCR Primers | Size (bp) | GDB ID |
|---|---|---|---|---|---|---|
| sWSS1767 | AFM324zg5 | D7S2527 | Genetic Marker | GCTACCTTTATCTATAAGACTC<br>ATCTTTCCTCTTGGTCTG | 165 | GDB:1105185 |
| sWSS1072 | AFM199zh4 | D7S2471 | Genetic Marker | ATGAATCATCTCAGTATTGGTCTGG<br>TGCTGATCTCAAGTTGTGGGAC | 241 | GDB:609093 |
| sWSS1406 | | D7S1917 | YAC End | CTGAAAACGACAAACTGG<br>CTTCCTTTTGCTGCAATAATTC | 61 | GDB:334047 |
| sWSS487 | | D7S2011 | Lambda | AAAAAATTCAAAAGGCTGGG<br>CTAGTATAGTAATAGCTGTGG | 88 | GDB:334392 |
| sWSS3254 | GATA67A05 | D7S2203 | Genetic Marker | GTAACCCTTTTTTGATCC<br>GAACCATGTCATACCTTG | 81 | GDB:1105176 |
| sWSS186 | CRI-pS94 | D7S87 | RFLP | GGAAGTGAGGAAATAGAAGAAG<br>TCTTTAGTAGGCAATCAACCTG | 155 | GDB:1105192 |
| sWSS2385 | | D7S2936 | YAC End | CTTCTAGCACACAATGAG<br>CGTGATAAGTAGTTCATAACC | 71 | GDB:1105170 |
| sWSS1126 | AFM323wd5 | D7S686 | Genetic Marker | TCAAAGCCTGGTGAGAAC<br>TGACCCCTATATAAAATGTAAAATG | 254 | GDB:199966 |
| sWSS1591 | | D7S2960 | YAC End | GAAAAACTGTGAAGTCTTG<br>GTGATGGATATGGTAATTG | 169 | GDB:1105194 |
| sWSS2772 | | D7S2946 | YAC End | CAATTTTGGGTTCACCTG<br>TCACCTTTCATACCTATC | 166 | GDB:1105180 |
| sWSS2852 | | D7S2952 | YAC End | CCAACCAAACTATCAATC<br>GCCTTACTGTCTCATATC | 119 | GDB:1105186 |
| sWSS3190 | EST20241 | D7S2378 | EST | TGTCAAAGCGCTAACCAGC<br>TCCAACAACAGAGAGAACATGC | 177 | GDB:591767 |
| sWSS3066 | AFMb340xh1 | D7S2501 | Genetic Marker | ATNCCCAGCATTTCTGTT<br>AGGTCTAATGTAAGATACTG | 138 | GDB:1105173 |
| sWSS3183 | 7B04H06 | D7S2940 | EST | AATTGTGACTTCTGGGAC<br>AAATGGAGAGAGGAAATG | 63 | GDB:1105174 |
| sWSS3221 | 7B24D06 | D7S2943 | EST | AATAGTACGTGCTGTCTG<br>ATTTGGATGACTATCTTGGG | 121 | GDB:1105177 |
| sWSS2975 | | D7S2954 | YAC End | CTCCAGAAATGAAACTAAC<br>GTTCCTTAGAGAGTTGTG | 70 | GDB:1105188 |
| sWSS804 | | D7S1461 | Alu PCR | ATAGCTGTTCAAGAGCTG<br>TTATGTGGGAAAAGTGAG | 82 | GDB:269314 |
| sWSS1037 | | D7S1884 | Lambda | TTTCCACACGTCTAAACC<br>CTAAGTGTTACTTCTAAGCC | 116 | GDB:333957 |
| sWSS3113 | HSC0GD092 | D7S2244 | EST | AAGTGGCACTGAGGTCATATACTT<br>GCCATCTGCTAAGCTCGACT | 152 | GDB:458833 |
| sWSS1338 | | D7S1908 | YAC Insert | GTTCATATCAGCCTTATTC<br>TTCTCCTGCCTTAGTTTC | 195 | GDB:334023 |
| sWSS2608 | | IMPDH1 | Gene | GTGAGTGTCCAGTGGGGTGATA<br>TGAATGAAACCAATACCTCC | 310 | GDB:128085 |
| sWSS2227 | | D7S2965 | YAC End | CCAAAGGAAATTGAGGAG<br>AACAGAGAGCAAAGAGAC | 100 | GDB:1105199 |
| sWSS824 | MIT-MS97 | D7S461 | Genetic Marker | GGGAAACTCAAAGGCAGAGA<br>AAACTCAGCATTGTCCTGCC | 177 | GDB:182225 |
| sWSS1325 | | BCP | Gene | TCACAGAGCCCAAGTGTTTTTAGAGGAGG<br>TGCCGCAACTTTTTGTAGCG | 289 | GDB:1105183 |

(*Table 2 continued on following pages.*)

**Table 2.** *(Continued)*

| STS Name | Alias | Locus | Source | PCR Primers | Size (bp) | GDB ID |
|---|---|---|---|---|---|---|
| sWSS2766 | | D7S2941 | YAC End | AAGCCCTGGTAAATACTC<br>TTGTCTGAAGATGTTTTCTG | 283 | GDB:1105175 |
| sWSS2977 | | D7S2956 | YAC End | CTTGTGAGTTAGTTACTG<br>ACATTCCTTTCTTCCCTTC | 226 | GDB:1105190 |
| sWSS375 | | D7S1645 | M13 Clone | AAATCTTTTCCCAGACCC<br>CCTGACTTTCATCCTATAC | 129 | GDB:334221 |
| sWSS2771 | | D7S2944 | YAC End | CATACAAAGTATGTTCTCTG<br>ATTCTCTTCGTTCCTTTTACTC | 135 | GDB:1105178 |
| sWSS1664 | | D7S2962 | YAC End | CTTACCAACAGCATAACC<br>TATTTGATTATCCCAGCAATCC | 118 | GDB:1105196 |
| sWSS538 | | D7S1541 | Lambda | GCTAATCACAGGCATAATC<br>TATAAGAGTCTAAGAAGTTGGG | 133 | GDB:334479 |
| sWSS2143 | | D7S2964 | YAC End | ATAGTAGTGGAGAAAAGG<br>CTCATCTTTCTTTTTCCC | 93 | GDB:1105198 |
| sWSS428 | | D7S1437 | Lambda | GGGATTGGCAAATTAACAG<br>CTTTGGATTTTCCCCAAC | 115 | GDB:269242 |
| sWSS1166 | AFM249xf9 | D7S530 | Genetic Marker | ATTAATAGTTTGGCTGGG<br>GAAAGCAAAACACAGTGG | 88 | GDB:307688 |
| sWSS2825 | | D7S2950 | YAC End | GGAATGAGATGTTGTAGG<br>ACAGATGTGAATCTTTGGG | 130 | GDB:1105184 |
| sWSS2339 | AFMa051ze9 | D7S2544 | Genetic Marker | TTAATACTTGCCTGTTCC<br>AACAAAACCCCCAAAACC | 132 | GDB:1105189 |
| sWSS3024 | AFMc024we9 | D7S2519 | Genetic Marker | GGAGGTTAAGATTTACAG<br>CACAAGTTCCTAAGTAGAG | 146 | GDB:1105191 |
| sWSS41 | | CPA1 | Gene | GATCAGCTTTCCAAGGCTGC<br>ATTGCCTTGATGATGCTGCC | 83 | GDB:1105181 |
| sWSS1578 | | D7S1958 | YAC End | ACAGAAGCAAATTCTCAC<br>CTTTGCCTCACATTTTACC | 60 | GDB:334155 |
| sWSS2819 | | D7S2948 | YAC End | GACCAGAGATTAGAAGAC<br>TGAGCTAAGACTTTTAGACC | 113 | GDB:1105182 |
| sWSS3224 | | D7S2945 | YAC End | AACAATTCCCTAAATGCC<br>TAGCCCAGAGATTTCAAC | 101 | GDB:1105179 |
| sWSS8 | | D7S786 | M13 Clone | GATCGCAAGTGTCCTCACAC<br>GGATCCTGTCTCAGTTACATC | 127 | GDB:205001 |
| sWSS3158 | | D7S2937 | YAC Insert | AGTCTTGGAAATGTCCAG<br>CCAGACATCAAGAAGTATC | 235 | GDB:1105171 |
| sWSS2308 | | D7S2966 | YAC End | TGACGGAAGTGATTTATG<br>GATTTCTCTATTCTCCTG | 230 | GDB:1105200 |
| sWSS2572 | | D7S2938 | YAC Insert | TAGCACAAAATGGGTATC<br>ATTTTCTCATCAACAGGG | 83 | GDB:1105172 |
| sWSS704 | | D7S1615 | Lambda | TCATTGTCTCAGATGAAC<br>TCTCTCATAGCACATTCC | 119 | GDB:334680 |
| sWSS1203 | AFM240xe9 | D7S649 | Genetic Marker | ATTTTGATCCCCAGCA<br>CAAGGAAAACACATATTCAC | 136 | GDB:307712 |
| sWSS1769 | AFM338wd5 | D7S2531 | Genetic Marker | GCTTTGGAAATAGATAATGGTG<br>CCATTTTGACCATCTTAAAGTG | 92 | GDB:1105187 |
| sWSS1606 | | D7S2961 | YAC End | TAATGGTAAGAAGAGGGG<br>CCTTCATCAGATTTACATAG | 60 | GDB:1105195 |

**Table 2.** (*Continued*)

| STS Name | Alias | Locus | Source | PCR Primers | Size (bp) | GDB ID |
|---|---|---|---|---|---|---|
| sWSS1536 | | D7S1946 | YAC End | ACATAATGTCTGGTTGTC<br>ATATTGTCACCTTAACCC | 66 | GDB:334125 |
| sWSS601 | | D7S1575 | Lambda | GAGTGATTATGGCATGTAG<br>CCTAATATCAGAGTATTTCC | 84 | GDB:334572 |
| sWSS349 | | D7S1418 | Lambda | TTTTACTCTTGCCCTCTC<br>CTTTCTTTCTGCCAAGAAAC | 116 | GDB:269185 |
| sWSS1334 | | D7S2959 | YAC End | AATCCTTGTGTTCTTCTG<br>AGTGTTGGCAATGATGTG | 136 | GDB:1105193 |
| sWSS1681 | | D7S2963 | YAC End | CAAAGTGTATGGCAAAAG<br>GAATGAGTAAAAAAGTGGG | 258 | GDB:1105197 |

Of the 72 STSs mapped to the YAC contig shown in Fig. 3, 19 were reported previously in Green et al. (1995b). Information about the remaining 53 STSs is provided here. In each case, the designated sWSS name, relevant alias, GDB-assigned locus name, STS source, PCR primer sequences, STS size, and GDB identification (ID) number are indicated. The sources of STSs are as follows: (YAC End) Isolated insert end of a YAC (Green 1993); (YAC Insert) random segment from YAC insert; (Genetic Marker) microsatellite marker (Green et al. 1994), (Lambda clone) random chromosome 7-specific λ clone (Green et al. 1991); (M13 Clone) random M13 clone derived from a flow-sorted chromosome 7 library (Green and Green 1991; Green 1993); (EST) expressed sequence tag-specific STS; (RFLP) restriction fragment length polymorphism; (Alu PCR) Alu–PCR product derived from a chromosome 7-containing human–hamster hybrid cell line (Green 1993). For some genetic marker-specific STSs, the PCR primers used for identifying YACs (listed here) are different from those used for performing genotype analysis, as the detection of YACs containing a genetic marker does not require amplification of the polymorphic tract itself.

using polynucleotide kinase (Promega) by incubating the primer at 37°C for 45 min followed by a 10-min incubation at 68°C to denature the kinase. PCR was then performed using 100 ng of genomic DNA under standard conditions (Weber and May 1989). The amplified products were electrophoresed on 6% denaturing polyacrylamide gels (Promega) for 2–4 hr and exposed to X-ray film overnight.

## Microsatellite Typing and Linkage Analysis

Allele types in individuals were assigned according to the molecular weights of their dinucleotide repeats. For linkage analysis, all unaffected at-risk individuals under the age of 16 were scored as "status unknown," with those over 16 given a remaining 2% chance of carrying an unexpressed RP mutation. The pedigree relationships were confirmed by appropriate segregation of all tested DNA markers. Multipoint linkage analysis was conducted on a Sun SPARCstation IPC using the MLINK program in the LINKAGE package optimized for fast sequential computations (Lathrop and Lalouel 1983; Cottingham et al. 1993).

## YAC-based STS-content Mapping

STS-specific PCR assays were developed and optimized essentially as described (Green et al. 1991, 1994; Green and Green 1991; Green 1993). Each STS is named using the prefix sWSS, followed by a unique number. Details about the 72 STSs are provided either in Green et al. (1995b) or in Table 2, with additional information (e.g., PCR assay conditions, complete DNA sequence) available in GenBank and/or the Genome Data Base (GDB). For the genetic marker-specific STSs, the oligonucleotide primers used for testing YAC clones corresponded either to those employed for genotype analysis or selected [most often with the computer program OSP (Hillier and Green 1991)] using the DNA sequence available in GenBank or provided by J. Weissenbach (pers. comm.).

Most of the YACs depicted in Figure 3 were derived from a collection of clones highly enriched for human chromosome 7 [the chromosome 7 YAC resource (Green et al. 1995a)], using a PCR-based screening strategy (Green and Olson 1990; Green et al. 1995a), which include YACs constructed from a chromosome 7-containing human–hamster hybrid cell line as well as clones derived from total genomic libraries. Each YAC is named using the prefix yWSS, followed by a unique number. The original library and locations of all YACs derived from total human genomic libraries are provided in Table 3.

## Mutation Screening of BCP

Oligonucleotide primers were chosen to amplify the exonic segments of the BCP gene (L. Shimmin, unpubl.). The amplified products (usually ~200 bp in length) were analyzed by single-strand conformational polymorphism (SSCP) analysis using 6% and 8% polyacrylamide gels with 10% glycerol. In addition, the coding sequences from two affected and two unaffected family members from each family were sequenced directly.

**Table 3. Original Well Locations of YACs Derived from Total Human Genomic Libraries**

| yWSS name | Library | Location |
|---|---|---|
| yWSS11 | WU | A64F1 |
| yWSS12 | WU | A177E4 |
| yWSS13 | WU | A196E3 |
| yWSS14 | WU | B1F10 |
| yWSS27 | WU | B58F12 |
| yWSS28 | WU | D34F10 |
| yWSS91 | WU | B103G11 |
| yWSS259 | WU | A76B8 |
| yWSS264 | WU | A62A5 |
| yWSS265 | WU | D90F3 |
| yWSS2547 | CEPH | 742E10 |
| yWSS2601 | CEPH | 760C10 |
| yWSS2618 | CEPH | 764E01 |
| yWSS2688 | CEPH | 773H12 |
| yWSS2798 | CEPH | 783C10 |
| yWSS2906 | CEPH | 795D02 |
| yWSS2935 | CEPH | 799C08 |
| yWSS2987 | CEPH | 805C08 |
| yWSS2990 | CEPH | 805E08 |
| yWSS3039 | CEPH | 812E03 |
| yWSS3051 | CEPH | 813F10 |
| yWSS3062 | CEPH | 814F06 |
| yWSS3131 | CEPH | 830D04 |
| yWSS3214 | CEPH | 848H08 |
| yWSS3247 | CEPH | 858E09 |
| yWSS3296 | CEPH | 872F01 |
| yWSS3319 | CEPH | 875F09 |
| yWSS4392 | CEPH | 752H4 |
| yWSS4393 | CEPH | 752H8 |
| yWSS4483 | CEPH | 784A6 |
| yWSS4495 | CEPH | 787E5 |
| yWSS4637 | CEPH | 857F2 |
| yWSS4741 | CEPH | 895B8 |
| yWSS4875 | CEPH | 928C01 |
| yWSS4922 | CEPH | 936F9 |
| yWSS4931 | CEPH | 938C6 |
| yWSS4932 | CEPH | 938C7 |
| yWSS4934 | CEPH | 938G5 |
| yWSS4970 | CEPH | 943F10 |
| yWSS5003 | CEPH | 950B1 |
| yWSS5004 | CEPH | 950B10 |
| yWSS5129 | CEPH | 744C2 |
| yWSS5180 | ICI | 92H3 |
| yWSS5181 | ICI | 308F10 |

Each yWSS name is listed along with the original library and precise well location from which the corresponding clone was isolated. The remaining YACs depicted in Fig. 3 were isolated from a human–hamster cell line-derived library (Green et al. 1995a), with their original names indicated in the contig.

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