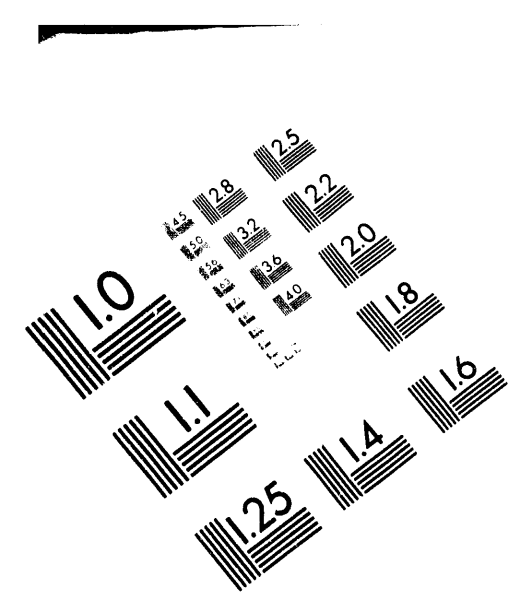

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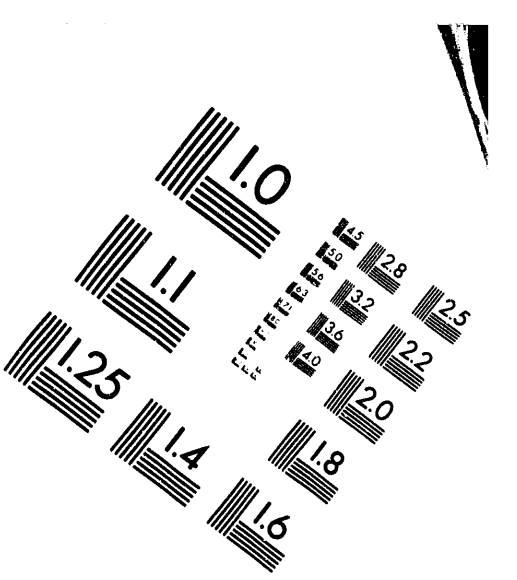

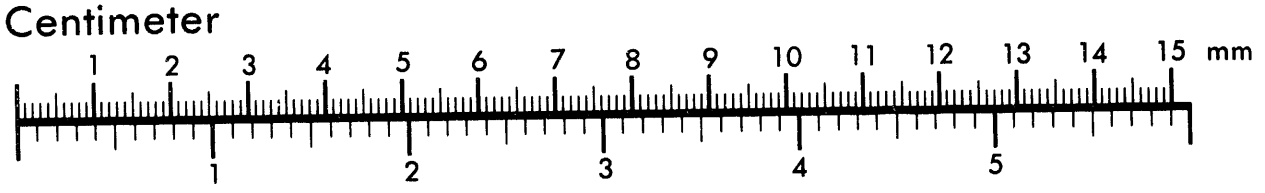

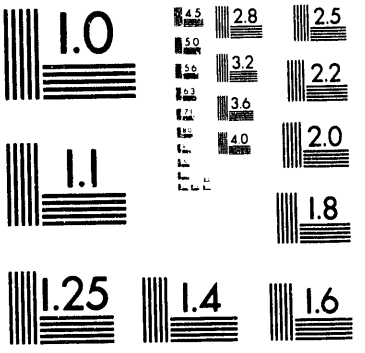

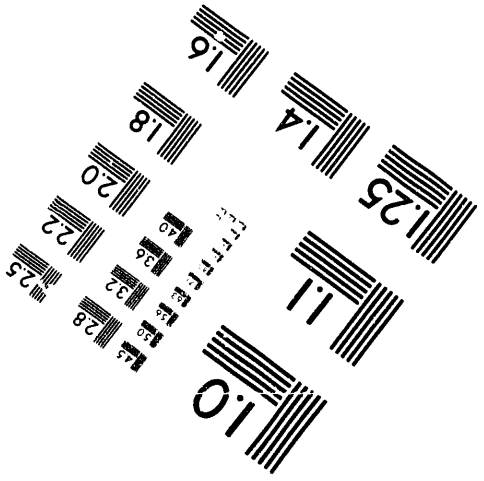

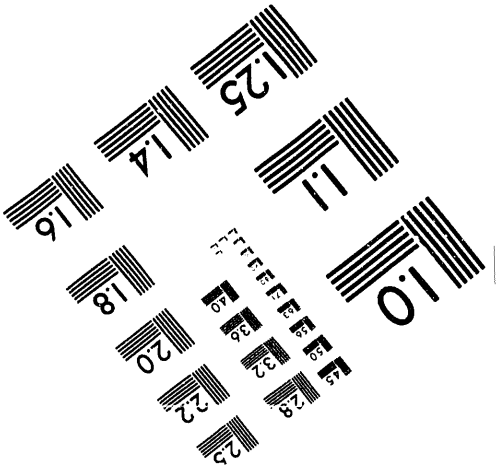

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PHYSICAL MAPPING OF HUMAN CHROMOSOME 16

ANNUAL REPORT FOR CURRENT PROJECT
PHYSICAL MAPPING OF HUMAN CHROMOSOME 16 (07.01.92 TO 06.30.93)

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Prepared for

THE U.S. DEPARTMENT OF ENERGY
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ANNUAL PROGRESS REPORT

TITLE :	**Physical Mapping of Human Chromosome 16**
PRINCIPAL INVESTIGATOR :	**Grant R. Sutherland**
SENIOR ASSOCIATES :	**D.F. Callen, J.C. Mulley, R.I. Richards**
GRANT ID :	**DE-FG02-89ER60863**

Physical Mapping

The construction of additional somatic cell hybrids containing breakpoints of chromosome 16 has continued. The integration of these breakpoints into the existing cytogenetic-based physical map and the mapping of a number of new DNA markers (principally STS based markers) has now enabled the euchromatin of this chromosome to be broken into segments of 1.4 Mb average size, Fig.1. The resolution of this physical map is now sufficient and construction of additional somatic cell hybrids will not be necessary. However, the mapping of DNA markers to this panel will continue with particular emphasis placed on the mapping of cDNA clones.

These somatic cell hybrids have proved to be a valuable and useful resource, not only for the physical mapping of chromosome 16 but of other chromosomes. Distribution of hybrids to groups working on chromosome 16 and other chromosomes will continue. A number of hybrids have now been lodged with the Camden Cell Repository (NIGMS).

Isolation and Mapping of cDNAs

We aim to isolate cDNAs mapping to human chromosome 16 and localise such cDNAs on the high resolution physical map. In collaboration with LANL PCR primers will be synthesised from cDNA sequences mapped to chromosome 16 and used as ESTs in the generation of mega-YAC contigs for this chromosome. Probing of high density cosmid grids will enable integration of the ESTs into cosmid contigs and location of the cosmid contigs on the YAC contig.

A hn-cDNA library has been constructed from the hybrid CY18 which contains chromosome 16 as the only human chromosome. The methods used were based on those published by Lui et al. (Science 245: 813-815, 1989). A modified screening protocol has been successfully developed and 15 hn-cDNA clones have been sequenced and localised on the hybrid map. Sequence analysis of four of these revealed that they were known cDNAs, which are now mapped to chromosome 16. Development of techniques to allow the isolation of longer cDNAs from the identified exons is in progress. This will depend on PCR amplification of cDNAs from a total human cDNA library. Future directions will be to investigate alternative methods based on an hn-cDNA approach.

...2/

Implementation and further development of technologies to identify cDNAs complementary to the human DNA of cosmids is in progress. Procedures involve the hybridization of biotinylated cosmid DNA (prehybridized with human Cot DNA to remove repeats) to PCR amplified cDNA from a human fetal brain cDNA library. Biotin-labelled DNA is separated by its binding to avidin-coated beads. By use of the PCR primers for amplification of the cDNA library, the cDNA which has hybridized to the biotinylated cosmid DNA can be amplified. This amplified cDNA is then screened for clones complementary to the original cosmid DNA. This procedure is based on methods published by Lovett et al. (PNAS 88: 9628-9632, 1991) and Parimos et al. (PNAS 88: 9623-9627, 1991). Further refinement of these procedures is in progress to remove the time-consuming cloning and screening step of the amplified cDNAs. Cosmids targeted for the isolation of cDNA clones are those with precise physical localisation. Therefore, any cDNAs isolated will also be precisely mapped and will be potential candidate genes for diseases mapping to the same region of the chromosome.

Several collaborative projects are in progress (Dr. M. Polymeropoulos and Dr. J. Korenberg, UCLA) to provide a detailed physical localisation of partially sequenced cDNA clones generated from various other sources.

High Resolution Physical Map of 16q24

A high resolution physical map of 16q24 (estimated size 10-15 megabases) is being constructed utilising all available resources. This region has been targeted due to the high density of genes and thus the potential biological interest, and the availability of somatic cell hybrids with this restricted region of chromosome 16. Utilising the LANL high density cosmid grids, approximately 100 cosmid contigs and singleton cosmids have been identified in this region by use of alu-PCR of the somatic cell hybrids which contain this region of chromosome 16 as their only human chromosome 16 genetic material. The localisation of each cosmid or contig has been confirmed by Southern analysis of somatic cell hybrids. In addition, 25 YACs from the Los Alamos sorted chromosome 16 library have been located in this region. Identification of CEPH mega-YACs in this region is in progress. All available DNA markers and genes, and sequence generated from representative cosmids, will be used to saturate this region with STSs to enable integration of cosmid, YAC and physical maps. Isolation of cDNAs from cosmids mapping to this region will be by use of the biotin-rescue technique. Emphasis will be on isolation of full-length cDNAs since these can be used as probes to the LANL high density cosmid grids to allow extension and merging of cosmid contigs.

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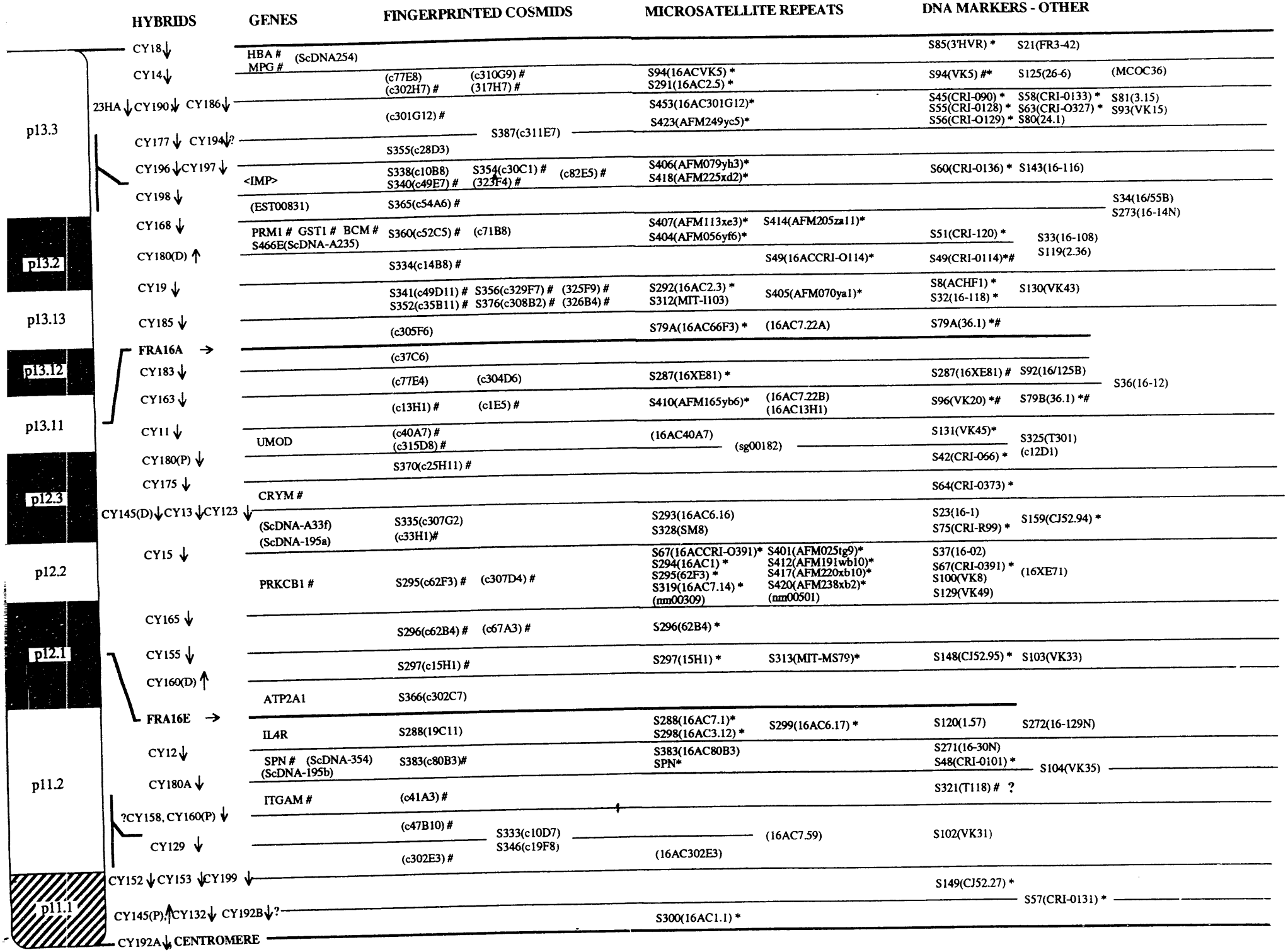
FIG. 1. Cytogenetic-based physical map of human chromosome 16. The portion of chromosome 16 present in each mouse/human hybrid cell line is delineated by a horizontal line with an arrow indicating the direction of the retained portion of chromosome 16 from the breakpoint. Breakpoints of fragile sites are indicated by horizontal arrows. The majority of hybrid cell lines contain the portion of chromosome 16 from the breakpoint to the tip of the long arm (qter) and have been derived from translocations involving chromosome 16. Exceptions are those derived from interstitial deletions (CY107, CY125, CY127, CY130, CY138, CY160, CY180); the hybrid CY18, which contains an intact chromosome 16; and CY18A and CY145, which contain one and two fragments of 16, respectively. The relative sizes of the idiograms for the short and long arms are not to scale. Hybrids CY2 and CY3 contain the der(X) and the der(16), respectively, from the same t(X;16) translocation. DNA markers are divided into columns corresponding to genes, cosmids that have been assembled into contigs by repetitive sequence fingerprinting by the Los Alamos National Laboratory, oligonucleotide primers that flank (AC)n microsatellite repeats and other anonymous DNA markers. When such (AC)n repeats have been identified in previously mapped DNA markers, the location of these markers is also given. All genes, cosmids or other DNA markers that have been converted to STS format by synthesis of oligonucleotide primers, are indicated by #. All polymorphic markers that have been typed in the CEPH pedigrees are indicated by *. The D numbers are abbreviated, e.g. *D16S3* to *S3*, and DNA marker names are given in brackets. A ? indicates a location which has not been completely resolved. *In situ* hybridisation to prophase banded chromosomes provided additional links between the ordered panel of hybrid breakpoints and the idiogram. The location of the hybridisation signal is indicated by a vertical line adjacent to the chromosome.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FINGERPRINTED COSMIDS
MICROSATELLITE REPEATS
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p13.13
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p13.11
p12.3
p12.2
p12.1
p11.2
p11.1
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CY14
23HA
CY190
CY186
CY177
CY194
CY196
CY197
CY198
CY168
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CY19
CY185
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CY183
CY163
CY11
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CY145(D)
CY13
CY123
CY15
CY165
CY155
CY160(D)
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CY12
CY180A
?CY158, CY160(P)
CY129
CY152
CY153
CY199
CY145(P)
CY132
CY192B
CY192A
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MPG #
(c77E8)
(c302H7) #
(c310G9) #
(317H7) #
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S291(16AC2.5) *
S85(3'HVR) *
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S94(VK5) #*
S125(26-6)
(MCOC36)
(c301G12) #
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S58(CRI-0133) *
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(c71B8)
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S321(T118) # ?
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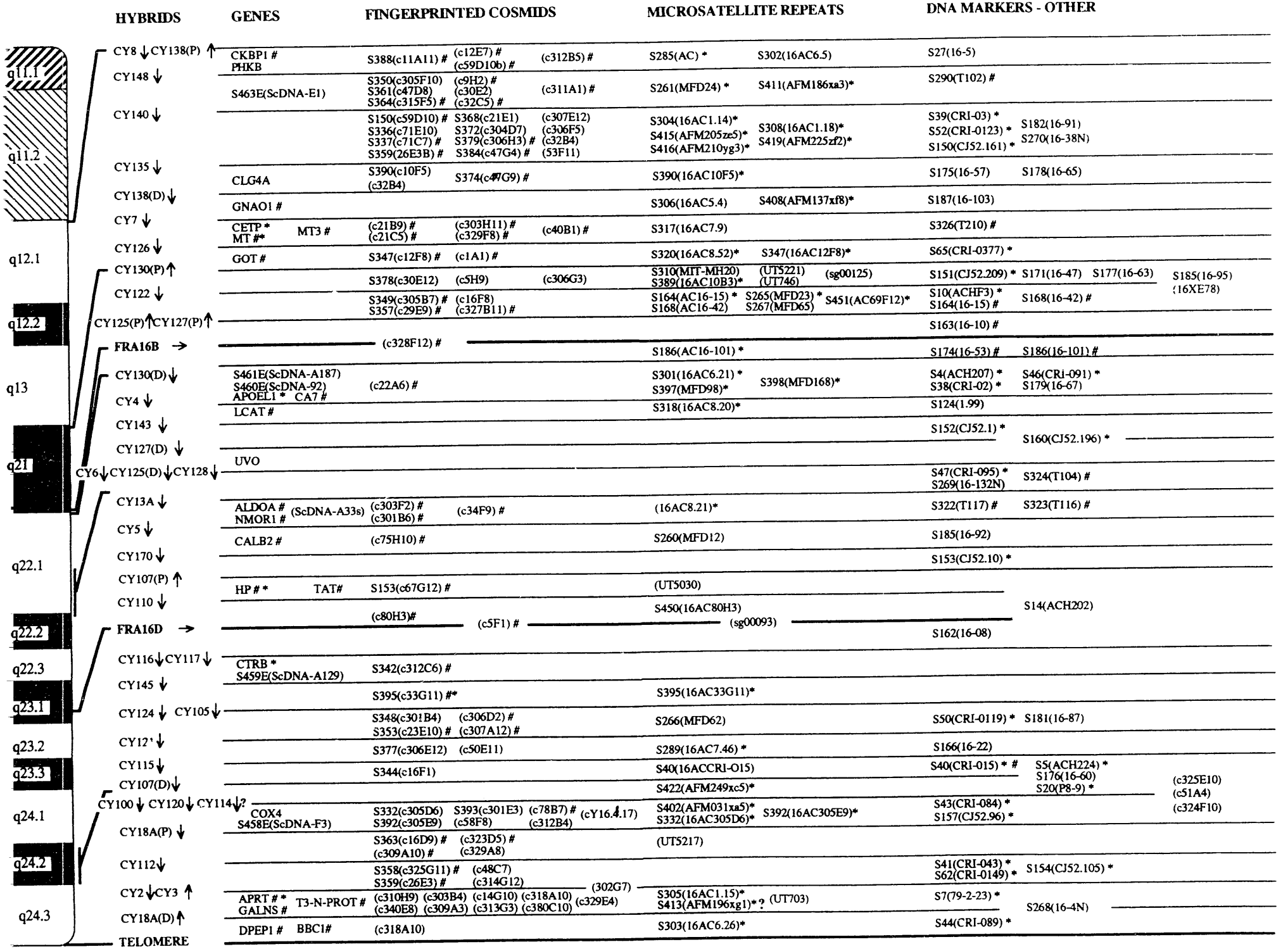
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FINGERPRINTED COSMIDS
MICROSATELLITE REPEATS
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q13
q21
q22.1
q22.2
q22.3
q23.1
q23.2
q23.3
q24.1
q24.2
q24.3
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CY130(D)
S461E(ScDNA-A187)
S460E(ScDNA-92)
APOEL1 *
CA7 #
(c22A6) #
S301(16AC6.21) *
S397(MFD98)*
S398(MFD168)*
S4(ACH207) *
S38(CRI-02) *
S46(CRI-091) *
S179(16-67)
CY4
LCAT #
S318(16AC8.20)*
S124(1.99)
CY143
S152(CJ52.1) *
S160(CJ52.196) *
CY127(D)
UVO
CY6 CY125(D) CY128
S47(CRI-095) *
S269(16-132N)
S324(T104) #
CY13A
ALDOA #
NMOR1 #
(ScDNA-A33s)
(c303F2) #
(c301B6) #
(c34F9) #
(16AC8.21)*
S322(T117) #
S323(T116) #
CY5
CALB2 #
(c75H10) #
S260(MFD12)
S185(16-92)
CY170
S153(CJ52.10) *
CY107(P)
HP # *
TAT#
S153(c67G12) #
(UT5030)
CY110
(c80H3)#
S450(16AC80H3)
S14(ACH202)
FRA16D
(c5F1) #
(sg00093)
S162(16-08)
CY116 CY117
CTRB *
S459E(ScDNA-A129)
S342(c312C6) #
CY145
S395(c33G11) #*
S395(16AC33G11)*
CY124
CY105
S348(c30!B4)
S353(c23E10) #
(c306D2) #
(c307A12) #
S266(MFD62)
S50(CRI-0119) *
S181(16-87)
CY12'
S377(c306E12)
(c50E11)
S289(16AC7.46) *
S166(16-22)
CY115
S344(c16F1)
S40(16ACCRI-O15)
S40(CRI-015) * #
S5(ACH224) *
S176(16-60)
S20(P8-9) *
(c325E10)
(c51A4)
(c324F10)
CY107(D)
S422(AFM249xc5)*
CY100 CY120 CY114 ?
COX4
S458E(ScDNA-F3)
S332(c305D6)
S392(c305E9)
S393(c301E3)
(c58F8)
(c78B7) #
(c312B4)
(cY16.4.17)
S402(AFM031xa5)*
S332(16AC305D6)*
S392(16AC305E9)*
S43(CRI-084) *
S157(CJ52.96) *
CY18A(P)
S363(c16D9) #
(c309A10) #
(c323D5) #
(c329A8)
(UT5217)
CY112
S358(c325G11) #
S359(c26E3) #
(c48C7)
(c314G12)
S41(CRI-043) *
S62(CRI-0149) *
S154(CJ52.105) *
CY2 CY3
APRT # *
GALNS #
T3-N-PROT #
(c310H9)
(c340E8)
(c303B4)
(c309A3)
(c14G10)
(c313G3)
(c318A10)
(c380C10)
(302G7)
(c329E4)
S305(16AC1.15)*
S413(AFM196xg1)* ?
(UT703)
S7(79-2-23) *
S268(16-4N)
CY18A(D)
DPEP1 #
BBC1#
(c318A10)
S303(16AC6.26)*
S44(CRI-089) *
TELOMERE

DATE
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9/14/93

END