Chapter 5
Evolutionary New Centromeres in Primates

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Abstract The centromere has a pivotal role in structuring chromosomal architecture, but remains a poorly understood and seemingly paradoxical "black hole." Centromeres are a very rapidly evolving segment of the genome and it is now known that centromere shifts in evolution are not rare and must be considered on a par with other chromosome rearrangements. Recently, unprecedented findings on neocentromeres and evolutionary new centromeres (ENC) have helped clarify the relationship of the

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centromere within the genome and shown that these two phenomena are two faces of the same coin. No prominent sequence features are known that promote centromere formation and both types of new centromeres are formed epigenetically, both clinical neocentromeres and ENC cluster at chromosomal "hotspots." The clustering of neocentromeres in 8p is probably the result of the relatively high frequency of non-canonical pairing. Studies on the evolution of the chromosomes 3, 13, and 15 help explain why there are clusters of neocentromeres. These domains often correspond to ancestral inactivated centromeres and some regions can preserve features that trigger neocentromere emergence over tens of millions of years. Neocentromeres may be correlated with the distribution of segmental duplications (SDs) in regions of extreme plasticity that often can be characterized as gene deserts. Further, because centromeres and associated pericentric regions are dynamically complex, centromere shifts may turbocharge genome reorganization by influencing the distribution of heterochromatin. The "reuse" of regions as centromere seeding-points in evolution and in human clinical cases further extends the concept of "reuse" of specific domains for "chromosomal events."

5.1 The "Black Hole"

The centromere, a term coined by Darlington 1936, is the primary constriction where the kinetochore forms and the spindle fiber attaches to ensure correct chromatid segregation during cell division. The centromere has always been given a pivotal role in structuring chromosomal architecture, and classical analyses emphasized Robertsonian fissions and fusions as well as pericentric inversions as the principle mechanisms in the transformation of species diploid ($2n$) and fundamental numbers (FN, number of chromosome arms). More recent investigations have also paid attention to deletions, duplications, tandem fusions, and centromere shifts, with both the deactivation and the activation of centromere playing a fundamental role. The pericentromeric regions of centromeres are regions rich in duplicons, transposons, retro elements, and even pseudogenes and expressed genes. They are hot spots of chromosome changes in both evolution and in disease (Villasante et al. 2007).

Clearly then, the centromere is a key structure in the evolution of eukaryotic chromosomes, yet remains poorly understood and seemingly paradoxical. Early work suggested that particular satellite sequences were involved in centromere formation but the comparative study of centromere DNA showed that it was highly variable across species (O'Neill et al. 2004) (see Chaps. 2–4 of this book).

In the last years, unprecedented findings on neocentromeres and evolutionary new centromeres (ENC) added additional oddities to this "black hole" of biology. On the other hand, they started to clarify the complex relationship of the centromere with the underlying sequences. Montefalcone et al. (1999) showed that a centromere, during evolution, can move along the chromosome without any accompanying

chromosomal rearrangements. This unusual centromere behavior is now well documented in a large array of taxa, in particular, primates. It was also shown that ENCs have an intriguing connection with a related phenomenon: human clinical neocentromeres. This chapter mainly addresses the evolutionary aspects of neocentromeres, but ENCs and neocentromeres are, very likely, two faces of the same phenomenon. For this reason, the clinical neocentromeres will be briefly summarized in the following paragraph. For an exhaustive review see Marshal et al. (2008)

5.1.1 Human Clinical Neocentromeres

Neocentromeres are analphoid centromeres that emerge in ectopic chromosomal regions. The emergence of a neocentromere most frequently occurs to provide mitotic stability to otherwise acentric chromosome fragments resulting from a rearrangement (Amor and Choo 2002; Warburton 2004; Marshall et al. 2008). The stabilized supernumerary chromosome has detrimental phenotypic consequences, and it is usually discovered when these clinical patients are examined cytogenetically.

Nearly 100 such cases were reported in the literature (cf. Marshall et al. 2008). Marshall et al. (2008) report that clinical neocentromere are noted once in every 70,000–200,000 live births, but these studies do not include the incidence of balanced rearrangements which have no phenotypic consequences and are not caught by the clinical filter (see Capozzi et al. 2008). Sometimes balanced neocentromeres are serendipitously found in normal individuals (see below). The chromosomal distribution of neocentromeres is reported in Fig. 5.1.

As mentioned, neocentromere emergence is usually an opportunistic, secondary event, concomitant to a rearrangement that generated an acentric fragment. This implies that human clinical neocentromeres are not the consequence of any kind of sequence transposition or mutational modification, and that, consequently, these events are epigenetic in nature (Alonso et al. 2003) (see also Chap. 1 of this book).

The chromosomal localization of neocentromeres (see Fig. 5.1) has usually been attained by fluorescence in situ hybridization (FISH) using BAC or similar DNA probes, with the aim of identifying clones mapped to opposite sides of the centromere. Occasionally, this approach for various reasons provided only an approximate mapping. One reason is that the neocentromere does not contain a heterochromatic block that can be very helpful in orienting the probe hybridization to one side or the other of the centromere. Additionally, several supernumerary, neocentromeric chromosomes have an inverted-duplication structure that makes characterization difficult. Neocentromeres in small ring chromosomes are also difficult to map because the primary constriction is not easily identified. These limitations explain why the mapping of chromosomal regions harboring neocentromeres was sometimes fairly approximate (see Fig. 5.1). In some instances, however, the neocentromere was mapped down to the sequence level using a ChIP-on-chip approach. In this method, living cells are crosslinked in situ by adding formaldehyde. DNA is then sheared by

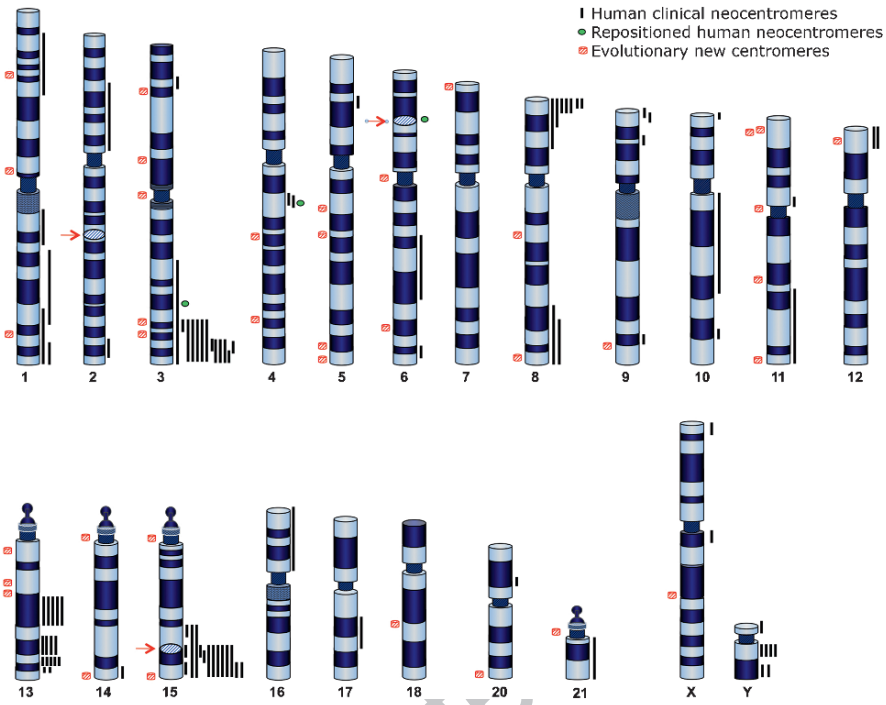

Fig. 5.1 ENCs and neocentromeres. The ideograms graphically report human clinical centromeres, represented by a *black bar* spanning the seeding point, on the right of each chromosome (modified from Marshall et al. 2008). The figure includes 1 new clinical centromere reported on chromosome 9 (Capozzi et al. 2008) and one repositioned centromere on chromosome 6 (Capozzi et al. submitted). The localization appears very approximate in some instances, for the reasons discussed in the text (Sect. 5.1.1). The three repositioned centromeres found in normal persons are represented by a small *green circle*. ENCs are indicated, *in red*, on the left of the chromosomes. *Red arrows* indicate inactivated ancestral centromeres. The Supplement Table 5.1 (see at the end of this chapter) reports in detail the data graphically summarized in this figure

sonication and immunoprecipitated using antibodies against centromeric proteins (CENP, usually CENP-A and CENP-C). Purified DNA fragments are then amplified, labeled, and hybridized to a high density BAC or oligo arrays (see Capozzi et al 2008). Thirteen neocentromeres were precisely mapped in this way (Lo et al. 2001a, b; Alonso et al 2003, 2007; Saffery et al. 2003; Sumer et al. 2003; Chueh et al. 2005; Cardone et al. 2006; Capozzi et al 2008). The CENP domain ranged from ~54 to 450 kb. The size can be occasionally over-estimated if BAC arrays are used. Sequence comparison among these regions did not show any prominent features that could be predictive of centromere-forming potential. In other words, it is not evident what makes a sequence "centromere competent." Another complication is the striking difference between a "normal" centromere, up to 3–4 Mb in size, and neocentromeres composed of as low as 50 kb of "plain" sequence. It has to be noted,

however, that the frequently reported mosaicism suggests that neocentromeres are not so efficient. This point will be further discussed below.

The phenotypic problems inherent in patients with neocentromeres also imply that they have no evolutionary future. It can be easily hypothesized that the fitness of these individuals is negligible. The neocentromere-ENC connection could therefore appear problematic. However, some recent lines of evidence suggested a surprisingly strong relationship. For instance, same chromosomal domain can be used as seeding-point for both neocentromeres and ENCs. A second line of evidence revealed that some seeding-point domains correspond to ancestrally inactivated centromeres (see below). Lastly, three familial cases of human neocentromeres were discovered segregating in perfectly normal people (Amor et al. 2004; Ventura et al. 2004; Capozzi et al. submitted). These three cases can be considered as repositioned centromeres "in progress." They are familiarly inherited and have no phenotypic implications; indeed their discovery was accidental.

5.2 Evolutionary Repositioned Centromeres in Primates

Karyotype evolution has been mainly studied using whole-chromosome painting probes. This approach has the advantage of mapping translocation differences between species, but does not usually provide information on intrachromosomal rearrangements or marker order differences. Recently, the availability of large cloned DNA collections of BACs and fosmids (see P. de Jong lab at http://bacpac.chori.org/home.htm; see also paragraph 9.6, Technical note) made it possible to study by FISH marker order changes during evolution in chromosomes of different species (molecular cytogenetic approach). The precise mapping of thousands of clones is graphically displayed in genome browsers (see the track "BAC End Pairs" or "Fosmid End Pairs" in UCSC, for instance). Two or more BAC clones can be simultaneously hybridized and their reciprocal order can be unequivocally defined. This cytogenetic approach to synteny definition complements other approaches that have been exploited to define genome organization: radiation hybrid mapping, linkage analysis, and sequencing (see Rocchi et al. 2006). Importantly, the molecular cytogenetic approach is sequence independent, and it can substantially aid sequence assembly, because the pure shot-gun approach, used for most genomes, is error prone (Green 1997; Roberto et al. 2008). For a fine synteny definition of complex genomes using the molecular cytogenetics technology, see Roberto et al. (2007) and Misceo et al. (2008) and the corresponding Web pages http://www.biologia.uniba.it/lar/ and http://www.biologia.uniba.it/gibbon/, respectively, provided as Supplemental Material to these publications.

Synteny arrangement comparisons allowed Montefalcone et al. (1999), as mentioned earlier, to disclose that some centromeres shifted along the chromosome during evolution. Studies over the last decade have amply demonstrated that centromere shifts in evolution are not rare and must be considered on a par with other chromosome rearrangements such as translocations, inversion, duplications, and

deletions. Ventura et al. (2007), comparing human and macaque, clarified how very frequent ENC are in primate evolution. In total, between macaque and humans there are 14 ENC; nine ENCs occurred in macaque lineage and five occurred in the human lineage. The last common ancestor of macaques and humans is estimated at about 25 million years ago (mya). So ENC in this case formed about once every three million years. Perhaps surprisingly, by comparison in the same arch of time, there are only four translocation differences (about one translocation every 12 million years). We might conclude from this example that ENC are four times more frequent than cytogenetically visible translocations and represent a significant facet of mammalian chromosomal evolution. ENCs were reported in the evolution of chromosome 3 (Ventura et al 2004), chromosome 6 (Eder et al. 2003), chromosome 10 (Carbone et al. 2002), chromosome 11 (Cardone et al. 2007), chromosome 13 (Cardone et al 2006), chromosome 14 and 15 (Ventura et al. 2003), chromosome 20 (Misceo et al. 2005), and chromosome X (Ventura et al. 2001). Figure 5.1 graphically reports, on the left of each chromosome, all the published ENCs. Supplement Table 5.1 (see at the end of this chapter) reports details of neocentromeres and ENCs literature data. It is interesting to note that the centromere is apparently a very rapidly evolving segment of the genome. Further, because centromeres and associated pericentric regions are dynamically complex, centromere shifts may turbocharge genome reorganization by influencing the distribution of heterochromatin (Ishii et al. 2008).

5.3 Hotspots of Neocentromere Formation

A clearly recognizable trend from the human clinical cytogenetic data is the clustering of neocentromere formation sites at chromosomal "hotspots." Certain regions of chromosomes – for example, 3q, 8p, 13q, and 15q telomeric regions – seem particularly prone to forming neocentromeres (Fig. 5.1). The survival of individuals with more distal inverted duplications will be favored (as such individuals possess a smaller region of partial trisomy or tetrasomy); it is therefore logical that neocentromeres cluster around the distal ends of chromosomes. It follows that some other regions with neocentromere-forming potentiality have never been described because of this bias. What becomes fixed in evolution is, therefore, the end result of mutation and the selectional filter. The neocentromere reported at 9q33.1 is paradigmatic in this respect (Capozzi et al 2008). The propositus, in fact, was found to carry an interstitial deletion of chromosome 9, of about 12 Mb (9q31.3-9q33.1). The parents were investigated because of the deletion in the son. The mother had a small ring chromosome that resulted from the excision of the 12 Mb from the chromosome 9. A neocentromere at 9q33.1 had stabilized the ring chromosome. The son had inherited the deleted chromosome but not the ring. This neocentromere would have been never detected if malsegregation had not occurred. No such neocentromere was detected in supernumerary chromosomes.

Table 5.1 ENC in a *red circle* indicates the emergence of an evolutionary new centromere. The *red arrows* point to inactivated centromeres. For details see text
Supplement Table 5.1 BAC clones used to delineate evolutionary history of chromosomes in primates

		HSA1			
BAC name	Acc.N.	UCSCMarch2006	Cytog.map	ENC or CNC	Reference
RP11-421C4	BES	chr1:1,247,484-1,432,829	1p36.33		
RP11-265F14	AL512883	chr1:15,630,693-15,735,006	1p32.21		
RP11-266K22	AL451070	chr1:31,512,242-31,645,345	1p35.2		
RP5-1154B21	(D1S3315)	chr1:47,605,724-47,806,009	1p33		
RP11-55M23	BES	chr1:55,214,018-55,384,910	1p32.3		
				LLA9ENC	
RP11-316C12	AL627317	chr1:71,621,580-71,715,216	1p31.1		
RP11-254E16	BES	chr1:84,539,796-84,689,450	1p31.1		
RP11-138K16	AC093559	chr1:99,728,340-99,904,318	1p21.2		
RP11-284N8	AL365361	chr1:110,897,276-111,090,458	1p13.3		
				LLA28ENC	Stanyon et al. (2008)
RP11-192J8	BES	chr1:118,333,066-118,333,600	1p12		
RP5-1042I8	AL359752	chr1:120,134,618-120,272,572	1p12		
CEN				APCEN	Stanyon et al. (2008)
HETEROCHRO	MATIN				
RP11-35B4	AL359093	chr1:143,999,789-144,166,609	1q21.1+…		
RP11-98F1	AL353760	chr1:153,539,660-153,543,654	1q22		
RP11-8D14	AC068728	chr1:158,524,306-159,027,864	1q23.3		
RP11-117F19	BES	chr1:160,616,094-160,786,583	1q23.3		
RP11-331H2	AL392003	chr1:161,033,791-161,225,664	1q23.3		
RP11-593N18	BES	chr1:165,733,633-165,896,797	1q24.2		
RP11-332H17	AL356475	chr1:168,242,366-168,350,412	1q24.2		
RP11-170H10	BES	chr1:177,378,863-177,558,311	1q25.2		
RP11-152A16	BES	chr1:177,339,824-177,521,905	1q25.2		
RP11-46A10	BES	chr1:179,093,659-179,296,257	1q25.3		
RP11-453M18	BES	chr1:179,309,603-179,489,310	1q25.3		

(*continued*)

Table 5.1 (continued)

		HSA1			
BAC name	Acc.N.	UCSCMarch2006	Cytog.map	ENC or CNC	Reference
RP11-382D12	AL445228	chr1:182,516,029-182,632,280	1q25.3		
RP11-134C1	BES	chr1:186,252,053-186,417,889	1q31.1		
RP11-92O4	BES	chr1:186,371,022-186,536,053	1q31.1		
RP11-13G5	BES	chr1:190,775,806-190,935,812	1q31.2		
RP11-173E24	AL138926	chr1:193,522,550-193,691,711	1q31.3		
RP11-44M20	BES	chr1:194,486,097-194,633,832	1q31.3		
RP11-112O19	BES	chr1:194,850,927-195,161,754	1q31.3		
RP11-192O22	BES	chr1:195,224,762-195,369,931	1q31.3		
RP11-553K8	AL157402	chr1:196,750,597-196,960,927	1q31.3		
RP11-57I17	AL137789	chr1:205,811,787-205,957,118	1q32.2		
RP11-2P2	BES	chr1:206,365,585-206,526,445	1q32.2		
RP11-167J2	BES	chr1:207,651,871-207,813,996	1q32.2		
RP11-345I23	BES	chr1:207,907,102-208,088,214	1q32.2		
RP11-237I23	BES	chr1:208,074,198-208,253,751	1q32.2		
RP11-168F20	BES	chr1:208,231,193-208,397,063	1q32.2		
RP11-123O6	BES	chr1:209,402,692-209,548,350	1q32.2-1q32.3		
RP11-74E6	BES	chr1:212,560,677-212,729,794	1q32.3-1q41		
RP11-324K19	AC118468	chr1:219,203,440-219,218,989	1q41		
RP11-351B5	BES	chr1:220,760,290-220,938,014	1q41		
RP11-18A13	BES	chr1:221,338,590-221,505,250	1q41		
				CJA19ENC	Unpublished data
RP11-122D22	BES	chr1:223,625,059-223,799,771	1q42.12		
RP11-3K22	AL359874	chr1:227,082,837-227,162,782	1q42.13		
RP11-108F13	BES	chr1:227,804,416-227,983,307	1q42.13		
RP11-543E8	FISH	chr1:228,724,827-228,906,001	1q42.13-42.2		
RP11-933K5	BES	chr1:228,896,382-229,080,653	1q42.2		
RP11-316N16	BES	chr1:229,077,217-229,258,402	1q42.2		

RP11-281B4	BES	chr1:229,444,660-229,608,742	1q42.2		
RP11-88N18	BES	chr1:229,794,597-229,951,130	1q42.2		
RP11-210E16	BES	chr1:230,558,779-230,738,335	1q42.2		
RP11-155C15	BES	chr1:232,687,473-232,687,928	1q42.2		
RP11-385F5	AL359921	chr1:234,752,824-234,966,581	1q43		
RP11-438F14	AC098483	chr1:246,754,133-246,932,000	1q44		

		HSA3			
BAC name	Acc.N.	UCSCMarch2006	Cytog.map	ENC or CNC	Reference
RP11-151A4(A)	BES	chr3:636,173-795,419	3p26.3		
RP11-183N22	AL512885	chr3:4,328,222-4,493,696	3p26.1		
RP11-48N24	BES	chr3:7,397,489-7,541,994	3p26.1		
RP11-732C9	BES	chr3:12,441,757-12,649,037	3p25.2		
RP11-316A10	AC090937	chr3:14,886,290-15,046,968	3p25.1		
RP11-616M11(B)	AC090954	chr3:15,045,785-15,213,797	3p25.1		
RP11-421B21(C)	AC090949	chr3:15,147,209-15,324,532	3p25.1		
RP11-109D5	BES	chr3:25,497,576-25,697,390	3p24.2		
			3p23	CNC	Maraschio et al. (1996)
RP11-627J17	AC112211	chr3:32,980,609-33,163,117	3p23		
RP11-240N7	BES	chr3:36,506,239-36,658,135	3p22.2		
RP11-607P24	BES	chr3:36,298,506-36,506,070	3p22.3-22.2		
RP11-491D6	AC006583	chr3:37,034,150-37,136,520	3p22.3		
RP11-713K14	AP006242	chr3:37,868,651-38,036,022	3p22.3		
RP11-409G11	BES	chr3:39,926,773-40,069,893	3p22.1		
RP11-465K13	BES	chr3:40,934,090-41,113,356	3p22.1		
RP11-756A10	AC099059	chr3:41,445,167-41,603,742	3p22.1		
RP11-626A1	AC137935	chr3:41,872,546-42,048,517	3p22.1		
RP11-12I10	BES	chr3:42,489,150-42,664,465	3p22.1		
RP11-1047D9	BES	chr3:42,688,112-42,792,912	3p22,1		
RP11-625B23	BES	chr3:43,135,351-43,328,084	3p22.1		

(*continued*)

Table 5.1 (continued)

		HSA3			
BAC name	Acc.N.	UCSCMarch2006	Cytog.map	ENC or CNC	Reference
RP11-353H3(D)	BES	chr3:43,377,484-43,547,690	3p22.1		
				CAE22ENC	Ventura et al. (2004)
RP11-395P16(E)	AC130472	chr3:47,584,747-47,778,906	3p21.31		
RP11-380J21	AC136275	chr3:64,182,355-64,200,696	3p14.1		
RP11-151M23	BES	chr3:67,043,727-67,181,276	3p14.1		
RP11-158P4	BES	chr3:73,574,165-73,731,870	3p13		
RP11-634L22(F)	BES	chr3:75,452,260-75,628,601	3p12.3		
				CJA21ENC	Ventura et al. (2004)
RP11-180C9(G)	BES	chr3:75,997,245-76,170,439	3p12.3		
RP11-536K4	AC016942	chr3:76,682,896-76,834,909	3p12.3		
RP11-655A17	BES	chr3:87,099,698-87,270,490	3p11.2-12.1		
RP11-547K2(H)	AC107028	chr3:89,543,932-89,670,647	3p11.1		
CEN		chr3:89,700,001-93,200,000		ANTHROPOIDEA ENC	Ventura et al. (2004)
RP11-124L3(I)	BES	chr3:94,987,545-95,112,295	3q11.2		
RP11-91M15	BES	chr3:96,443,224-96,627,928	3q11.2		
RP11-117C10	BES	chr3:99,602,287-99,735,761	3q11.2		
RP11-454H13	AC084198	chr3:102,798,688-102,993,642	3q12.3		
RP11-305I9	AC092981	chr3:120,465,181-120,625,176	3q13.32-13.33		
RP11-757I12	AC092908	chr3:123,727,494-123,901,752	3q21.1		
RP11-257B7	BES	chr3:125,091,060-125,253,000	3q21.1		
RP11-98E19	BES	chr3:126,496,462-126,666,036	3q21.2		
RP11-26M12(J)	BES	chr3:130,089,343-130,276,597	3q21.3		
RP11-787P10(K)	BES	chr3:131,347,364-131,500,312	3p21.3-22.1		
RP11-21N8	BES	chr3:131,810,630-131,961,198	3q22.1		
RP11-58H13	BES	chr3:134,587,560-134,765,508	3q22.1		
RP11-45B17	BES	chr3:139,765,789-139,942,541	3q22.3		
RP11-13N24	BES	chr3:145,950,523-146,100,894	3q24		

RP11-505J9	BES	chr3:149,845,223-150,049,901	3q24		
			3q24	HRC	Ventura et al. (2004)
RP11-36G5	BES	chr3:151,686,443-151,861,594	3q25.1		
RP11-484J9	BES	chr3:154,596,424-154,771,945	3q25.2		
RP11-142B1	BES	chr3:162,044,658-162,223,487	3q26.1		
			3q26.1	CNC	Ventura et al. (2004)
RP11-498P15	AC112906	chr3:163,530,323-163,648,093	3q26.1		
RP11-355I21	AC025826	chr3:163,822,353-164,122,697	3q26.1		
RP11-498P15	AC112906	chr3:163,530,323-163,648,093	3q26.1		
RP11-355I21(L)	AC025826	chr3:163,822,353-164,122,697	3q26.1		
				OWMENC	Ventura et al. (2004)
RP11-418B12(M)	AC079910	chr3:164,539,721-164,707,127	3q26.1		
RP11-526M23	AC048332	chr3:166,898,263-167,089,798	3q26.1		
RP11-114M1	BES	chr3:178,755,562-178,913,002	3q26.32		
RP11-121O16(N)	BES	chr3:179,246,025-179,381,716	3q26.32		
				CJA15,LLA22, CJA17ENCs	Ventura et al. (2004)
RP11-160P8(O)	BES	chr3:179,811,584-179,969,664	3q26.32		
RP11-102M21	BES	chr3:180,795,920-180,965,055	3q26.33		
RP11-218A22	AC108670	chr3:186,915,322-187,078,478	3q27.2		
			3q27	CNC	Papenhausen et al. (1995)
RP11-709J22	BES	chr3:187,553,921-187,754,882	3q27.3		
RP11-42D20	AC007690	chr3:188,091,027-188,272,557	3q27.3		
RP11-298A18	AC063932	chr3:189,731,862-189,918,758	3q28		
RP11-153K2	BES	chr3:192,086,117-192,227,844	3q28		
RP11-6E10	BES	chr3:197,556,002-197,719,622	3q29		
RP11-313F11(P)	FISH	chr3:198,844,624-198,845,224	3q29		
				CMO20ENC	Ventura et al. (2004)

(*continued*)

Table 5.1 (continued)

		HSA4			
BAC name	Acc.N.	UCSCMarch2006	Cytog.map	ENC or CNC	Reference
RP11-61B7	BES	chr4:39,428-230,148	4p16.3		
RP11-167K22	BES	chr4:15,166,060-15,327,998	4p15.32-15.33		
RP11-102K4	BES	chr4:18,302,511-18,440,696	4p15.32		
RP11-585D5	BES	chr4:21,855,149-22,058,506	4p15.31		
RP11-156A17	BES	chr4:21,620,803-21,774,876	4p15.31		
RP11-362I16	AC093814	chr4:22,002,309-22,165,509	4p15.31		
RP11-157B23	BES	chr4:23,884,053-24,050,372	4p15.2		
RP11-125D22	BES	chr4:26,121,547-26,273,493	4p15.2		
RP11-100L2	BES	chr4:28,768,899-28,923,065	4p15.1		
RP11-164K20	BES	chr4:29,841,543-30,016,485	4p15.1		
RP11-124E24	BES	chr4:32,539,454-32,710,916	4p15.1		
RP11-135M12	AC096735	chr4:35,206,808-35,400,317	4p15		
RP11-108H14	BES	chr4:36,852,417-37,053,575	4p14		
RP11-103K10	BES	chr4:38,660,498-38,852,024	4p14		
RP11-473D12	AC108149	chr4:43,753,530-43,839,853	4p13		
RP11-317G22	AC020593	chr4:48,589,290-48,773,495	4p12		
CEN					
RP11-365H22	AC027271	chr4:52,354,875-52,532,859	4q11		
RP11-669F1	BES	chr4:68,763,265-68,894,804	4q13.2		
			4q21.1-21.3	CNC	Grimbacher et al. (1999); Warburton et al. (2003)
RP11-458G13	BES	chr4:87,075,729-87,246,734	4q21.23-21.3		
RP11-209G6	BES	chr4:88,092,435-88,250,275			
				HRC	Amor et al. (2004)

RP11-204I22	BES	chr4:89,693,568-89,857,513			
RP11-499E18	AC098487	chr4:103,434,684-103,598,599	4q24		
				LLA19, LLA4ENC	Stanyon et al. (2008)
RP11-510D4	AC092661	chr4:118,641,765-118,817,790	4q26		
RP11-45L5	AC015631	chr4:135,278,394-135,423,531	4q28.3		
RP11-780M14	AC104090	chr4:144,875,612-144,976,649	4q31.21		
RP11-663M18	AC109823	chr4:159,961,327-160,041,534	4q32.1		
RP11-493C20	AC098867	chr4:164,688,088-164,830,401	4q32.3		
RP11-808H17	AC079240	chr4:165,338,176-165,541,022	4q32.3		
RP11-443J23	AC093842	chr4:166,667,434-166,780,458	4q32.3		
RP11-511B7	AC080079	chr4:166,778,459-166,890,974	4q32.3		
RP11-371E22	AC097507	chr4:167,055,229-167,222,260	4q32.3		
RP11-624O16	AC093874	chr4:167,220,261-167,378,505	4q32.3		
RP11-436G13	AC107055	chr4:167,376,506-167,521,299	4q32.3		
RP11-368M2	BES	chr4:167,334,183-167,519,234	4q32.3		
RP11-13P1	BES	chr4:167,405,757-167,570,823	4q32.3		
RP11-662N23	BES	chr4:167,405,729-167,578,663	4q32.3		
				NWMCEN	Stanyon et al. (2008)
RP11-455K3	BES	chr4:167,510,402-167,693,775	4q32.3		
RP11-638N11	BES	chr4:167,724,651-167,927,839	4q32.3		
RP11-662D13	AC068989	chr4:169,331,047-169,528,540	4q32.3		
RP11-648O9	AC106878	chr4:170,815,533-170,950,874	4q33		
RP11-51M24	BES	chr4:175,426,843-175,581,124	4q34.1		
RP11-99E17	BES	chr4:180,594,112-180,767,207	4q34.3		
RP11-104E20	BES	chr4:185,808,872-185,994,898	4q35.1		
RP11-45C13	BES	chr4:187,655,959-187,810,778	4q35.2		
RP11-138B4	BES	chr4:188,409,307-188,555,694	4q35.2		
RP11-242B20	BES	chr4:190,768,122-190,931,965	4q35.2		

(*continued*)

Table 5.1 (continued)

		HSA5			
BAC name	Acc.N.	UCSCMarch2006	Cytog.map	ENC or CNC	Reference
RP11-58A5	BES	chr5:4,965,694-5,123,154	5p15.32		
RP11-5N8	BES	chr5:14,926,850-15,108,182	5p15.2		
			5p14-p15.1	CNC	Fritz et al. (2001)
RP11-12C2	AC114298	chr5:23,056,186-23,144,972	5p14.3		
RP11-94E6	BES	chr5:33,737,270-33,926,014	5p13.3		
RP11-159F24	BES	chr5:43,509,883-43,672,607	5p12		
CEN					
RP11-160F8	BES	chr5:53,365,254-53,520,258	5q11.2		
RP11-298P6	AC109465	chr5:64,070,008-64,258,158	5q12.3		
RP11-172K14	BES	chr5:74,490,650-74,673,873	5q13.3		
				CMO11-CMO14CEN	Stanyon et al. (2008)
RP11-258M21	BES	chr5:85,443,998-85,587,282	5q14.3		
RP11-297G19	AC093268	chr5:93,288,262-93,463,665	5q15		
				LLA3ENC	Stanyon et al. (2008)
RP11-326M11	BES	chr5:105,194,174-105,355,876	5q21.3		
RP11-81C5	BES	chr5:115,183,047-115,366,698	5q23.1		
RP11-209F21	BES	chr5:124,786,541-124,969,874	5q23.2		
RP11-42M12	BES	chr5:127,176,898-127,327,842	5q23.2		
RP11-186F1	BES	chr5:130,344,505-130,522,404	5q31.1		
RP11-4E3	BES	chr5:133,085,104-133,272,697	5q31.1		
RP11-1030O9	BES	chr5:133,318,962-133,507,753	5q31.1		
RP11-737P20	BES	chr5:133,455,828-133,650,405	5q31.1		
RP11-21C10	BES	chr5:133,880,133-134,045,963	5q31.1		
RP11-114H21	BES	chr5:135,739,999-135,916,051	5q31.2		
RP11-365D10	BES	chr5:144,529,859-144,719,618	5q32		
RP11-170L13	BES	chr5:155,123,977-155,288,472	5q33.2		
RP11-367N22	BES	chr5:156,258,929-156,421,855	5q33.3		

RP11-52L13	BES	chr5:156,420,347-156,582,237	5q33.3		
RP11-92E20	BES	chr5:156,594,140-156,769,665	5q33.3		
RP11-631N12	BES	chr5:157,000,006-157,172,852	5q33.3		
RP11-82E8	BES	chr5:157,616,481-157,787,918	5q33.3		
RP11-678F4	BES	chr5:158,490,847-158,671,405	5q33.3		
RP11-90N23	FISH	chr5:159,983,909-159,984,761	5q34		
RP11-114D4	BES	chr5:160,424,255-160,577,512	5q34		
RP11-569B13	AC091984	chr5:161,495,046-161,702,085	5q34		
RP11-88J19	BES	chr5:162,047,143-162,237,277	5q34		
RP11-653G7	BES	chr5:163,166,056-163,341,883	5q34		
RP11-308N24	AC109466	chr5:164,314,289-164,468,103	5q34		
RP11-90C21	BES	chr5:165,262,926-165,426,112	5q34		
RP11-436K21	BES	chr5:166,079,437-166,247,339	5q34		
RP11-69K7	BES	chr5:167,097,350-167,258,685	5q34		
RP11-14K9	BES	chr5:168,358,455-168,532,236	5q35.1		
				LLA11CEN	Stanyon et al. (2008)
RP11-170N13	BES	chr5:168,433,613-168,593,223	5q35.1		
RP11-270N4	BES	chr5:168,532,261-168,704,355	5q35.1		
RP11-486H5	BES	chr5:168,909,435-169,092,364	5q35.1		
RP11-15F10	BES	chr5:169,073,440-169,267,747	5q35.1		
RP11-117L6	BES	chr5:170,679,528-170,854,638	5q35.1		
RP11-48K2	BES	chr5:172,952,607-173,131,912	5q35.2		
RP11-125L2	BES	chr5:173,447,703-173,616,629	5q35.2		
RP11-298C7	BES	chr5:176,032,557-176,197,535	5q35.2		
RP11-452O4	BES	chr5:177,234,210-177,410,189	5q35.3		
				CJA2-SSC20-SSC1CEN	Stanyon et al. (2008)

		HSA6			
BAC name	Acc.N.	UCSCMarch2006	Cytog.map	ENC or CNC	Reference
RP11-328C17	AL365272	chr6:213,636-346,084	6p25.3		

(*continued*)

Table 5.1 (continued)

		HSA6			
BAC name	Acc.N.	UCSCMarch2006	Cytog.map	ENC or CNC	Reference
RP11-391F23	AL589203	chr6:929,025-940,528	6p25.3		
RP11-125I8	BES	chr6:10,001,499-10,140,467	6p24.3		
RP11-15I14	BES	chr6:10,459,005-10,639,794	6p24.3		
RP11-147C6	BES	chr6:10,622,819-10,774,675	6p24.2		
RP11-48D18	BES	chr6:10,937,982-11,123,046	6p24.2		
RP11-4A24	AL137221	chr6:12,238,011-12,244,433	6p24.1		
RP11-27M22	BES	chr6:14,673,843-14,829,605	6p23		
RP11-61I16	BES	chr6:15,365,240-15,519,994	6p23		
RP11-17L3	BES	chr6:15,716,005-15,896,106	6p22.3		
RP11-90O12	BES	chr6:16,445,725-16,630,424	6p22.3		
RP11-59N15	BES	chr6:26,015,628-26,168,053	6p22.2		
		chr6:26,407,000-26,491,000		HRC(ChIP-on-chip)	Capozzi et al. (2008a)
RP11-911D8	BES	chr6:27,069,535-27,253,168	6p22.1		
RP11-297M4	BES	chr6:29,016,624-29,189,711	6p22.1		
RP11-99D3	BES	chr6:29,049,490-29,222,060	6p22.1		
				ANCESTRALCEN.	Capozzi et al. (2008a)
RP11-261L19	BES	chr6:29,259,359-29,405,414	6p22.1		
RP11-751N3	BES	chr6:29,555,726-29,748,946	6p22.1		
RP11-351O4	BES	chr6:30,258,900-30,456,684	6p21.33		
RP11-1021F13	BES	chr6:30,304,165-30,524,743	6p21.33		
RP11-349M22	BES	chr6:30,802,793-30,972,043	6p21.33		
RP11-754H10	BES	chr6:33,960,388-34,137,229	6p21.31		
RP11-61E9	BES	chr6:34,202,278-34,379,674	6p21.31		
RP11-481A14	BES	chr6:34,820,453-34,979,774	6p21.31		
RP11-615A19	BES	chr6:34,993,942-35,155,751	6p21.31		
RP11-10I8	BES	chr6:39,087,900-39,254,420	6p21.2		
RP11-25I3	BES	chr6:41,836,819-42,004,320	6p21.1		

RP1-139D8	AL096814	chr6:42,208,853-42,375,930	6p21.1		
RP11-397G17	BES	chr6:50,026,694-50,190,757	6p12.3		
RP11-346L9	BES	chr6:57,351,232-57,548,984	6p11.2		
RP11-791F20	BES	chr6:57,500,124-57,690,152	6p11.2		
RP11-343D24	BES	chr6:57,644,937-57,835,610	6p11.2		
RP11-799H20	BES	chr6:57,787,081-58,000,708	6p11.2		
RP11-484F20	BES	chr6:58,720,610-58,883,743	6p11.1		
CEN				HOMINOIDEA ENC	Eder et al. (2003)
RP11-346M3	BES	chr6:62,456,388-62,630,578	6q11.1		
RP11-474L11	BES	chr6:76,244,412-76,429,104	6q14.1		
RP3-494K13	AL136312	chr6:85,740,159-85,796,186	6q14.3		
RP11-451P21	BES	chr6:96,988,167-97,146,901	6q16.1		
RP11-117A20	AL589920	chr6:119,888,999-119,906,826	6q22.31		
RP11-472E5	AL138828	chr6:136,464,198-136,605,737	6q23.3		
RP11-478J9	BES	chr6:140,333,714-140,416,207	6q24.1		
				OWMENC	Ventura et al. (2007)
RP11-474A9	BES	chr6:145,651,644-145,845,896	6q24.3		
RP11-64M7	AL589705	chr6:149,289,814-149,303,728	6q25.1		
			6q26	CNC	Sala et al. (2005)
RP1-230L10	AL137005	chr6:164,038,658-164,142,336	6q27		
RP11-37D8	BES	chr6:168,661,593-168,825,471	6q27		
RP11-302L19	AL596442	chr6:170,264,380-170,375,196	6q27		

		HSA7			
BAC name	Acc.N.	UCSCMarch2006	Cytog.map	ENC or CNC	Reference
				CJA2ENC; LLA11ENC	Unpublished data
RP11-713A20	AC093686	chr7:106,471-298,664	7p22.3		
RP11-416J17	AC069288	chr7:1,911,784-2,057,495	7p22.3		
RP11-792G24	BES	chr7:2,339,107-2,562,885	7p22.2		
RP11-400E7	BES	chr7:2,600,022-2,778,338	7p22.2		

(*continued*)

Table 5.1 (continued)

		HSA7			
BAC name	Acc.N.	UCSCMarch2006	Cytog.map	ENC or CNC	Reference
RP11-96L18	BES	chr7:2,825,887-2,981,935	7p22.2		
RP11-166P10	BES	chr7:3,369,663-3,531,934	7p22.2		
RP11-160E17	BES	chr7:4,751,001-4,913,015	7p22.1		
RP11-1080O3	BES	chr7:6,392,079-6,613,748	7p22.1		
RP11-1119G2	BES	chr7:6,991,152-7,134,725	7p22.1		
RP11-1061P7	BES	chr7:7,043,428-7,227,820	7p22.1		
RP4-755G17	AC004879	chr7:10,151,763-10,286,666	7p21.3		
RP11-486P11	AC007001	chr7:20,042,179-20,150,596	7p15.3		
RP11-112E16	BES	chr7:30,108,214-30,275,845	7p15.1		
RP11-585N13	BES	chr7:31,423,410-31,589,333	7p15.1		
RP11-714H18	BES	chr7:31,716,263-31,853,656	7p15.1		
RP11-638B17	BES	chr7:32,687,412-32,896,865	7p14.3		
RP11-420P20	BES	chr7:40,248,240-40,427,560	7p14.1		
RP11-653O17	AC073424	chr7:48,207,950-48,399,090	7p12.3		
RP11-339F13	AC073324	chr7:55,222,879-55,348,131	7p11.2		
CEN					
RP11-72B17	BES	chr7:65,153,654-65,319,685	7q11.21		
RP11-105P18	BES	chr7:68,481,085-68,640,842	7q11.22		
RP5-1102A12	AC004963	chr7:70,212,578-70,386,204	7q11.22		
RP11-243I17	BES	chr7:75,622,228-75,783,726	7q11.23		
RP11-982E3	BES	chr7:76,687,499-76,879,221	7q11.23		
RP11-580C19	BES	chr7:83,150,102-83,328,226	7q21.11		
RP11-215P16	AC006036	chr7:90,317,020-90,473,859	7q21.13		
RP11-908F6	BES	chr7:97,256,389-97,437,527	7q21.3		
RP11-150J17	BES	chr7:97,536,166-97,711,886	7q21.3		
RP11-163E9	BES	chr7:101,687,461-101,859,446	7q22.1		
RP11-803J14	BES	chr7:101,984,409-102,291,257	7q22.1		

RP11-282M13	BES	chr7:102,291,028-102,457,862	7q22.1		
RP11-418B19	AC073208	chr7:103,221,699-103,293,304	7q22.1		
RP11-328M22	AC018464.9	chr7:112,279,363-112,435,224	7q31.1		
RP11-22K23	BES	chr7:115,242,134-115,391,679	7q31.2		
RP11-108L6	BES	chr7:116,588,336-116,756,666	7q31.2		
RP11-55P11	BES	chr7:119,167,923-119,349,966	7q31.31		
RP11-3L10	BES	chr7:120,824,541-120,989,241	7q31.32		
RP11-329I5	AC018642.7	chr7:130,598,383-130,792,905	7q32.3		
RP5-839B19	AC006347	chr7:140,157,573-140,227,347	7q34		
RP11-422E4	AC024730.7	chr7:153,750,370-153,901,567	7q36.2		
RP11-764O12	AC006476	chr7_random:1-112,804			

		HSA8			
BAC name	Acc.N.	UCSCMarch2006	Cytog.map	ENC or CNC	Reference
RP11-18D5	AC090135	chr8:381,182-484,890	8p23.3		
RP11-59B16	BES	chr8:5,798,863-5,947,994	8p23.2		
RP11-737E8	BES	chr8:11,580,455-11,789,912	8p23.1		
RP11-247B12	BES	chr8:11,819,908-11,980,152	8p23.1		
RP11-98O19	BES	chr8:12,259,223-12,433,476	8p23.1		
RP11-45O16	BES	chr8:12,919,224-13,073,779	8p22		
RP11-460L9	BES	chr8:19,538,527-19,705,748	8q21.3		
RP11-583M2	AC051642	chr8:23,420,721-23,595,169	8q21.2		
RP11-120K21	BES	chr8:25,830,685-25,965,011	8q21.2		
RP11-51H24	BES	chr8:30,654,547-30,835,886	8p12		
RP11-10D7	AC013603	chr8:33,487,657-33,665,495	8p12		
RP11-262I23	BES	chr8:39,846,706-40,045,213	8p11.22		
CEN					
RP11-1134I14	BES	chr8:48,063,873-48,241,291	8q11.1-q11.21		
RP11-80E22	BES	chr8:52,787,115-52,932,670	8q11.22-q11.23		
RP11-151B2	BES	chr8:56,450,221-56,608,976	8q12.1		

(*continued*)

Table 5.1 (continued)

		HSA8			
BAC name	Acc.N.	UCSCMarch2006	Cytog.map	ENC or CNC	Reference
RP11-36P16	BES	chr8:60,277,203-60,460,134	8q12.1		
RP11-45G14	BES	chr8:62,325,810-62,478,435	8q12.2-q12.3		
RP11-280G9	BES	chr8:62,716,900-62,859,102	8q12.3		
				LLA7ENC	Stanyon et al. (2008)
RP11-382J12	AC022731	chr8:71,614,507-71,778,503	8q13.3		
RP11-75P23	BES	chr8:72,585,391-72,768,280	8q13.3		
RP11-232D14	BES	chr8:73,793,280-73,983,779	8q13.3		
RP11-361C12	BES	chr8:74,618,561-74,774,512	8q21.11		
RP11-300E4	AC100782	chr8:76,034,751-76,219,873	8q21.11		
RP11-706J10	BES	chr8:77,470,475-77,644,774	8q21.11		
RP11-91P17	AC084706	chr8:79,158,450-79,305,261	8q21.12		
RP11-14D5	BES	chr8:86,064,185-86,255,578	8q21.2		
RP11-353O11	AC091184	chr8:90,077,451-90,220,326	8q21.3		
RP11-703K20	BES	chr8:90,220,321-90,398,370	8q21.3		
RP11-179G18	BES	chr8:90,294,331-90,433,195	8q21.3		
RP11-18K20	AC099816	chr8:90,469,419-90,620,876	8q21.3		
RP11-15J4	BES	chr8:92,022,248-92,211,470	8q21.3		
RP11-14G13	BES	chr8:96,166,084-96,341,103	8q22.1		
RP11-122P10	BES	chr8:97,321,450-97,488,017	8q22.1		
RP11-452M24	BES	chr8:98,106,600-98,303,112	8q22.1		
RP11-35A21	BES	chr8:98,181,249-98,335,203	8q22.1		
RP11-828L5	BES	chr8:98,534,473-98,760,672	8q22.1		
RP11-958K24	BES	chr8:98,760,678-98,948,597	8q22.1		
RP11-640O15	BES	chr8:99,070,433-99,228,183	8q22.1-q22.2		
RP11-410L14	AC104986	chr8:99,944,884-100,098,300	8q22.2		
RP11-697C18	AC024996	chr8:113,395,877-113,573,740	8q23.3		
RP11-269I24	AC090987	chr8:131,641,435-131,795,238	8q24.21		

RP11-349C2	AC087337	chr8:145,586,068-145,770,875	8q24.3		
RP4-698E23	AF186192	chr8:145,807,985-145,953,950	8q24.3		
				CMO17ENC	Stanyon et al. (2008)

		HSA9			
BAC name	Acc.N.	UCSCMarch2006	Cytog.map	ENC or CNC	Reference
RP11-59O6	BES	chr9:188,713-373,816			
RP11-130C19	AL136979	chr9:615,148-812,246	9p24.3		
RP11-341G2	BES	chr9:1,121,123-1,241,689			
RP11-472F14	BES	chr9:6,427,961-6,601,707	9p24.1		
RP11-77E14	AL354694	chr9:7,671,919-7,825,210	9p24.1		
RP11-44k8	BES	chr9:10,913,827-11,089,825	9p23		
	BES	~chr9:10,913,827-11,341,974	9p23	CNC	Satinover et al. (2001)
RP11-23D5	BES	chr9:11,170,427-11,341,974	9p23		
RP11-115I23	BES	chr9:13,017,706-13,186,522	9p23	tumor	Italiano et al. (2006)
RP11-58K1	BES	chr9:15,874,140-16,051,195	9p22.3		
RP11-340N12	FISH	chr9:17,136,369-17,298,494	9p22.2		
RP11-57I14	BES	chr9:19,650,027-19,797,139	9p22.1		
RP11-393P6	AL513317	chr9:23,950,338-24,092,705	9p21.3		
RP11-1006E22	BES	chr9:27,142,243-27,331,367	9p21.2		
RP11-976P13	BES	chr9:30,838,876-31,023,309	9p21.1		
RP11-562M8	AL353717	chr9:32,871,544-32,992,078	9p21.1		
RP11-58A20	BES	chr9:36,392,186-36,539,166	9p13.2		
RP11-3J10	AL138752	chr9:37,745,972-37,935,175	9p13.2		
RP11-168J7	BES	chr9:38,261,095-38,421,467	9p13.1		
RP11-788E5	BES	chr9:38,558,002-38,723,846	9p13.1		
			9p13	CNC	Vance et al. (1997)
CEN					
RP11-203L2	BES	chr9:70,447,920-70,642,602	9q21.11		
RP11-876N18	BES	chr9:70,831,740-71,036,759	9q21.11		

(*continued*)

Table 5.1 (continued)

		HSA9			
BAC name	Acc.N.	UCSCMarch2006	Cytog.map	ENC or CNC	Reference
RP11-63P12	AL135924	chr9:74,048,190-74,211,174	9q21.12		
RP11-522I20	AL354920	chr9:85,375,435-85,544,238	9q21.32		
RP11-30C23	AL451131	chr9:87,314,024-87,468,183	9q21.33		
RP11-507D14	AL137849	chr9:87,988,837-88,120,520	9q21.33		
RP11-155P1	BES	chr9:88,673,580-88,845,643	9q21.33		
RP11-107G16	BES	chr9:89,315,407-89,492,510	9q21.33		
RP11-164I22	BES	chr9:89,981,566-90,160,305	9q22.1		
RP11-875O18	BES	chr9:92,518,647-92,715,334	9q22.2		
RP11-714A6	BES	chr9:94,105,260-94,266,691	9q22.31		
RP11-240L7	BES	chr9:98,020,526-98,190,156	9q22.32		
RP11-330M2	AL158827	chr9:98,730,413-98,744,393	9q22.32		
RP11-106N7	BES	chr9:99,884,782-100,053,954	9q22.33		
RP11-208F1	BES	chr9:102,010,490-102,158,124	9q31.1		
RP11-354J3	BES	chr9:105,921,994-106,093,407	9q31.1		
RP11-714K8	BES	chr9:108,383,090-108,577,624	9q31.2		
RP11-18A3	AL359963	chr9:111,085,649-111,220,627	9q31.3		
RP11-243H16	BES	chr9:111,103,930-111,282,692	9q31.3		
RP11-16A3	BES	chr9:116,448,704-116,610,074	9q32		
RP11-336A17	AL160272	chr9:119,493,517-119,640,494	9q33.1		
		chr9:121.261.000-121,315,000		CNC(ChIP-on-chip)	Capozzi et al. (2008b)
RP11-100H1	BES	121.315	9q33.1		
RP11-160J24	BES	chr9:124,090,783-124,264,726	9q33.2		
RP11-542K23	AL359636	chr9:124,189,785-124,383,720	9q33.2		
				MMUENC	Ventura et al. (2004)
RP11-64P14	AL162254	chr9:124,304,812-124,493,132	9q33.2		
RP11-465F21	AC006313	chr9:124,622,045-124,630,661	9q33.2		
RP11-85O21	AC006450	chr9:125,657,313-125,834,867	9q33.3		

RP11-30A13	BES	chr9:131,628,209-131,798,961	9q34.11		
RP11-469E24	AL353636	chr9:137,865,306-137,936,843	9q34.3		

		HSA10			
BAC name	Acc.N.	UCSCMarch2006	Cytog.map	ENC or CNC	Reference
RP11-387K19	BES	chr10:149,098-312,071	10p15.3		
RP11-10D13	BES	chr10:214,415-366,376	10p15.3		
RP11-15D19	BES	chr10:835,011-1,011,342	10p15.3		
RP11-363N22	AL359878	chr10:854,871-1,039,159	10p15.3		
RP11-61P15	BES	chr10:13,747,461-13,911,792	10p13		
RP11-142F1	AL391334	chr10:17,555,784-17,653,214	10p12.3		
RP11-109I13	AL390783	chr10:18,510,777-18,688,434	10p12.3		
RP11-383B4	AL450384	chr10:18,842,308-18,966,878	10p12.4		
RP11-110M17	BES	chr10:24,276,209-24,449,403	10p12.1		
RP11-39E10	BES	chr10:31,183,319-31,363,437	10p11.23		
RP11-92J19	BES	chr10:36,759,042-36,945,342	10p11.21		
RP11-56L6	BES	chr10:38,038,398-38,212,095	10p11.21		
RP11-162G10	AL135791	chr10:38,123,110-38,190,084	10p11		
CEN					
RP11-351D16	AC010864	chr10:42,817,197-43,022,992	10q11.21		
RP11-285G1	AL353801	chr10:44,640,088-44,862,577	10q11.21		
RP11-90N8	BES	chr10:51,442,402-51,622,394	10q11.23		
RP11-1001A13	BES	chr10:52,032,752-52,229,644	10q11.23		
RP11-6J8	BES	chr10:57,715,995-57,887,658	10q21.1		
RP11-749A7	BES	chr10:63,201,531-63,375,079	10q21.2		
RP11-615M13	BES	chr10:78,282,179-78,448,176	10q22.3		
RP11-717O2	BES	chr10:84,795,601-84,982,857	10q23.1		
RP11-830J13	BES	chr10:88,357,707-88,550,596	10q23.2		
RP11-659F22	BES	chr10:89,246,763-89,428,545	10q23.3		
RP11-829M16	BES	chr10:92,526,680-92,680,990	10q23.31		

(*continued*)

Table 5.1 (continued)

		HSA10			
BAC name	Acc.N.	UCSCMarch2006	Cytog.map	ENC or CNC	Reference
RP11-166O7	BES	chr10:110,714,067-110,884,778	10q25.1		
		chr10:116,837,988-117,377,461	10q25.3	CNC(ChIP-ob-chip)	Lo et al. (2001a)
RP11-296H2	AL135793	chr10:123,907,460-124,121,322	10q26.13		
RP11-92A10	BES	chr10:132,010,830-132,165,851	10q26.3		
RP11-1022E21	BES	chr10:134,703,784-134,906,603	10q26.3		

		HSA11			
BAC name	Acc.N.	UCSCMarch2006	Cytog.map	ENC or CNC	Reference
RP11-401C19	AC083984	chr11:896,316-1,008,135	11p15.5		
RP11-650F7	BES	chr11:3,297,781-3,455,204	11p15.4		
RP11-749O23	BES	chr11:3,501,436-3,690,087	11p15.4		
RP11-661M13	BES	chr11:5,856,181-6,043,020	11p15.4		
				OWMENC	Cardone et al. (2007)
RP11-625D10	BES	chr11:5,667,339-5,864,725	11p15.4		
RP11-645I8	AC021935	chr11:6,072,745-6,229,122	11p15.4		
				PPY8ENC	Cardone et al. (2007)
RP11-56J22	BES	chr11:20,180,424-20,332,556	11p15.1		
RP11-103P20	BES	chr11:36,021,057-36,180,792	11p13		
RP11-150D18	BES	chr11:41,858,282-42,020,207	11p12		
			11p11	CNC	
RP11-29O22	BES	chr11:46,582,988-46,583,429	11p11.2		
RP11-318O24	BES	chr11:50,545,853-50,719,949	11p11.12		
CEN				GGOPTRHSAENC	Cardone et al. (2007)
RP11-217G11	BES	chr11:56,609,801-56,610,186	11q12.1		
RP11-75H24	BES	chr11:58,632,233-58,632,565	11q12.1		
RP11-160L9	BES	chr11:67,190,649-67,191,077	11q13.2		

RP11-955G14	BES	chr11:71,190,153-71,377,632	11q13.4		
RP11-757C15	AP000719	chr11:71,236,122-71,432,551	11q13.4		
RP11-807H22	AP000812	chr11:71,481,809-71,602,336	11q13.4		
RP11-7H7	BES	chr11:78,034,240-78,206,818	11q14		
RP11-119M23	BES	chr11:85,346,396-85,346,523	11q14.2		
RP11-529A4	AP004607	chr11:89,286,313-89,446,995			
			11q14.3	HLA11/NLE15ENC	Roberto et al. (2007)
RP11-692G6	BES	chr11:89,719,943-89,890,899			
RP11-732A21	AP001527	chr11:101,397,613-101,564,917	11q22.1		
RP11-864G5	AP000942	chr11:101,600,598-101,786,581	11q22.1		
RP11-1044B1	BES	chr11:105,109,962-105,322,691	11q22.3		
RP11-276O11	BES	chr11:105,262,409-105,262,775	11q22.3		
RP11-100J10	BES	chr11:112,570,375-112,735,819	11q23.1		
RP11-90A13	BES	chr11:130,889,654-131,037,422	11q25		
RP11-265F9	BES	chr11:134,272,267-134,441,179	11q25		
				APCEN	Cardone et al. (2007)

		HSA12			
BAC name	Acc.N.	UCSCMarch2006	Cytog.map	ENC or CNC	Reference
RP11-283I3	BES	chr12:153,051-329,683	12p13.33		
RP11-691J6	BES	chr12:5,200,006-5,384,129	12p13.32		
RP11-62G3	BES	chr12:6,121,261-6,298,431	12p13.31		
RP11-20D14	BES	chr12:8,690,273-8,864,148	12p13.31		
RP11-157L2	BES	chr12:9,788,001-9,945,600	12p13.31		
				NWMENC	Stanyon et al. (2008)
RP11-316E18	BES	chr12:9,916,001-10,122,368	12p13.31-p13.2		
RP11-13C13	BES	chr12:10,122,517-10,291,047	12p13.2		
RP11-502N13	BES	chr12:14,521,905-14,648,407	12p13.1		
RP11-1018J8	FISH	chr12:15,049,657-15,261,830	12p12.3		
RP11-489N6	FISH	chr12:16,084,282-16,171,229	12p12.3		

(*continued*)

Table 5.1 (continued)

		HSA12			
BAC name	Acc.N.	UCSCMarch2006	Cytog.map	ENC or CNC	Reference
RP11-871F6	FISH	chr12:17,423,813-17,640,601	12p12.3		
RP11-678N14	BES	chr12:19,566,006-19,721,709	12p12.3		
RP11-157I19	BES	chr12:20,050,190-20,206,352	12p12.2		
RP11-120A19	BES	chr12:20,325,423-20,486,337	12p12.2		
RP11-57F15	BES	chr12:20,863,387-21,018,324	12p12.2		
RP11-125O5	BES	chr12:21,151,769-21,303,642	12p12.2.p12.1		
RP11-12D15	BES	chr12:22,210,387-22,369,559	12p12.1		
RP11-877E17	BES	chr12:25,986,021-26,163,998	12p12.1		
RP11-666F17	FISH	chr12:26,671,081-26,857,010	12p11.23		
RP11-485K18	FISH	chr12:28,287,829-28,467,827	12p11.22		
RP11-517B23	BES	chr12:31,362,925-31,533,973	12p11.21		
RP11-956A19	BES	chr12:32,174,154-32,364,169	12p11.21		
RP11-460N10	FISH	chr12:33,170,516-33,333,493	12p11.1		
CEN					
RP11-152M7	BES	chr12:37,365,174-37,556,018	12q12		
RP11-490D11	BES	chr12:40,112,781-40,280,202	12q12		
RP11-618L22	AC079906	chr12:45,523,783-45,704,447	12q13.11		
RP11-23J18	BES	chr12:45,755,429-45,925,226	12q13.11		
RP11-241O10	BES	chr12:46,000,294-46,169,804	12q13.11		
RP11-47A12	BES	chr12:46,070,299-46,235,151	12q13.11		
RP11-19H5	BES	chr12:46,299,642-46,450,387	12q13.11		
RP11-254E3	BES	chr12:46,507,219-46,672,845	12q13.11		
RP11-30N17	BES	chr12:46,672,928-46,875,216	12q13.11		
RP11-159H4	BES	chr12:46,744,342-46,895,584	12q13.11		
RP11-204C20	BES	chr12:46,894,555-47,075,616	12q13.11		
RP11-94F1	BES	chr12:50,496,385-50,663,940	12q13.13		

RP11-699F3	BES	chr12:50,919,590-51,102,247	12q13.13		
RP11-4K11	BES	chr12:52,417,124-52,574,796	12q13.13		
RP11-631N16	BES	chr12:61,280,212-61,458,292	12q14.1-q14.2		
RP11-680F18	BES	chr12:63,441,896-63,614,208	12q14.3		
RP11-63J20	BES	chr12:80,424,584-80,582,696	12q21.31		
RP11-900F13	FISH	chr12:87,374,561-87,546,806	12q21.32-q21.33		
RP11-205I24	BES	chr12:102,490,342-102,647,694	12q23.3		
RP11-1G17	BES	chr12:110,393,571-110,596,386	12q24.12		
RP11-344G11	BES	chr12:125,063,337-125,209,987	12q24.32		
RP11-394D10	BES	chr12:132,034,089-132,208,159	12q24.33		

		HSA13			
BAC name	Acc.N.	UCSCMarch2006	Cytog.map	ENC or CNC	Reference
CEN					
RP11-110K18	AL137119	chr13:19,404,216-19,568,080	13q12.11		
RP11-45B20	AL445985	chr13:23,305,109-23,483,639	13q12.12		
				CMO18-CMO21ENCs	Cardone et al. (2006)
RP11-64I8	AL158065	chr13:30,406,381-30,571,172	13q12.3		
RP11-142E9	BES	chr13:33,252,754-33,451,136	13q13.2		
RP11-29G24	AL161718	chr13:34,851,469-34,910,004	13q13.3		
RP11-477C5	BES	chr13:41,599,503-41,760,120	13q14.11		
RP11-413N19	AL592523	chr13:41,969,072-41,973,065	13q14.11		
			LLA8ENC	Cardone et al. (2006)	
RP11-14553	BES	chrl13:45,340,029,42,504,601	13q14.11		
RP11-443J2	BES	chr13:45,279,141-45,450,817	13q14.12		
RP11-719B12	BES	chr13:45,408,175-45,579,860	13q14.12		
RP11-939G7	BES	chr13:45,754,269-45,939,953	13q14.13		
RP11-945G11	BES	chr13:45,928,366-46,127,167	13q14.13		
RP11-417C20	FISH	chr13:46,020,378-46,185,497	13q14.13		
RP11-103J18	AL138875	chr13:48,654,460-48,818,895	13q14.2		

(*continued*)

Table 5.1 (continued)

		HSA13			
BAC name	Acc.N.	UCSCMarch2006	Cytog.map	ENC or CNC	Reference
RP11-10O23	AC013618	chr13:55,430,704-55,602,978	13q21.1		
RP11-1043D14	BES	chr13:61,282,357-61,458,258	13q21.31		
				OWMENC	Cardone et al. (2006)
RP11-187E23	AL136999	chr13:66,092,979-66,264,337	13q21.32		
RP11-51P14	AL356006	chr13:67,146,127-67,174,887	13q21.32		
RP11-543G6	AC162212	chr13:70,669,808-70,794,225	13q21.33		
			13q21.33	CNC(ChIP-on-chip)	Cardone et al. (2006)
RP11-512J14	AL354995	chr13:70,797,636-70,947,217	13q21.33		
RP11-138N13	BES	chr13:74,311,795-74,458,502	13q22.2		
RP11-188A23	AL354831	chr13:77,153,157-77,297,742	13q22.3		
RP11-115N13	BES	chr13:82,035,688-82,200,947	13q31.1		
RP11-120L14	BES	chr13:83,766,483-83,924,544	13q31.1		
RP11-351H1	BES	chr13:84,396,772-84,582,561	13q31.1		
RP11-780G3	BES	chr13:85,161,451-85,333,456	13q31.1		
RP11-30L8	BES	chr13:85,529,117-85,655,956	13q31.1		
RP11-29P20	BES	chr13:86,954,636-87,112,529	13q31.2		
RP11-143O10	BES	chr13:88,496,254-88,673,921	13q31.2		
RP11-210E23	FISH	chr13:93,776,946-93,877,017	13q32.1		
RP11-721F14	BES	chr13:96,392,847-96,575,482	13q32.1		
RP11-46I10	BES	chr13:101,854,484-102,028,829	13q33.1		
RP11-261F2	AL445226	chr13:103,364,122-103,420,360	13q33.1		
RP11-245B11	AL161774	chr13:113,770,458-113,932,864	13q34		
RP11-569D9	FISH	chr13:113,930,807-114,103,243	13q34		
			13q34	CNC	Depinet et al. (1997)(case4)

		HSA14			
BAC name	Acc.N.	UCSCMarch2006	Cytog.map	ENC or CNC	Reference
CEN				HOMINOIDEA ENC	Ventura et al. (2003)
RP11-246M13(A)	BES	chr14:19,547,383-19,702,125	14q11.2		
RP11-68M15	BES	chr14:22,546,692-22,722,266	14q11.2		
RP11-3K11	BES	chr14:25,676,157-25,851,493	14q12		
RP11-96N22	BES	chr14:30,522,558-30,688,098	14q12		
RP11-642G19	BES	chr14:32,380,196-32,540,929	14q13.1		
RP11-918D6	BES	chr14:36,404,852-36,569,960	14q13.3		
RP11-94J22(B)	BES	chr14:41,623,645-41,782,055	14q21.1		
RP11-453F20	BES	chr14:44,679,792-44,872,979	14q21.3		
RP11-631K15	BES	chr14:48,752,711-48,915,809	14q22.1		
RP11-316E4	BES	chr14:50,001,799-50,183,814	14q22.1		
RP11-841O20	BES	chr14:52,073,343-52,285,417	14q22.1		
RP11-312M17	BES	chr14:54,251,694-54,407,050	14q22.2-.3		
RP11-81D11	BES	chr14:64,128,328-64,294,463	14q23.3		
RP11-886F16	BES	chr14:67,619,469-67,780,148	14q24.1		
RP11-204P19(C)	BES	chr14:71,001,855-71,164,272	14q24.2		
RP11-606A3	BES	chr14:73,138,310-73,312,477	14q24.2		
RP11-92H20	BES	chr14:74,381,660-74,551,240	14q24.2		
RP11-89I23	BES	chr14:79,486,870-79,652,196	14q31.1		
RP11-4E24	BES	chr14:85,025,843-85,182,785	14q31.3		
RP11-91C7	BES	chr14:90,549,220-90,692,115	14q32.12		
RP11-45E1	BES	chr14:96,885,041-97,054,197	14q32.2		
RP11-90G22	BES	chr14:100,210,924-100,389,009	14q32.2		
RP11-417P24	AL122127	chr14:105,267,349-105,437,150	14q32.33		
RP11-51P11(D)	BES	chr14:106,049,593-106,211,962	14q32.33		
				CMO13ENC	Ventura et al. (2003)

(*continued*)

Table 5.1 (continued)

		HSA15			
BAC name	Acc.N.	UCSCMarch2006	Cytog.map	ENC or CNC	Reference
CEN				HOMINOIDEA ENC	Ventura et al. (2003)
RP11-441B20(A)	AC080077	chr15:22,905,050-23,073,407	15q11.2		
RP11-570N16	AC019229	chr15:24,960,279-25,129,482	15q12		
RP11-11J16(B)	BES	chr15:29,518,297-29,691,905	15q13.3		
RP11-106G20(C)	BES	chr15:30,609,233-30,773,871	15q13.3		
RP11-50O2	BES	chr15:31,571,307-31,737,827	15q13.3		
RP11-747K21	BES	chr15:33,683,242-33,871,801	15q14		
RP11-720L8	AC068875	chr15:35,362,749-35,529,454	15q14		
RP11-133K1	AC020658	chr15:38,241,120-38,400,125	15q15.1		
RP11-729O24	BES	chr15:40,156,888-40,332,058	15q15.1		
RP11-753P14	BES	chr15:40,820,559-40,988,584	15q15.1		
RP11-594K13	BES	chr15:44,037,250-44,241,054	15q21.1		
RP11-846K6	BES	chr15:48,810,360-48,983,766	15q21.2		
RP11-316P21	AC025041	chr15:50,971,951-51,145,337	15q21.2		
RP11-126E3	BES	chr15:52,895,739-53,061,863	15q21.3		
RP11-450G20	BES	chr15:53,782,464-53,963,923	15q21.3		
RP11-294K12	BES	chr15:54,112,385-54,286,277	15q21.1		
RP11-844G16	BES	chr15:54,271,610-54,460,838	15q213		
RP11-829F13	BES	chr15:54,481,897-54,676,988	15q21.3		
RP11-323F24	BES	chr15:54,901,235-55,123,208	15q21.3		
RP11-44G18	ends	chr15:55,902,848-56,062,565	15q21.3		
RP11-93I17	BES	chr15:56,226,257-56,226,689	15q21.3		
RP11-236P11	AC087632	chr15:62,366,899-62,510,409	15q22.31		
RP11-282M16	AC022254	chr15:65,872,233-66,060,841	15q22.33		
RP11-1107A19(D)	ends	chr15:72,073,586-72,217,438	15q24.1		
			15q24.1	CNC	Ventura et al. (2003)
RP11-247C2(E)	AC010931	chr15:72,201,386-72,358,658	15q24.1		

RP11-624N5	ACO24552	chr15:72,158,366-72,251,969	15q24.1		
RP11-20M10	AC016276	chr15:75,965,634-76,127,696	15q24.2		
			15q25		Depinet et al. (1997)(case1)
RP11-1001M11(F)	BES	chr15:76,752,817-76,966,382	15q25.1		
				APCENNWMENC	Ventura et al. (2003)
RP11-16K12(G)	BES	chr15:76,939,472-77,105,720	14q25.1		
RP11-635O8	BES	chr15:80,103,012-80,257,524	15q25.2		
RP11-127F21	AC044907	chr15:81,186,929-81,348,689	15q25.2		
RP11-19E5(H)	AC027605	chr15:82,473,051-82,637,127	15q25.2		
			15q25.2	CNC	Ventura et al. (2003)
RP11-182J1(I)	AC048382	chr15:82,835,478-83,006,963	15q25.2		
			15q26.1	CNC	Rowe et al. (2000); Depinet et al. (1997)(case2)
RP11-90E5(J)	AC022710	chr15:98,163,252-98,349,768	15q26.3		

		HSA18			
BAC name	Acc.N.	UCSCMarch2006	Cytog.map	ENC or CNC	Reference
RP11-78H1	BES	chr18:2,136,811-2,307,213	18p11.32		
RP11-96I11	BES	chr18:12,904,782-12,904,961	18p11.21		
CEN					
RP11-10G8	BES	chr18:17,274,438-17,431,001	18q11.2		
RP11-104N11	BES	chr18:33,436,610-33,608,704	18q12.2		
RP11-61D1	AC090897	chr18:50,155,761-50,313,129	18q21.1		
				OWMENC	Ventura et al. (2007)
RP11-289E15	AC091135	chr18:50,360,135-50,526,341	18q21.2		
RP11-153B11	BES	chr18:52,818,203-52,977,905	18q21.2		
RP11-53N15	BES	chr18:70,195,436-70,195,693	18q22.3		
RP11-87C15	BES	chr18:75,965,206-75,965,502	18q23		

(*continued*)

Table 5.1 (continued)

		HSA20			
BAC name	Acc.N.	UCSCMarch2006	Cytog.map	ENC or CNC	Reference
RP11-371L19	AL118502	chr20:659,205-785,463	20p13		
RP5-1187M17	AL121891	chr20:3,013,541-3,139,396	20p13		
RP5-1068F16	AL023913	chr20:10,155,017-10,295,322	20p12.2		
		chr20:10,662,941-11,127,046	20p12.2	CNC(ChIP-on-chip)	Lo et al. (2001b)
RP4-813H11	AL079337	chr20:11,379,732-11,417,054	20p12.2		
RP5-1069O1	AL049633	chr20:15,126,843-15,219,199	20p12.1		
RP11-922G6	BES	chr20:22,887,406-23,046,035	20p11.21		
RP11-661H1	BES	chr20:23,454,246-23,627,064	20p11.21		
RP5-966J20	AL121925	chr20:24,698,120-24,737,379	20p11.21		
CEN					
RP11-1036L7	BES	chr20:28,048,230-28,206,006	20q11.1		
RP5-836N17	AL049539	chr20:30,126,905-30,238,598	20q11.21		
RP5-954P9	AL359828	chr20:34,046,335-34,084,879	20q11.23		
RP11-888D20	BES	chr20:34,932,840-35,111,176	20q11.23		
RP11-1152L20	BES	chr20:35,084,554-35,209,548	20q11.23		
RP11-192N1	BES	chr20:35,209,599-35,358,886	20q11.23		
RP11-826B14	BES	chr20:35,332,463-35,548,961	20q11.23		
RP11-138A15	BES	chr20:35,595,079-35,595,342	20q11.23		
RP5-906C1	AL133342	chr20:46,828,731-46,939,544	20q13.13		
RP5-1059L7	AL121913	chr20:55,665,561-55,815,784	20q13.32		
RP11-476I15	AL137028	chr20:62,376,540-62,435,964	20q13.33		
				CMO22ENC	Misceo et al. (2005)

		HSA20			
BAC name	Acc.N.	UCSCMarch2006	Cytog.map	ENC or CNC	Reference
RP11-800K15	BES	chrX:483,105-664,235	Xp22.33		
		chrX:6,000,001-9,500,000	Xp22.31	CNC	
RP11-458E23	BES	chrX:10,007,515-10,251,587	Xp22.2		
RP11-450P7	AL772392	chrX:21,383,521-21,507,706	Xp22.12		
RP11-450E21	AL591591	chrX:33,274,317-33,378,433	Xp21.1		
RP11-64P15	BES	chrX:33,376,434-33,542,431	Xp21.1		
RP11-1078G21	BES	chrX:33,512,076-33,704,078	Xp21.1		
RP11-825L2	BES	chrX:33,920,685-34,107,259	Xp21.1		
RP11-281B1	BES	chrX:33,989,930-34,174,295	Xp21.1		
RP11-910L4	BES	chrX:34,033,952-34,208,856	Xp21.1		
RP11-831J15	BES	chrX:34,148,053-34,301,011	Xp21.1		
RP11-384A17	BES	chrX:43,240,049-43,392,966	Xp11.3		
RP11-552J9	AL450023	chrX:52,556,131-52,566,971	Xp11.22		
CEN					
RP11-978L24	BES	chrX:61,470,646-61,691,665	Xq11.1		
RP11-148E15	BES	chrX:62,253,894-62,418,154	Xq11.1		
RP11-135B16	BES	chrX:62,460,317-62,628,230	Xq11.1		
RP11-213M6	BES	chrX:62,791,311-62,954,364	Xq11.1		
RP11-151C15	BES	chrX:62,874,379-63,050,505	Xq11.1		
RP11-754F6	BES	chrX:63,033,136-63,192,316	Xq11.1		
RP11-346J4	BES	chrX:63,171,662-63,365,730	Xq11.1		
		chrX:65,100,001-67,700,000	Xq12	CNC	
RP11-625B4	BES	chrX:69,721,202-69,884,160	Xq13.1		
RP11-395L12	AL157933	chrX:81,134,235-81,183,002	Xq21.1		
RP11-483J19	BES	chrX:92,542,566-92,694,921	Xq21.32		
			Xq21.2	LCAXENC	Ventura et al. (2001)
RP11-449F11	FISH	chrX:96,896,057-97,059,042	Xq21.33		
RP11-426L6	BES	chrX:104,850,550-105,005,976	Xq22.3		

(*continued*)

Table 5.1 (continued)

		HSA20			
BAC name	Acc.N.	UCSCMarch2006	Cytog.map	ENC or CNC	Reference
RP5-874H6	AL078580	chrX:111,900,664-111,922,369	Xq23		
RP11-243N2	BES	chrX:115,064,564-115,228,958	Xq23		
RP11-488B15	BES	chrX:124,895,774-125,047,349	Xq25		
RP11-535K18	AL078638	chrX:134,948,985-135,131,392	Xq26.3		
RP11-478P19	BES	chrX:143,327,218-143,502,603	Xq27.3		
RP11-402H20	AC016977	chrX:153,772,076-153,951,934	Xq28		

The table reports, for each chromosome, a panel of BAC clones used to delineate its evolutionary history in primates, essentially as reported by Stanyon et al. (2008), in the Supplementary files. Chromosomes not showing any ENC or finely-mapped human clinical centromeres are not reported. For these chromosomes the reader can refer to Fig. 5.1 and to Marshall et al. (2008). The first column shows the BAC name; a letter in parenthesis after the BAC name, occasionally reported, indicates the BAC code utilized in Figs. 5.3 and 5.5. The second column indicates the method used for placing the BAC on the human sequence (BES = BAC End Sequence; see Sect. 5.6), reported in the third column, while its cytogenetic position is shown in column four.

In this frame, the table reports, in the fifth column:

1. The ENCs (hatched red row) and the corresponding reference (sixth column). Usually, reiterative FISH experiments have been performed to characterize at the maximal resolution the mapping of each ENC. The closest BACs on each side of the ENC are reported. The acronyms of the species in which the ENC has been discovered are reported below.
2. The clinical neocentromeres (hatched light-blue rows) that have been mapped at least at a cytogenetic band resolution. The annotation "ChIP" indicates that they have been mapped by ChIP-on-chip technology (see text). In this case the CENP-A or -C domains is reported.
3. The three human repositioned centromeres (HRC)
4. The normal human centromere (blue rows)

ENC Evolutionary new centromere, *CNC* Clinical neoCentromere, *HRC* Human repositioned centromere, *AC* Ancestral centromere, *AP* Ancestral primate
Literature not reported in the main paper is reported below.
Species' acronyms: CJA Callithrix jacchus (common marmoset) (NWM), *CMO Callicebus moloch,* also indicated as *Callicebus pallescens* (dusky titi) (NWM), *GGO Gorilla gorilla* (gorilla), *HLA Hylobates lar* (lar gibbon), *LCA Lemur catta* (ring-tailed lemur), *MMU Macaca mulatta* (rhesus monkey), *PPY Pongo pygmaeus* (orangutan)

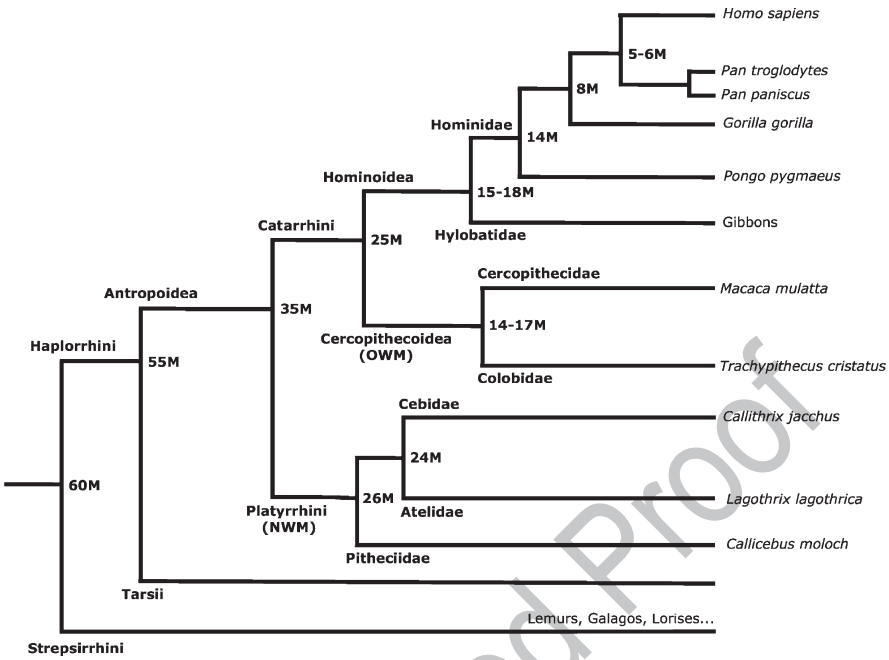

Fig. 5.2 Phylogeny of primates. Summary of the phylogenetic relationship among extant primates. Branching time is according to Raaum et al. (2005) and Opazo et al. (2006). The bars' length is not proportional to elapsed time. The figures indicate the branching time in million years

Studies on the evolution of the chromosomes where clustering of neocentromeres were reported (3q, 13q, and 15q) put these regions in a completely new light. These chromosomes were investigated in detail, and each of these clusters disclosed distinct, intriguing aspects of the relationship between human clinical neocentromeres and ENCs. For this reason they will be described in detail later.

The full appreciation of these data presupposes a basic knowledge of primate phylogeny, which is summarized in Fig. 5.2. It is also important that the reader is acquainted with the concept of the "outgroup" in phylogenetic studies. A brief description is reported in the Sect. 5.6.

5.3.1 Evolution of Chromosome 15

Human chromosomes 15 and 14 derive from the fission of an ancestral chromosome in the Hominoidea ancestor. Comparison with outgroup species confirms that the fission is the derivative rearrangment. Figure 5.3 reports the study of the evolution of these chromosomes through the use of BAC clones that showed that the marker

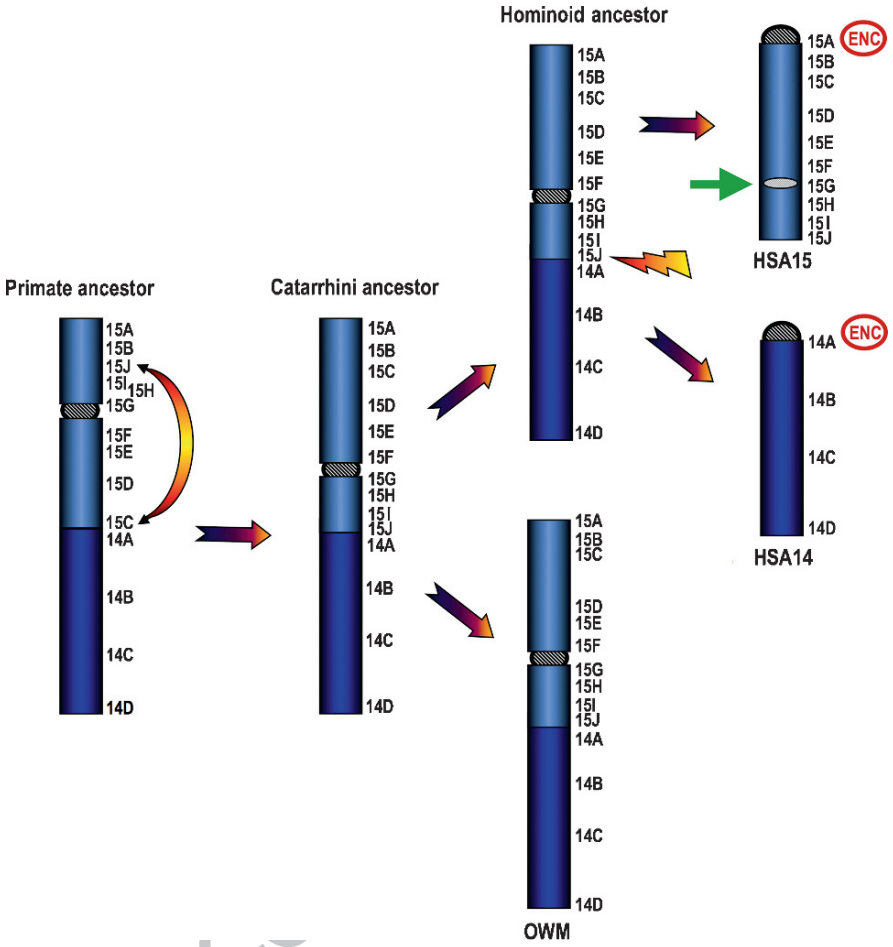

Fig. 5.3 Evolution of human chromosomes 15 and 14. The figure delineates the evolutionary history of chromosomes 15 and 14 in OWMs and Hominoidea. BAC clones used in the synteny investigation are represented by letters on the right of the chromosomes. The letter-BAC correspondence is reported in Supplement Table 5.1. Chromosomes 15 and 14 in Hominoidea were generated by fission of an ancestral chromosome, which appears to be composed of these two chromosomes arranged head-tail. ENC in a *red circle* indicates the emergence of an evolutionary new centromere. The *green arrow* points to the inactivated centromere. For details see text

order was perfectly conserved between macaque chromosome 7 (*Macaca mulatta*, MMU) and the two human chromosomes. To derive the two independent human chromosomes, 14 and 15, you only need to fission between markers F and G (Ventura et al 2003) (Fig. 5.3). One novel centromere emerged in human chromosome 15, corresponding to the telomeric region of the short arm of MMU7 (Fig. 5.3). A second centromere emerged on chromosome 14 and corresponded to the fission point of MMU7. The ancestral centromere, precisely mapped by the apparent split

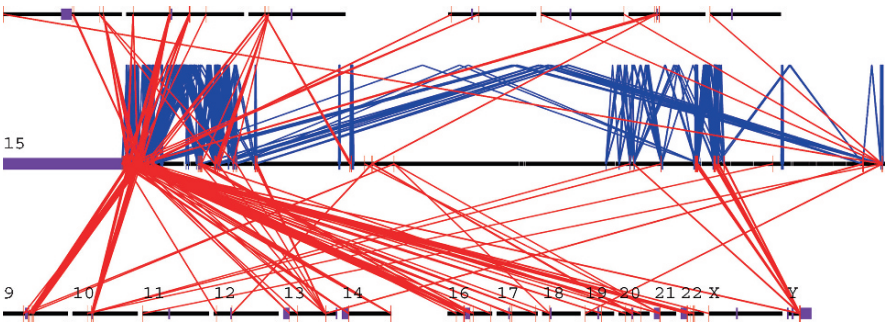

Fig. 5.4 Segmental duplication analysis of chromosome 15. The figure illustrates the interchromosomal (*red lines*) and intrachromosomal (*blue lines*) segmental duplications of chromosome 15 (Courtesy of Dr. E.E. Eichler; from Bailey et al. 2002)

of marker E (chr15:82,835,478-83,006,963, UCSC, March 2006 release), got inactivated.

Segmental duplications (SDs) are biased against pericentromeric regions (She et al. 2004). The graphic representation of the distribution of SDs of chromosome 15 shows a clear clustering of SDs at 15q24-26 (Fig. 5.4). In light of the evolutionary analysis of chromosome 15 we have reported, they represent the remains of the pericentromeric SDs that flanked the ancestral centromere. No alphoid sequences are present in this domain, suggesting that the loss of this satellite DNA, typical of primate centromeres, was relatively rapid. The most interesting observation, however, is that human clinical neocentromeres clustering at 15q24-26 perfectly overlap the distribution of SDs. Apparently, the region has preserved features that trigger neocentromere emergence. This potentiality has been conserved for approximately 25 MY, the time of divergence between Hominoidea from Cercopithecoidea (Old World Monkeys, OWM) (Raaum et al. 2005).

Main conclusions are as follows: (i) neocentromeres can emerge in domains corresponding to ancestral inactivated centromeres; (ii) neocentromeres are scattered over a fairly relatively large area (15q24-26), overlapping the dispersion of SDs; (iii) apparently, centromere forming latency is not linked to a specific sequence.

5.3.2 Evolution of Chromosome 3

The evolutionary history of chromosome 3 is relatively complex in comparison to that of 15/14 (Ventura et al. 2004). Figure 5.5 shows how the human chromosome 3 can be derived from the primate ancestor by fission of the 21 synteny and several inversions. Marker order comparison among selected primate species revealed that the centromeres in both Hominoidea and OWM are ENCs. The paucity of SDs

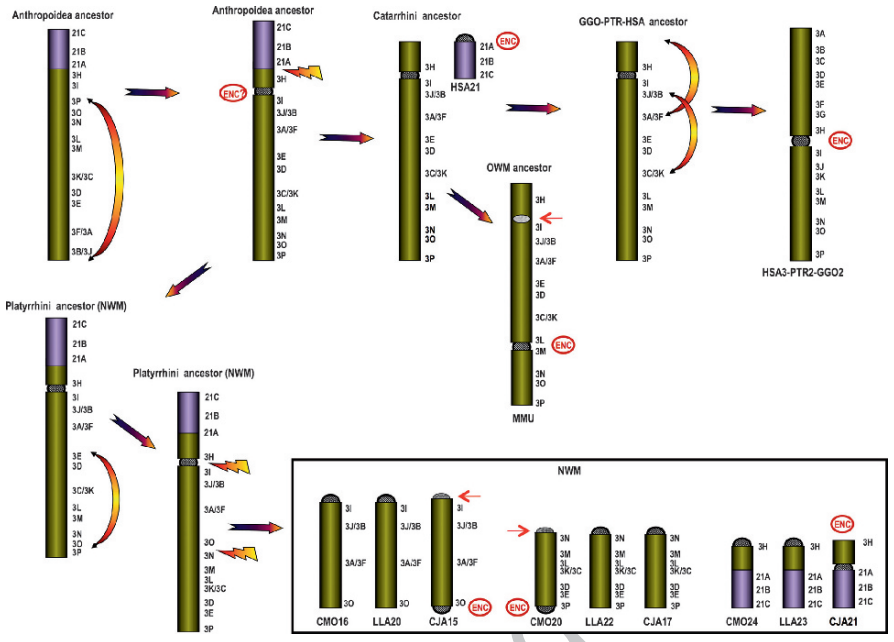

Fig. 5.5 Evolution of chromosome 3. Delineation of the chromosomal changes of chromosome 3 during primate evolution, modified from Ventura et al. (2004). BAC clones used in the synteny investigation are represented by letters on the right of the chromosomes. The letter-BAC correspondence is reported in Supplement

around this ENC (She et al 2004) could be interpreted as the consequence of its recent origin. We had the opportunity to study one case of a neocentromere that resulted from the excision of a small region, including the centromere, to form a small autonomous chromosome (Wandall et al. 1998). The neocentromere appeared located in a domain almost overlapping with the ENC described in macaque (Ventura et al 2004).

Main conclusion: the same chromosomal domain was used as a seeding point for an ENC and for a human clinical neocentromere.

5.3.3 Evolution of Chromosome 13

Contrary to chromosome 3, chromosome 13 can be regarded as one of the most evolutionary conserved chromosomes. The human form very likely corresponds to that of the primate ancestor, which in turn differs from the mammalian ancestor form just for a small inversion (Cardone et al 2006). The same syntenic arrangement of the mammalian ancestor was found in chicken (Consortium 2004) that diverged

from mammals about 310 mya. In OWMs, a novel centromere emerged in a region in the middle of the long arm (13q21). Interestingly, a similarly located, independent ENC emergence was detected in pigs. Additionally, some human neocentromeres reported on chromosome 13 mapped close to the same chromosomal domain. These findings resemble the results reported for chromosome 3. The study, however, exposed some important additional aspects of the centromere repositioning phenomenon: (i) this region maintained centromere forming potential for a very long time of about 95 my, that is, the divergence time of Cetartiodactyla and Primates; (ii) human probes mapping in the seeding region had a very variable results on different OWM species (MMU, *Papio hamadryas*, *Trachypithecus cristatus*, and *Cercopithecus aethiops*), indicating that the region is extremely plastic; (iii) the ENC was seeded in a very large gene-desert region (4.88 Mb) (Lomiento et al. 2008). This last feature will be discussed in detail later.

5.3.4 Neocentromere Clustering at 8p

Contrary to chromosomes 15, 3, and 13, the evolutionary history of chromosome 8 did not reveal any feature that could be of help in interpreting the clustering of clinical neocentromeres at 8p (personal unpublished data). Recent studies published by Dr. Zuffardi's group on cytogenetic anomalies of 8p can be helpful to interpret this clustering. They found that parents of patients carrying de novo 8p chromosomal rearrangement, usually the mother, were heterozygous for an 8p23.1 inversion, delimited by two large clusters of olfactory receptor genes (Giglio et al. 2001). The noncanonical meiotic pairing, consisting in the refolding of one chromosome onto itself, favors the formation of derivative 8p chromosomes, including inv dup(8p) (see Fig. 5.4 of Giglio et al 2001). The inversion is relatively common: 26% of the studied population appears heterozygous for the inversion and the neocentromere reports in literature are all acentric inv dup(8p) rescued by a neocentromere which insured their mitotic survival. Main conclusion: the reason for the clustering of neocentromeres in 8p is probably the result of the relatively high frequency of noncanonical pairing in individuals heterozygous for the 8p inversion.

An alternative hypothesis, discussed below, is that the potential restructuring of chromatin at the break that generated the inv dup(8) could be a concurrent epigenetic cause of neocentromere emergence.

5.3.5 Reuse of Sites of "Chromosomal Events" in Evolution

It is well known that the mouse genome accumulated a large number of chromosomal rearrangements during evolution (Waterston et al. 2002). Subsequent independent bioinformatic studies have shown, in humans, an extensive "reuse" of breakpoints (Pevzner and Tesler 2003; Murphy et al. 2005), and, additionally, an

enrichment of segmental duplications in regions of synteny breaks between the human and mouse genomes (Armengol et al. 2003; Bailey et al. 2004). The SD in humans, however, occurred in the lineage leading to humans long after rodent/primates divergence. The conclusion was that the analysis "supports a nonrandom model of chromosomal evolution that implicates specific regions within the mammalian genome as having been predisposed to both recurrent small-scale duplication and large scale evolutionary rearrangements." The "reuse" of regions as centromere seeding-points in evolution and in human clinical cases further extends the concept of "reuse" of specific domains for "chromosomal events."

5.4 Human Repositioned Centromeres "in Progress"

A crossover inside the region encompassed by the normal and the repositioned centromere results in the formation of dicentric or acentric fragments. In contrast with the expectation that heterozygous carriers of neocentromeres have diminished fitness, the number of repositioned centromeres is relatively high and many repositioned centromeres have been fixed in different species. Meiotic drive in females, as reported for Robertsonian fusions in humans, in favor of the repositioned chromosome might be a possible explanation (Pardo-Manuel de Villena and Sapienza 2001). Meiotic drive has also been invoked to account for the progressive acquisition of heterochromatin in the neocentromeric regions (Henikoff et al. 2001). The progression towards normal centromere complexity, composed of large satellite DNA arrays, is presumed to stabilize neocentromere function. Most clinical neocentromeres are relatively unstable, as suggested by the fact that they are often found as mosaics. Population structure and genetic drift can also be hypothesized to have played an important role in neocentromere fixation.

It can be reasonably supposed, furthermore, that repositioned centromeres that reach fixation are only a minority of those that have emerged in the population. Repositioned centromeres have no clinical consequences. They therefore escape, in humans, the clinical filter that intercepts most of the neocentromeres present as supernumerary chromosomes. Prenatal cytogenetic analyses are most often performed without parental clinical indication. Further, centromere repositioning events can easily be misinterpreted as pericentric inversions. In non-human species, no cytogenetic population data are available, but the number of neocentromere that become fixed ENC is surely a minority. As a consequence, the number of centromere repositioning events in both clinical and evolutionary cytogenetics must be much higher than that noted in the literature.

Examples of balanced centromere repositioning events with no obvious phenotypic effect do exist. The first instances were reported on the Y chromosome (Bukvic et al. 1996; Rivera et al. 1996; Tyler-Smith et al. 1999). The large block of heterochromatin present in this chromosome, however, hampered a full characterization of these repositioned centromeres, in which the satellite DNA could have played a nonminor role. More recently, three autosomal examples of repositioned

centromeres have been reported at 3q24 (Ventura et al 2004), 4q21.3 (Amor et al 2004), and 6p22.1 (Capozzi et al. submitted). They were serendipitously found (two because of a prenatal diagnosis). We will focus on the last case because it showed unprecedented features.

5.4.1 Repositioned Centromere at 6p22.1

The variant chromosome was discovered during a prenatal diagnosis (Capozzi et al. submitted). Molecular cytogenetic analysis showed that the centromere was located in the middle of the short arm, at 6p22.1, without marker order changes. The analysis was extended to the family. The repositioned centromere was found in six individual in three generations. The segregation in three generation and the absence of any phenotypic problem suggested that the repositioned centromere was perfectly functional. In some metaphases, however, extra copies of chromosome 6 indicated that the functionality was not identical to a normal centromere. The precise position of the neocentromere was investigated using ChIP-on-chip analysis that indicated that it was located at chr6:26,407–26,491 kb. The evolutionary history of chromosome 6 had been already delineated by Eder et al. (2003), but the position of the centromere in the ancestor of primates could not be defined with certainty. New data accumulated in the literature allowed us to establish that the ancestral form of chromosome 6 in primates had the same marker order as in humans, but the centromere was located at 6p22.1. This centromere repositioned to the present-day location in the Hominoidea ancestor before gibbon branching, that is at least 17 mya (Raaum et al. 2005). The repositioned centromere was found about 2 Mb apart from the ancestral centromere. In our family case, therefore, it appears as if the centromere jumped back to the ancestral position, where it was located about 17 mya.

5.5 Evolutionary Fate of Novel Centromeres

The organization of a "mature" centromere is complex. In primates, the central core is composed of a large array of alpha satellite DNA, usually surrounded by a cluster of SDs. Occasionally, other types of satellite DNA flank the alphoid core. Similarities with human clinical neocentromeres and human "repositioned" centromeres (see above) strongly suggest that the seeding event is epigenetic in nature, not accompanied by any sequence changes. In macaque, nine of 22 chromosomes are ENCs (Ventura et al 2007). This subset of centromeres, however, is indistinguishable from the "normal" ones: all autosomal macaque centromeres possess a large block of alphoid DNA (Ventura et al 2007). The same applies to the humans ENCs (Ventura et al 2007). It appears as if the progression of these centromeres, from a "plain" sequence, obligatory ends in the acquisition of complexity. To better understand this process, it is worth noting that, as already mentioned, many human

clinical neocentromeres and repositioned centromeres have been found to be mitotically unstable, with mosaicism, especially in supernumerary chromosomes (Marshall et al 2008). Altogether, these observations suggest that rapid progression stabilizes the functionality of the centromere.

Data on pericentromeric SDs of repositioned centromeres are contrasting. Human centromeres of chromosome 3, 6, 11, 14, 15, and 21 are evolutionary new. While acrocentric chromosomes 14, 15, and 21 show large clusters of pericentromeric SDs, the centromere of chromosome 3 and 6 are relatively poor in SDs. Data on non-human primates are scarce, specifically because the shot-gun sequence approach is inefficient to spot SDs, especially if they are duplicated in tandem (Eichler 2001). Their characterization requires meticulous assembly efforts because of the homology, occasionally very high, of SDs. Using a combination of BAC library screening, FISH experiments, and STS sequencing, we were able to characterize the pericentromeric region of macaque ENC of chromosome 6. It appeared as if a 250-kb segment was imperfectly duplicated seven times around the macaque centromere (Ventura et al 2007). Several deletions were supposed to have occurred during the process, because STSs failed several time to amplify the DNA of some macaque BACs.

Studies on the expression of genes embedded in human neocentromeres have shown that they are not affected by their unusual position (Wong and Choo 2001; Saffery et al 2003; Capozzi et al. submitted). However, the deep restructuring that accompanies neocentromere progression, as deduced from the results on MMU6 ENC, can be supposed to physically disrupt the sequence integrity of these genes and that a purifying selection would negatively affect the fixation in the population of these ENC. We tested this hypothesis by checking the gene density in the regions where ENC were seeded (Lomiento et al 2008). The regions of ENCs seeding were significantly depleted of genes. It can be concluded that this circumstance had played a crucial role in their fixation in the population.

Further, we examined the occurrence of SDs around the ENCs present in humans and OWM. SDs in human have been characterized in great detail (She et al 2004), but the macaque assembly is relatively poor in this respect. Using appropriate macaque BAC clones, we investigated SDs located pericentromerically to macaque ENCs. We found that all the examined regions have a certain level of SDs, but, as in humans, the amount varied considerably. The differences could not be attributed, in macaque, to the tempo of their seeding. All of them have been seeded in the common ancestor of OWM, between 16 and 25 mya (Raaum et al 2005). It could be hypothesized that the amount of SDs proceeds as a cascade process. In this case, pericentromeric regions with a higher amount of SDs should contain older SDs. To test this hypothesis would require, however, a substantial effort in sequencing these complex regions.

An additional interesting point of discussion is provided by the unusual findings reported on the pericentromeric region of macaque chromosome 13 (Cardone et al 2006). The comparison of the different duplication pattern in three OWM species (*Macaca mulatta*, MMU, Cercopithecinae), sacred baboon (*Papio hamadryas*, PHA, Cercopithecinae), and silvered-leaf monkey (*Trachypithecus cristatus*, TCR,

Colobinae) showed an unprecedented plasticity. The involved region spans about 3.7 Mb (from marker H2 to marker H8 in Fig. 5.2b of Cardone et al. (2006)). Importantly, this ENC was seeded in a vast gene desert as reported by Lomiento et al. (2008), and appears to involve almost the entire gene-desert, that is about 4.88 Mb. It could be hypothesized that the size of the gene desert defines the degree of plasticity of the pericentromeric region.

5.5.1 Telomeres, Centromeres, and Breakpoint Regions

Evolutionary studies of karyotypes have shown that chromosomes frequently result from the fission of ancestral chromosomes. In humans, chromosomes 15 and 14 and chromosome 21 among others were generated in this way (see above). In such instances at least one new centromere emerged at one telomere or at the breakpoint of the fission. One hypothesis on the origin of centromeres in eukaryotes is that they derived from telomeres. According to this hypothesis, telomeres existed before centromeres and that the recurrent appearance of unstable dicentric chromosomes through the formation of new centromeres (from telomeres) may have had a role in the origin of multiple chromosomes (Villasante et al 2007). The evolution of chromosome 3 in NWM shows several examples of the centromere-telomere functional interchange that may be a remnant of the evolutionary origin of centromeres. The studied species were wooly monkey (*Lagothrix lagothricha*, LLA, Atelinae), common marmoset (*Callithrix jacchus*, CJA, Callitrichinae), dusky titi (*Callicebus moloch*, CMO, Callicebinae). The three segments of chromosome 3 in these NWM species had a similar marker content and orientation, but the centromere position was puzzling (Fig. 5.5b). The orthologous chromosomes LLA20 and CMO16 had the centromere telomerically located, close to marker I, while CJA15 centromere mapped at the opposite telomere, close to marker O. Similarly, the centromeres of CJA17 and LLA22 were located at one telomere, close to marker N, while in CMO the centromere was located at the opposite telomere, close to 3P. The three chromosomes were generated by two successive fissions. The first one occurred at the ancestral centromere, while the second mapped between the markers O and N. It is worth noting that both ends generated by the second fission accommodated a centromere, and that the novel centromere in CJA21 appears to be located at the breakpoint region that, in Hominoidea, generated the human chromosome 21.

The two human clinical neocentromeres reported by Ventura et al. (2003) are invdup(15). It was hypothesized that breaks, through chromatin reorganization, could favor the emergence of neocentromeres. Literature data on breaks that generated the acentric fragments and neocentromere seeding-points, however, are relatively approximate. Precise mapping at the sequence level is mandatory to clarify this question. In the case of a neocentromere that stabilized the ring chromosome excised from chromosome 9, both the neocentromere and the breaks were precisely mapped (Capozzi et al 2008; see above). They turned out to be about 2.1 Mb apart, which is in the range of the neocentromere-ENC correspondence reported so far.

5.5.2 ENCs in Non-Primate Mammals and in Other Taxa

The ENC phenomenon appears widespread in a large number of different taxa. In addition to primates, clear examples of ENCs are available for cattle (Larkin et al. 2003; Everts-van der Wind et al. 2005), pig (Cardone et al 2006), rat (Kobayashi et al. 2008), birds (Kasai et al. 2003), and rice (Nagaki et al. 2004). For marsupials, see Chap. 4. One of the most interesting species, in this context, is the donkey. Comparison of donkey and zebra, using the horse as outgroup, revealed that at least five ENCs emerged in donkey (Carbone et al. 2006) but, because we were able to analyze only larger chromosomes for which marker order could be unequivocally established, there may be additional ENCs. These data are impressive if one considers that donkey and zebra diverged less than 1 mya (Oakenfull and Clegg 1998; Oakenfull et al. 2000).

5.5.3 Concluding Remarks

Centromeres, the "black hole" of the genome, even in the sequencing era resist easy explanation. Yet over the last decade, notable progress has been made especially using molecular cytogenetics. It has become increasingly clear that neocentromere formation and ENCs must be considered as important modes of genome evolution. Perhaps even more remarkable is that the mechanisms in the formation of both types of centromere are intimately related. The "reuse" of regions as centromere seeding-points in evolution and in human clinical cases further extends the concept of "reuse" of specific domains for "chromosomal events." Centromere-forming domains often correspond to ancestral inactivated centromeres and some regions can preserve features that trigger neocentromere emergence over tens of millions of years of evolutionary time. In 2009, we will celebrate the 200th birthday of Charles Darwin and 150 years since the publication of his monumentus book "On the Origin of Species." We now can appreciate that centromeres have an origin, live, and go extinct. Many of the findings we have described in this chapter clearly show how evolutionary perspectives can provide compelling underlying explicative grounds for contemporary genomic phenomena.

5.6 Technical Note

5.6.1 "Outgroup" Concept

When two species display a difference (in our case a chromosomal difference), it is important to know which of the two forms is ancestral and which is derivative to resolve the polarity of the difference. The solution is to introduce into the analysis

of one or multiple closely related species chosen from those that diverged from the common ancestor before the two species under study. More technically, an outgroup species is defined as species or group of species closely related to but not included within the taxon.

5.6.2 *Synteny Studies Exploiting BAC or Fosmid Clones in FISH Experiments*

The conspicuous number of mapped human clones, as can be graphically seen in genome browsers (see the track "BAC End Pairs" in UCSC, for instance), is a side effect of the hierarchical approach utilized to sequence the human genome. As a first step toward sequencing, a very large number of BAC clones were ordered in contigs by characterizing their STS content, by fingerprinting, and by BAC end sequencing (BES). Then, a minimal number of overlapping BACs (or, occasionally, cosmid clones) were fully sequenced. This subset of clones constituted the "golden path."

Following the completion of the human genome sequencing, all non-sequenced BACs were precisely placed on the sequence itself by BLASTing their BES against the human genome. This was possible only for the subset of BAC clones whose ends were both single copy. The complete set of BES data is present in the "Trace archive" database at the NCBI (http://www.ncbi.nlm.nih.gov/Traces/). Note that the fully sequenced BACs of the "golden path" are not present in the "BAC end pairs" track, but present in the "Clone coverage" and "Assembly from Fragments" tracks (UCSC) according to their accession number. It is anyway possible to discover the name of the clone that contributed that sequence by querying the accession number at NCBI (http://www.ncbi.nlm.nih.gov/sites/entrez?cmd=search&db=nucleotide). Recently, the ends of several fosmid libraries (~40 kb insert) were sequenced as part of a copy number variation research projects (Kidd et al. 2008; Tuzun et al. 2005). The fosmids of the first library are present in the track "Fosmid End Pairs" of UCSC. Many of these resources are available from the P. de Jong Laboratory (http://bacpac.chori.org/).

Human BAC clones can be successfully FISHed on apes and Old World monkeys. The success rate decreases in New World monkeys. A rule of thumb for sequence homology comparison among species says that it approximately diminishes by 1% every 5 million years of divergence. Hybridization efficiency can be improved by decreasing the hybridization stringency conditions and increasing the hybridization time. Additionally, pools of 2–4 overlapping BACs can be hybridized together, and gene-rich BACs should be preferred, because gene domains can be supposed to be more conserved. At the present, with several mammal genomes sequenced, the evolutionary conservation of a region can be easily checked by visually inspecting the "Conservation" track at UCSC browser.

The genome sequencing of non-human species was usually achieved using a pure shotgun method, which is less time- and money-consuming, but has a higher risk of mis-assembly as compared to the hierarchical approach (Green 1997). The BES

pairs of a specific BAC library are usually utilized to improve the shot-gun assembly. As a consequence, a species-specific BAC library is usually available for a sequenced genome. These BACs can be very helpful. Appropriate BAC clones can be identified by their BES, present in the "Trace archive" at the NCBI (see above).

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