

1 **The evolution of human synteny 4 by mapping sub-chromosomal specific probes in Primates**

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9 **Abstract**

10 Comparative cytogenetic data concerning the ortholog to human chromosome 4 in primates shows that this
11 chromosome is conserved between humans and non-human primates. However, the degree of conservation is not
12 as high as previously estimated. In primates it is a large submetacentric chromosome but many exceptions are
13 known especially in taxa characterized by a high level of chromosomal rearrangements. The rearrangements that
14 have been visualized by chromosome painting so far, mostly interchromosomal changes, are only a fraction of
15 the actual chromosomal changes that have occurred during evolution. Intrachromosome changes can be analyzed
16 through classical cytogenetic approach or by mapping sub-chromosomal specific probes. In order to study
17 human synteny 4 evolution we mapped diverse subchromosomal specific probes, on chromosomes of
18 representative species of the main Primates taxa , with the aim to verify markers order conservation along the
19 orthologues to human chromosome 4 allowing us the detection of possible intra-chromosomal rearrangements.
20 The mapping of these probes permitted us to test previous cytogenetic hypothesis on human synteny 4
21 evolution, and to show a markers order conservation between orthologues to human synteny 4 in
22 Catarrhini and Platyrrhini, but with a different position of the centromeres. This data permitted us to
23 hypothesize the occurrence of a new centromeres evolution in one of the two lineages. Moreover we
24 analysed literature data regarding HSA4 homologous in Primates with particular attention to Platyrrhini
25 allowing us the reconstruction of the changes that synteny 4 has undergone during evolution. Lastly we
26 highlight the value of the subchromosomal specific probes mapping approach in the detection of
27 intrachromosomal rearrangements that can be crucial for a more refined comparative mapping and for
28 phylogenetic reconstruction.
29

30 **Keywords:** human chromosome 4; chromosomal rearrangements; Platyrrhini, Phylogeny; Evolution.
31

32 **Introduction**

33 Molecular cytogenetics by chromosomal painting provides a tentative reconstruction of ancestral
34 genomes for the major branching of Mammals trees. Starting from the proposed ancestral genome of
35 Primates (Ferguson-Smith and Trifonov 2007; Robinson and Ruiz-Herrera 2008) it has been possible to

1 reconstruct the most important steps leading to the formation of human chromosomes over the last 100
2 million years (Stanyon *et al.* 2008). However, chromosome painting gives considerable data on inter-
3 chromosomal rearrangements (translocations) but the knowledge of intra-chromosomal rearrangements
4 in the different lineages remains limited. This creates several problems on interpretation of results
5 applied to phylogeny. Intrachromosomal rearrangement can be hypothesized through the study of
6 classical cytogenetics data such as G-band patterns and can be confirmed, at the molecular level, using
7 subchromosomal probes (Sineo *et al.* 2007, Dumas and Sineo 2010) obtained or by cloning DNA in
8 vectors such as Yeast Artificial Chromosomes (YACs) and Bacterial Artificial Chromosomes (BACs)
9 or by microdissection. This approach is a useful tool as it allows researchers the definition of markers
10 order along chromosomes and eventually detect inversions and the occurrence of evolutionary new
11 centromeres (Stanyon *et al.* 2008), which are considered important genomic structures promoting
12 chromosomal evolution (Villasante *et al.* 2007). Indeed, it has been possible to appreciate that the
13 pericentromeric regions are rich in duplicons, transposons, retroelements, all currently considered to
14 be characteristic of "hot spots" of chromosomes in both evolution and in diseases. Evolutionary new
15 centromeres (ENC) arise in a novel chromosomal region without any change in marker order and are
16 accompanied by the inactivation of the old centromere (Marshall *et al.* 2008; Rocchi *et al.* 2009).

17 One of the most debated topics of evolutionary history involves human chromosome 4. The human
18 synteny 4 evolution has been recently studied in Eutherian mammals by comparative karyological and
19 genomic data analysis (Picone *et al.* 2010, Dumas, 2012b). In most mammals the homologues to
20 human chromosome 4 are associated with the small arm of the human chromosome 8 (4/8p) (Richard *et al.*
21 *et al.* 2001; Svartman *et al.* 2004; Wienberg *et al.* 2005; Dumas *et al.* 2012). For this reason, and because
22 of the 4/8 association is present in the marsupial *Monodelphis domestica*, (Mikkelsen *et al.* 2007) and
23 the bird *Gallus gallus* (Murphy *et al.* 2005; Robinson and Herrera 2008), it has been considered as an
24 ancestral association in the reconstruction of the ancestral karyotype of all eutherian mammals
25 (Ferguson-Smith and Trifonov, 2007; Stanyon *et al.* 2008). As already demonstrated (Graphodasky *et al.*
26 *et al.* 2011), the 4/8 association has been subject to numerous rearrangements forming new associations
27 with other (human) syntenies in Muridae and Canidae or it has even been diversely disrupted in
28 Primates (Stanyon *et al.* 2008), Sirenia (Kellogs *et al.* 2007) and Proboscidea (Yang *et al.* 2003).

29 The ortholog to human synteny 4 in the ancestral primate karyotype is derived from the fission of
30 the ancestral 4/8 association. In Primates, the HSA 4 homolog has been considered a conserved single
31 submetacentric chromosome (Haig *et al.* 1999), but many exceptions are known especially in taxa
32 characterized by a high level of chromosomal rearrangements such as Strephirrhini (Nie *et al.* 2006),

1 New World monkeys (De Oliveira *et al.* 2002, 2012) Cercopithecini (Dumas and Sineo 2010; Moulin *et*
 2 *al.* 2008) and Hylobatidae (Muller *et al.* 2003).

3 In order to refine the dynamic of human synteny 4 in Primates, we hybridized a panel of sub-
 4 chromosomal specific probes, (arm probes, BACs and single locus probes) on the orthologous to
 5 human chromosome 4 in a representative group of haplorrhini species (table 1). The mapping of these
 6 probes permits us to test previous cytogenetic hypothesis on human synteny 4 evolution, and to analyse
 7 markers order and intrachromosomal rearrangements. The results, compared and associated with
 8 previously published data regarding HSA4 homologous in Primates, allowed us to propose the changes
 9 that synteny 4 has undergone during evolution, with a special focus on Platyrrhini.

10

11 Materials and methods

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13 Following the standard protocol (Small *et al.* 1985) metaphases of the taxa listed in table 1, were
 14 obtained from primary cultures of lymphoblast or fibroblast cell lines and successively fixed on slides:

15

Infraorder	Scientific name of Taxa	abbreviations of names	Common name	Sample Source
Platyrrhini				
	<i>Saimiri sciureus</i>	SSC	Common squirrel monkeys	National Cancer Institute, United States of America
	<i>Saguinus oedipus</i>	SOE	Cotton top-tamarins	University of Bari, Italy
	<i>Callimico goeldii</i>	CGO	Goeldi's tamarin	National Cancer Institute, United States of America
	<i>Cebuella pygmaea</i>	CPY	Pygmy marmoset	National Cancer Institute, United States of America
	<i>Callithrix jacchus</i>	CJA	Common marmoset white-tufted-ear	National Cancer Institute, United States of America
	<i>Aotus lemurinus griseimembra</i>	ALE	Owl monkeys	Tokyo University, Japan
	<i>Lagothrix lagotricha</i>	LLA	Woolly monkeys	National Cancer Institute, United States of America
Catarrhini				
	<i>Chlorocebus aethiops</i>	CAE	Grivet monkey	National Cancer Institute, United States of America
	<i>Erythrocebus</i>	EPA	Patas monkey	National Cancer Institute,

	<i>patas</i>			United States of America
	<i>Cercopithecus albogularis labiatus</i>	CAL	Afromontane samango monkey	Fort Hare University, South Africa
	<i>Macaca arctoides</i>	MAR	Bear macaca	National Cancer Institute, United States of America
	<i>Pongo p. pygmaeus</i>	PPY	Borneo orangutan	National Cancer Institute, United States of America
	<i>Gorilla gorilla</i>	GGO	Gorilla	National Cancer Institute, United States of America
	<i>Pan troglodytes</i>	PTR	Common chimpanzee	National Cancer Institute, United States of America

1 Table 1. list of platyrrhini and catarrhini taxa analysed in the present study and samples source.
2 Primates species classification follows Perelman et al. [2011].

3

4 The human BAC clones, kindly provided by Prof. M. Rocchi from Bari University, were chosen on
5 the UCSC browser (hg18 assembly, UCSC March 2006 release) and previously used in FISH
6 experiments on human metaphases to validate their mapping. The validated BACs were co- hybridized
7 in FISH experiments.

8 Two supplementary subchromosomal specific probes commercially available have been mapped on
9 the Primates taxa analysed: the human 4(HSA) p-arm probe (Q-BIOgene – rhodamine labeled/
10 PlatinumBright) and the single locus probe FIP1L1-CHIC2-DDGFRA –HSA 4 q12, (Q-BIOgene –
11 rhodamine labeled/ PlatinumBright).

12

13 Fluorescence *in situ* hybridization (FISH) using subchromosomal probes on primates metaphases
14 fixed on slides.

15

16 FISH with HSA, BACs probe

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18 Metaphases fixed on slides were performed in 50% formamide (v/v), 10% dextran sulphate, 2 × SSC
19 at 37°C, in the presence of human Cot1 DNA (Gibco-BRL). Hybridization of BACs probes on
20 Primates Post-hybridization washing included 50% formamide, 2 × SSC at 42°C, or 50% formamide, 1
21 × SSC at 37°C, followed by three washes in 1 × SSC at 42°C. The chromosomes were stained with
22 DAPI (4',6-diamidino-2- phenylindole).

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3 FISH with HSA 4p-arm probe
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5 Primates metaphases fixed on slides where incubated in 2X SSC 0.5% NP-40, pH 7.0 at 37°C for 15
6 minutes and dehydrated in ethanol series (70%, 85%, 100%) at room temperature for 2 minutes each.
7 Metaphases were denatured in 70% formamide/ 2X SSC, pH 7.0 at 72°C (\pm 2°C) for 2 minutes;
8 dehydrated in a 4°C ethanol series (70%, 85% and 100%) for 2 minutes each. The probe was denatured
9 at 90°C for 5-10 minutes and hybridized. Slides after hybridization where incubated overnight at 37°C
10 in a wet chamber. After hybridization slides were washed in 1X Wash buffer (0,4X SSC/0,3% NP-40)
11 for 2 minutes at 72°C without agitation followed by a wash of 2XSSC/0,1% Igepal for a minute at
12 room temperature. Slides were then dehydrated in ethanol series (70%, 85%, 100%) at room
13 temperature for 1 minutes each. On the wet slides was applied 15 μ l DAPI antifade (final concentration
14 0.02 μ g/ml) or PI/antifade (0.3 μ g/ml), and a glass cover slip.

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16 FISH with HSA, FIP1L1-CHIC2-DDGFRA, 4p12 probe
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18 Primates metaphases fixed on slides where incubated in 2X SSC 0.5% NP-40, pH 7.0 at 37°C for 30
19 minutes and a dehydrated in ethanol series (70%, 80%, 95%) at room temperature for 2 minutes each.
20 Metaphases were denatured in 70% formamide/ 2X SSC, pH 7.0 at 72°C (\pm 2°C) for 2 minutes; and
21 dehydrated in a 4°C ethanol series (70%, 80% and 95%) for 2 minutes each. The probe was denatured
22 at 75°C for 5-10 minutes and hybridized. Slides after hybridization were incubated overnight at 37°C in
23 a wet chamber. After hybridization slides were washed in 1X Wash buffer (0,5 X SSC/ 0,1% SDS) for
24 5 minutes at 65°C without agitation. On the wet slides was applied 15 μ l DAPI antifade (final
25 concentration 0.02 μ g/ml) or PI/antifade (0.3 μ g/ml), and a glass cover slip.
26

27 All digital images were obtained using a Leica DMRXA2 epifluorescence microscope equipped
28 with a cooled CCD camera (Princeton Instruments). Cy3-dCTP, FluorXdCTP, Cy5-dCTP, and DAPI
29 Pseudocoloring; merging of images were performed using Adobe Photoshop software.
30

31 **Results**

32 All the probes mapped in the present study and the taxa on which they were appropriately
33 hybridized are listed in Table 2. Hybridization are in agreement with painting data regarding the

1 orthologous to human chromosome 4 in Primates. The primates syntenies reconstructed in this work
 2 have been done using the homologies with humans as reference.
 3

HSA chromosomal arm international code	Alphabetic order and labelling of probes	Clone name	UCSC browser position of HSA, DNA probes	Human banding position of probes	Taxa and chromosomes on which probes map
p	A	RP11-1150B4	chr4:1,850,027-2,000,642	4p16	SSC1, CJA3, SOE7, CGO9, CPY9, LLA19, MAR4, CAE27, EPA2, GGO10, PPY3, PTR3, CAL 24
		4p- arm probe (Whole arm)			CJA3, SSC1, SOE7, ALE 9
c	centromere				
q	F	FIP1L1-CHIC2-DDGFRA		4q12	SSC1,SOE7, ALE9
	I	RP11-637n1	chr4:135,127,036-135,329,748	q28.3	PPY3, SSC1
	L	RP11-166k6	chr4:145,428,129-145,602,514	q31.22	PPY3, SSC1
	M	RP11-70L18	chr4:157,931,025-158,098,577	q32.1	PPY3, SSC1
	N	RP11-433J23	chr4:166,447,984-66,559,008	q32.3	SSC1, SOE7

1 Table 2. list of probes used to track HSA 4 evolution. In the first column are listed in order and
2 portioned by the indication of the centromeres position the HSA probes in p and q arms. In the second
3 column are reported the alphabetic letters labelling the probes used in text and figures of the work for
4 the sake of simplicity. In third and fourth columns are listed the specific BAC clones name and their
5 HSA sequence position reported in the UCSC browser. The fifth column is listed the G banding
6 regions from which HSA probes derive. Acronyms in the last column refer to the taxa and the
7 chromosome on which the probes were mapped.

8
9 The results can be resumed as follow:

10 1) Probes A has been mapped on various catarrhini and platyrrhini species (Fig1a-d); it
11 falls in a p-terminal position on submetacentric chromosomes of all Old World monkeys (PPY 3,
12 PTR 3, GGO 10, MAR 5, EPA 2) with the exception of CAE, where it falls on the acrocentric
13 chromosome 27 and CAL, where it maps in a terminal position of the acrocentric chromosome
14 24. Probe A falls on New World monkeys in a q terminal position on the submetacentric
15 chromosomes (SSC 1, SOE 7, CPY 9, CGO 9) but on LLA acrocentric chromosome 19, and on
16 ALE submetacentric chromosome 9 where synteny 4 is fissioned and associated with HSA
17 synteny 15 (images not shown in the picture).

18 2) The human 4 p-arm (Fig 1 e-h) and F (HSAq12) probes (Fig 1 i-l) on Platyrrhinae maps
19 in a q position on the submetacentric chromosome of CJA (ch. 3), SSC (ch. 1), SOE (ch.7) and
20 ALE (ch. 9),

21 3) The hybridization of probes I, L, M, N was repeated on *Saimiri sciureus* (Platyrrhini)
22 and *Pongo pygmaeus* (Catarrhini) (Fig. 1 m). A co-hybridization of L, M, N probes were
23 performed to assess the relative order of markers with certainty. They mapped respectively in a
24 q arm position on chromosome 3 of PPY and in the p arm position on chromosome 1 of SSC
25 with an opposite orientation (Dumas and Sineo 2011). The obtained data have been compared
26 with BAC probes previously mapped on the homolog to human chromosome 4 in CJA (ch. 3)
27 (Stanyon *et al.* 2008).

28 29 **Discussion**

30 *Sinteny 4 evolution in Primates*

31 On the basis of previous molecular cytogenetics results present in literature, mainly painting data,
32 we reconstruct a scenario regarding chromosome 4 evolution in Primates (Fig 2). The ortholog to

1 human synteny 4 in the ancestral primate karyotype, a submetacentric chromosome, is derived from the
2 fission of the ancestral 4/8 association. In Strepsirrhini, synteny 4 was divided into two segments
3 (Stanyon et al., 2002, Stanyon et al., 2006), which in various species have been subject to translocations
4 (Rumpler et al., 2008). In Platyrrhini the orthologous segments are conserved as a single
5 submetacentric chromosome in Cebidae and Pitheciidae (Stanyon et al., 2000, Neusser et al., 2001,
6 Dumas et al., 2007) except in Atelidae. In this last family synteny 4 exhibits a high degree of
7 reshuffling and presents up to three fragments (Dumas et al., 2005, De Olivera et al., 2012). In
8 Catarrhini infraorder the ortholog to human chromosome 4 is a submetacentric chromosome in all the
9 species analysed through painting (Ruiz Herrera *et al.* 2002; Stanyon *et al.* 2005; Bigoni *et al.* 1997a,b,
10 2003, 2004) with the exception of Cercopitheciinae and Hylobatidae (Finelli *et al.* 1999; Moulin et al
11 2008; Muller *et al.* 2003; Dumas and Sineo 2010).

12 *Marker order along synteny 4 in Primates*

13 The mapping of subchromosomal specific probes in a few representative of Primates and the
14 comparison with an outgroup (*Felis catus*) leads researchers to hypothesized a markers order
15 conservation in the ancestral form of human synteny 4 in Primates (Stanyon *et al.* 2008) with some
16 exception in Catarrhini; indeed, through subchromosomal probes mapping in various Old World
17 monkeys such as *Macaca* (Ventura *et al.* 2007), *Pongo pygmaeus*, *Gorilla gorilla* and *Pan troglodytes*
18 (Marzella *et al.* 2000; Clemente *et al.* 1990) those exception have been demonstrate as previously
19 suggested (Yunish and Prakash 1982) on the base of high resolution GTG banding analysis, probably
20 as result of peri-centromeric inversions.

21 Our BACs mapping is in agreement with the chromosome painting results, as all probes fall on the
22 orthologous to human chromosomes. The ortholog to human chromosome 4 in the species here
23 considered is a submetacentric chromosome with the exception of *Chlorocebus aethiops* and
24 *Cercopithecus albogularis labiatus* (Catarrhini), where it is fissioned (Finelli *et al.* 1999, Moulin *et al.*
25 2008) with *Lagothrix lagotricha* and *Aotus lemurinus griseimembra* (Platyrrhini), where respectively
26 human paint 4 maps on two or more chromosomes in association with others syntenies (Neusser et al,
27 2001; Stanyon *et al.* 2011).

28 We found probe A on apparent opposite location in the species analyzed (Fig. 3a): in a terminal
29 position of the short arm (4p), in Catarrhini (*P. pygmaeus* 3, *P. troglodytes* 3, *M. arctoides* 5, *E. patas* 2
30 and *G. gorilla* 10), and in a terminal position of the long arm (4q) in Platyrrhini (*S. sciureus* 3, *S.*
31 *oedipus* 7, *C. goeldii* 9 and *C. pygmaea* 9); even in *C. albogularis labiatus*, *C. aethiops* (Catarrhini), *A.*
32 *lemurinus griseimembra* and *L. lagotricha* (Platyrrhini) where human synteny 4 has been split in two or

1 more fragments and, and in association with synteny 15, the probe maintained its original location.
2 Indeed probe A falls in a terminal position, on acrocentric chromosomes of *L. lagotricha* 19, *C.*
3 *aethiops* 27, *C. albogularis labiatus* 24, and in the q arm position on a submetacentric chromosome of
4 *A. lemurinus griseimembra* 9, without other evident rearrangements. The different position of the probe
5 signal in the two lineages can be explained as the result of a large pericentromeric inversion or of the
6 occurrence of a new centromeres activation as it was previously hypothesized through classic banding
7 analysis for the homologues to human chromosome 4 in *Cebus capucinus* (Platyrrhini) (Dutrillaux *et*
8 *al.* 1976). To test the two hypothesis we hybridized human 4 p-arm (including the HSA 4 p16.3 region-
9 probe A) and probe F (being in a region close to the centromeres in HSA chromosome -4 but on the
10 other arm, q) in Platyrrhini (*C. jacchus* 3, *S. sciureus* 1, *S. oedipus* 7 and *A. lemurinus griseimembra* 9).
11 We show that both the probes map on the q arm in platyrrhini species; furthermore both in a region far
12 from their centromere position but maintaining their reciprocal position and orientation (Fig. 3b); this
13 evidence shows that the HSA markers order is conserved in the species analysed and the different
14 position seen for probe A in Platyrrhini and Catarrhini is only apparent Those results allow us to
15 support the hypothesis of a conservation of markers orders as any inversion of the markers occurred,
16 supporting the previous results reported for a few platyrrhini 4 orthologs analysed (Stanyon *et al.* 2008)
17 and furthermore to suggest that the different apparent position of the A probe signals in New and Old
18 World monkeys considered, as like the differences of 4p-arm and F probes signal position in Platyrrhini
19 and Catarrhini is due to a new centromere activation occurred in one of the two lineages and the two
20 form of chromosome are inverted (upside down). In evaluating the orientation of synteny segments in
21 non-human primates with respect to humans, it is important to note that chromosomes are usually
22 represented with the short arm (p) on top and for each chromosomes the base-pair count conventionally
23 starts from the tip of the short arm; In several Primates chromosomes (Roberto *et al.*, 2008) the
24 centromere index in the genome release could be incorrect because of rearrangements or simply
25 because of centromere repositioning events as it is possible to appreciate in the CCJ chromosome 4
26 homologues released in the UCSC browser when compared with the present evidences.
27 In the present work we repeated a previous BACs hybridizations [(4q 28.3), L (4q 31.22), M (4q32.1),
28 N (4q32.3)] on *S. sciureus* and *P. pygmeus* (Dumas and Sineo 2010). The results has been compared
29 with data present in literature regarding *C. jacchus* (Ch.3), where marker order had been demonstrate to
30 be conserved (Stanyon *et al.* 2008, Rocchi *et. Al.* 2009). The comparison permitted us to show a
31 different position and orientation of the probes (block I to N), along the chromosomes homologues of
32 the two platyrrhini species, explainable as result of a large pericentric inversion occurred in *S. sciureus*

1 1 (Fig 3c). This data permits us to underline that there are exceptions respect the conservative status of
2 synteny 4 even in Platyrrhini.

3 Furthermore based on cytogenetic data present in literature (Stanyon et al. 2008, Dumas and Sineo
4 2011, Ruiz Herrera *et al.* 2005; Stanyon *et al.* 2011, Stanyon *et al.* 2001; Stanyon *et al.* 2008) we
5 define the chromosomes rearrangements occurred during evolution in New World monkeys (Fig 3d).
6 We recognize, through classic banding pattern analysis (Dutrillaux *et al.* 1979), a first genomic
7 organization of synteny 4 in cebidae species such as *Cebus capucinus* from which derived the others
8 forms by: a new centromere formation in *C. jacchus* 3 as demonstrate by Stanyon and Collegues
9 (2008); a large pericentric inversion in *S. sciureus* 1 (Cebidae) (present work); a robertsonian fission
10 and successive traslocation to form a new syntenic association with human synteny 15 (4a, 4bc/15) in
11 *A. lemurinus griseimembra* (Aotinae- Cebidae); two non centromeric fissions with the production of
12 tree fragments and a traslocation to form the 4/15 association (4a, 4b/15, 4c) in *L. lagotricha* (Atelidae)
13 with chromosome LLA19 (4c) showing a new centromeres. Note that the association 4/15 in *L.*
14 *lagotricha* has different breakpoints if compared with the one in *Aotus* and does not represent a
15 synapomorphy linking the two species (Picone and Sineo 2010) as supposable even in the *A. lemurinus*
16 *griseimembra* subspecies.

17

18 **Conclusion**

19 We investigated the evolutionary steps of human synteny 4 by performing original hybridizations
20 and interrogating our data with respect to previous findings on orthologous to human chromosome 4 in
21 primates. We mapped sub-chromosomal probes of interesting critical points on chromosomes of
22 representative group of Anthropeida (Primates), in order to define and verify marker clustering and
23 possible chromosomal rearrangements. We performed banding pattern and BACs pattern study together
24 that are of great help in joint analysis. Furthermore we reconstructed the evolutionary steps that synteny
25 4 has undergone during primate evolution with particular attention to Platyrrhini by analysing literature
26 data on painting and BACs probes mapping.

27 Through the mapping of different probes of critical interest we tested previously cytogenetics
28 hypothesis on synteny 4 in New and Old word monkeys allowing us to support the general conservative
29 status of the synteny but with some exceptions. In particular:

30 1) the mapping of A, p-arm and F probes in catarrhini and platyrrhini species analysed permit us to
31 exclude the hypothesis of a pericentromeric inversion as responsible of the apparent differences in
32 between the syntenies 4 in Neotropical and Old word monkeys; conversely we support the hypothesis

1 about the markers order conservation in the orthologues to human chromosome 4 in anthropoidea
2 species; indeed we single out that the chromosomes homologous to human synteny 4 in the two
3 lineages are just inverted and they differ merely in the position of the centromeres; this evidence
4 stimulates a innovative hypothesis in which the activation of a new centromere occurred in one of the
5 two lineages;

6 2) Our analysis of cytogenetic data present in literature regarding human synteny 4 allow us to show
7 the main evolutionary steps that synteny 4 has undergone during Primates evolution with particular
8 attention to Platyrrhini. In New World monkeys we show a high level of genomic changes including
9 inter and intrachromosomes rearrangements such as traslocation, fissions, pericentromeric inversion
10 and new centromere activation; rearrangements potentially useful in phylogenetic and genomic studies
11 of sequence assembly.

12

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