

Legend for Supplementary Figure 3

Extended metaphase FISH analysis to place and orient contigs 1-5 and to place aqua palindromes 1 and 2.

Note: Contig 5 (containing X-degenerate region and pseudoautosomal region (PAR) was already known to be on long arm with PAR most distal (Glazer, et al. *Simian Y chromosomes: Species-specific rearrangements of DAZ, RBMY, and TSPY versus contiguity of PAR and SRY*. Mammalian Genome, 9:226-231, 1998).

All probes are chimpanzee genomic clones except 18E8, which is a human genomic clone (Saxena, et al. *The DAZ gene cluster on the human Y chromosome arose from and autosomal gene that was transposed, repeatedly amplified and pruned*. Nature Genetics, 14:292-299, 1996).

Distal and proximal are defined on each arm relative to the centromere.

a. Contig 1 is on the short arm shown by three-color FISH using the following probes:

Clone ID	Probe Type	Target	Color
CH1251-6881E11	Fosmid	Unique sequence in contig 1	Red
CH251-541M1	BAC	Centromere	Green
CH1251-4421N16	Fosmid	Distal Yq	Yellow

Results:

red-green-yellow 18 times
red/green-yellow (red and green overlapped) 17 times

Red (contig 1) was never seen closer to yellow (Yq) than green (centromere).

b. Contig 2 is on the short arm shown by three-color FISH using the following probes:

Clone ID	Probe Type	Target	Color
CH1251-4029M17	Fosmid	Unique sequence in contig 2	Red
CH251-541M1	BAC	Centromere	Green
CH1251-4421N16	Fosmid	Distal Yq	Yellow

Results:

red-green-yellow 5 times
red/green-yellow (red and green overlapped) 14 times

Red (contig 2) was never seen closer to yellow (Yq) than green (centromere).

c. Contig 1 is more distal than contig 2 shown by three-color FISH using the following probes:

Clone ID	Probe Type	Target	Color
CH251-4029M17	Fosmid	Unique sequence in contig 2	Red
CH1251-6076G10	Fosmid	Centromere adjacent	Green
CH1251-6687A05	Fosmid	Unique sequence in contig 1	Yellow

Results:

yellow-red-green	24 times
yellow-red/green (red and green overlapped)	16 times
yellow-green-red and yellow/green-red (yellow and green overlapped)	2 times

Yellow (contig 1) was always most distal.

d. Arm placement/orientation of contig 3 shown by three-color FISH using the following probes:

Clone ID	Clone Type	Target	Color
CH1251-6085F22	Fosmid	Unique sequence in contig 3	Red
CH1251-4428N05	Fosmid	Teal palindrome loop	Green
CH1251-4221N16	Fosmid	Distal Yq	Yellow

Results: Sequencing indicates that the unique sequence in contig 3 is adjacent to the centromere, but the arm position was unknown. There are three copies of the teal palindrome loop on contig 3. There are no additional copies of this sequence on the chromosome. The following patterns were observed:

red-green-yellow	15 times
green-red-yellow	1 time

This indicates that contig 3 is on the long arm and the unique sequence probe is more centromere proximal.

e. Contig 4 is on the long arm shown by two-color FISH analysis using the following probes:

Clone ID	Probe Type	Target	Color
18E8	Cosmid	Red palindrome in contigs 2 and 4	Red
CH1251-6076G10	Fosmid	Centromere adjacent	Green

Results: Contig 4 has no unique sequence, so we could only determine its position in relation to contig 2. Only contigs 2 and 4 contain the red palindrome. The pattern green-red-green was seen 16 times. No alternate patterns were seen.

Since contig 2 is on the short arm (part b), contig 4 must be on the long arm. The only location for contig 4 is between contig 3 (adjacent to centromere) and contig 5 (adjacent to PAR at distal Yq).

f. Orientation of contig 1 shown by two-color FISH using the following probes:

Clone ID	Probe Type	Target	Color
CH1251-6687A05	Fosmid	Unique sequence in contig 1 (near Yptel)	Red
19708/9	PCR	Pink amplicon	Green

Results: Contig 1 contains one copy of the pink amplicon. The following patterns were observed:

red-green-green-green	18 times
green-red-green-green	2 times

We conclude that the unique sequence in contig 1 is more distal than the pink amplicon.

g. Orientations of contigs 2 and 4 shown by three-color FISH using the following probes:

Clone ID	Clone Type	Target	Color
CH251-387H09	BAC	Orange amplicon	Red
18E8	Cosmid	Red palindrome	Green
CH1251-4221N16	Fosmid	Distal Yq	Yellow

Results: Contigs 2 and 4 both have copies of the orange amplicon and the red palindrome. There is an additional copy of the orange amplicon in contig 1, which is the most distal contig on Yq. The following patterns were observed:

red-green-red-green-red-yellow	14 times
pattern red-green-red-???-yellow	18 times
???-green-red-yellow	13 times

“???” indicates overlapping signals that could not be interpreted. In each pattern, the order of the probes in both contigs 2 and 4 is green-red indicating that the red palindrome is more distal than the orange amplicon in contig 2 (i.e. closer to the short-arm telomere) and the red palindrome is more proximal than the orange amplicon in contig 4 (i.e. closer to the centromere on the long arm).

h. Placement of the aqua palindromes on the short and long arm shown by three-color FISH using the following probes:

Clone ID	Clone Type	Target	Color
CH1251-6076G10	Fosmid	Centromere adjacent	Red
CH1251-6332E22	Fosmid	Aqua palindrome arm	Green
CH1251-4221N16	Fosmid	Distal Yq	Yellow

Results: One copy of the aqua palindrome is on contig 3 (located on the long arm – see part g). The other two copies are not anchored to a contig. The pattern green-red-green-yellow was seen 24 times. No other patterns were seen. We can conclude that at least one of the unplaced copies of the aqua palindrome is on the short arm.

i. Placement of aqua palindrome on the short arm between contigs 1 and 2 shown by three-color FISH using the following probes:

Clone ID	Clone Type	Target	Color
CH1251-387H09	BAC	Orange amplicon	Red
CH1251-4694J22	Fosmid	Aqua palindrome arm	Green
CH1251-4221N16	Fosmid	Distal Yq	Yellow

Results: Contigs 1 and 2 both have copies of the orange amplicon. There is an additional copy of the orange amplicon in contig 4 (long arm). The following patterns were observed:

red-green-red-green-red-yellow	6 times
red-green-red-??-yellow	9 times
red-green-red-red-green-yellow	4 times
??-red-green-red-yellow	2 times

In all cases where the short arm signals were interpretable, the green signal is surrounded by red signals. This indicates that there is at least one copy of the aqua palindrome between contigs 1 and 2.

j. Placement of aqua palindrome on the long arm distal to contig 4 shown by three-color FISH using the following probes:

Clone ID	Clone Type	Target	Color
CH11251-5291I08	Fosmid	Aqua palindrome loop	Red
18E8	Cosmid	Red palindrome	Green
CH1251-4221N16	Fosmid	Distal Yq	Yellow

Results: Contig 2 (short arm) and contig 4 (long arm) both have copies of the red palindrome (green signal). There are no additional copies of the red palindrome. The following patterns were observed:

red-green-red-green-red-yellow	24 times
red-green-???-yellow	21 times
???-red-green-red-yellow	9 times
green-red-???-yellow	2 times
red-green-green-red-red-yellow	1 time

In all cases where the long arm signals were interpretable, the red signal (aqua palindrome loop) is more distal than the green signal (red palindrome). This observation indicates that there is at least one copy of the aqua palindrome between contigs 4 and 5.

k. Repeat sequence at distal end of contig 1 extends to Yp telomere.

Clone ID	Clone Type	Target	Color
CH11251-6881E11	Fosmid	Unique sequence in contig 1	Red
CH251-270L13	BAC	Repeat sequence in contig 1	Green

Results: Top – BAC from repeat sequence at terminal gap on Yp extends to the end of the chromosome. Bottom – Same repetitive BAC hybridizes to many autosomes.