Cell, Volume *138*

Supplemental Data

Isodicentric Y Chromosomes and Sex Disorders as Byproducts of Homologous Recombination that Maintains Palindromes

Julian Lange, Helen Skaletsky, Saskia K.M. van Daalen, Stephanie L. Embry, Cindy M. Korver, Laura G. Brown, Robert D. Oates, Sherman Silber, Sjoerd Repping, and David C. Page

Table of Contents

Supplemental Experimental Procedures

Fluorescence In Situ Hybridization (FISH) Analysis of IdicY and IsoY Structure

To obtain metaphase spreads for FISH, actively proliferating lymphoblastoid cells were incubated for 15 min with 200 ng/ml colcemid (KaryoMAX, Invitrogen), harvested by centrifugation, resuspended for 10 min in 75 mM KCl at 37°C, and fixed in 3:1 methanol/acetic acid. To obtain interphase spreads, cells were first starved for four days prior to incubation in 75 mM KCl and fixation, without undergoing colcemid treatment. Preparations were dropped onto slides, treated for 60 min with 100 μg/ul RNase A in 2X SSC solution at 37°C and for 10 min with 0.01% pepsin in 10 mM hydrochloric acid at 37°C, fixed for 10 min with 10% formaldehyde, dehydrated by successive two-minute incubations in 70%, 96%, and 100% ethanol, and air-dried.

FISH probes were labeled by nick translation with biotin-dUTP (Roche), digoxigenin-dUTP (Roche), Cy3-dUTP (Amersham), or FITC-dUTP (Amersham). For hybridizations to single- or low-copy-number euchromatic sites, purified probes were resuspended in 50% formamide, 2X SSC, 50 mM sodium phosphate buffer, 10% dextran sulfate, pH7.0. Slides were denatured for 5 min in 60% formamide, 2X SSC, 50 mM sodium phosphate buffer, pH7.0 at 80°C, dehydrated by successive incubations in 70%, 96%, and 100% ethanol, and air-dried. Labeled probe (70-400 ng) was denatured for 5 min at 80°C, pre-hybridized with human COT DNA (Roche) for 30 min at 37°C, and hybridized overnight to slides under sealed coverslips at 37°C in a humid chamber. For hybridizations to high-copy-number heterochromatic repeats, purified probes were resuspended in 60% formamide, 2X SSC, 50 mM sodium phosphate buffer, pH7.0. Under sealed coverslips, 16-400 ng labeled probe and slides were denatured together for 5 min at 80°C, then hybridized overnight at 37°C in a humid chamber.

Slides were then subjected to three washes of 5 min each in 50% formamide, 2X SSC at 42°C, two washes of 5 min each in 2X SSC at 42°C, and a final wash of 5 min in TNT solution (TN buffer [100 mM Tris-HCl, pH7.5, 150 mM NaCl] plus 0.05% Tween 20) at room temperature. For hybridizations of biotin-labeled or digoxigenin-labeled probes (including all co-hybridizations), preparations were blocked by incubating them for 10 min with TNB (TN buffer plus 0.5% blocking reagent [Roche] and 0.02% thimerosal) at room temperature. Biotin-labeled probes were detected by incubation with Cy3-conjugated avidin (Jackson ImmunoResearch) diluted 1:5000 in TNB. Digoxigenin-labeled probes were detected by incubation with mouse anti-digoxigenin antibody (Sigma) diluted 1:100 in TNB followed by incubation with FITC-labeled rabbit anti-mouse IgG antibody (Sigma) diluted 1:500 in TNB. These incubations were performed in a humid chamber at 37°C for 20 min. Slides were then washed in TNT for 5 min at room temperature. (Exceptionally, preparations hybridized with biotin-labeled probe 18E8 underwent blocking with NFDM solution [4X SSC plus 5% non-fat dry milk and 0.02% thimerosal] for 10 min at room temperature, biotin detection with FITC-conjugated avidin diluted 1:125 in NFDM for 20 min at room temperature, and washing with 4X SSC plus 0.05% Tween 20 for 5 min at room temperature.) Slides were dehydrated by successive two-minute incubations in 70%, 96%, and 100% ethanol, and air-dried. Chromosomes were counterstained with DAPI or propidium iodide in mounting medium (Vector Laboratories).

Slides were viewed though a Zeiss Axioskop fluorescence microscope equipped with a CCD camera (ER-3339, Applied Imaging). Images were captured with Applied Imaging Cytovision software and processed with Adobe Photoshop. In each interphase experiment that involved the counting of sites of hybridization, at least 100 nuclei were scored. See Table S5 for specific information on individual FISH probes and assays.

Immunofluorescence (IF) – FISH Counting of Active Centromeres

To obtain metaphase spreads for IF-FISH, actively proliferating lymphoblastoid cells were incubated with 25 ng/ml colcemid for 75 min, harvested by centrifugation, resuspended at a concentration of $1x10^5$ cells/ml in hypotonic solution (0.4% sodium citrate, 25 mM KCl, prewarmed to 37°C) for 15 min, and centrifuged onto glass slides for 10 min at 2000 rpm with high acceleration (Shandon Cytospin 4, Thermo Scientific). Spreads were fixed for 10 min at room temperature in 4% formaldehyde in PBS plus 0.1% Triton X-100, and then rinsed with PBS.

For IF, preparations were first blocked by incubating with 3% BSA in PBS-0.1% Tween 20 for 1 hr at room temperature. Anti-CENP-E antibody (Harrington et al., 1997) was diluted to 1:200 in PBS-0.1% Tween 20 plus 1% BSA, added to slides, and covered with glass coverslips. Slides were incubated for 60 min at room temperature, and then washed three times for 5 min each with PBS-0.1% Triton X-100 at room temperature. Primary antibody was detected by incubating slides for 30 min at room temperature with Cy3-conjugated donkey anti-rabbit IgG antibody (Jackson ImmunoResearch) diluted 1:200 in PBS-0.1% Tween 20 plus 1% BSA. All incubations were performed in a humid chamber. Preparations were then washed three times for 5 min each in PBS-0.05% Tween 20 at room temperature, fixed for 10 min in 10% formalin solution (Sigma) at room temperature, washed once for 5 min in PBS-0.05% Tween 20 at room temperature, and either processed immediately or stored overnight in PBS-0.05% Tween 20 at 4°C.

For FISH, probe pDP97 was labeled by nick translation with SpectrumGreen dUTP (Abbott Molecular), purified, and resuspended at a concentration of 20 ng/μl in hybridization mix (65% formamide, 2X SSC, 10% dextran sulfate, 100 μg/ml salmon sperm DNA). Chromosomal DNA was denatured in 70% formamide, 2X SSC, pH7.0 at 75°C for 12 min, and then rinsed in 2X SSC at RT for 5 min. Labeled probe (200 ng) was denatured at 72°C for 7 min and hybridized to chromosomes under sealed coverslips at 37°C overnight in a humid chamber. Slides were washed twice for 8 min each in 50% formamide, 2X SSC, pH7.0 at 42°C and once for 8 min in 2X SSC at 37°C. Chromosomes were counterstained with DAPI in mounting medium (Vector Laboratories).

Slides were examined under a Zeiss Axiovert 200M microscope fitted with a Hamamatsu ORCA-ER camera. Images were captured with OpenLab software (Improvision) and processed with Adobe Photoshop. For each cell line, at least 20 idicYp-chromosome-bearing spreads of good morphology were scored.

Figure S1. Triangular dot plot of the MSY reference sequence shows intrachromosomal sequence similarities
Triangular intra-arm dot plot of the MSY reference sequence (adapted from Skaletsky et al., 2003). In this plot, each dot represents a match of >98% within a window of 1000 bp. Repeat elements have been masked. Euchromatic repeats are shown in black, heterochromatic repeat arrays in red. Inverted repeats such as palindromes appear as vertical lines, direct repeats as horizontal lines.

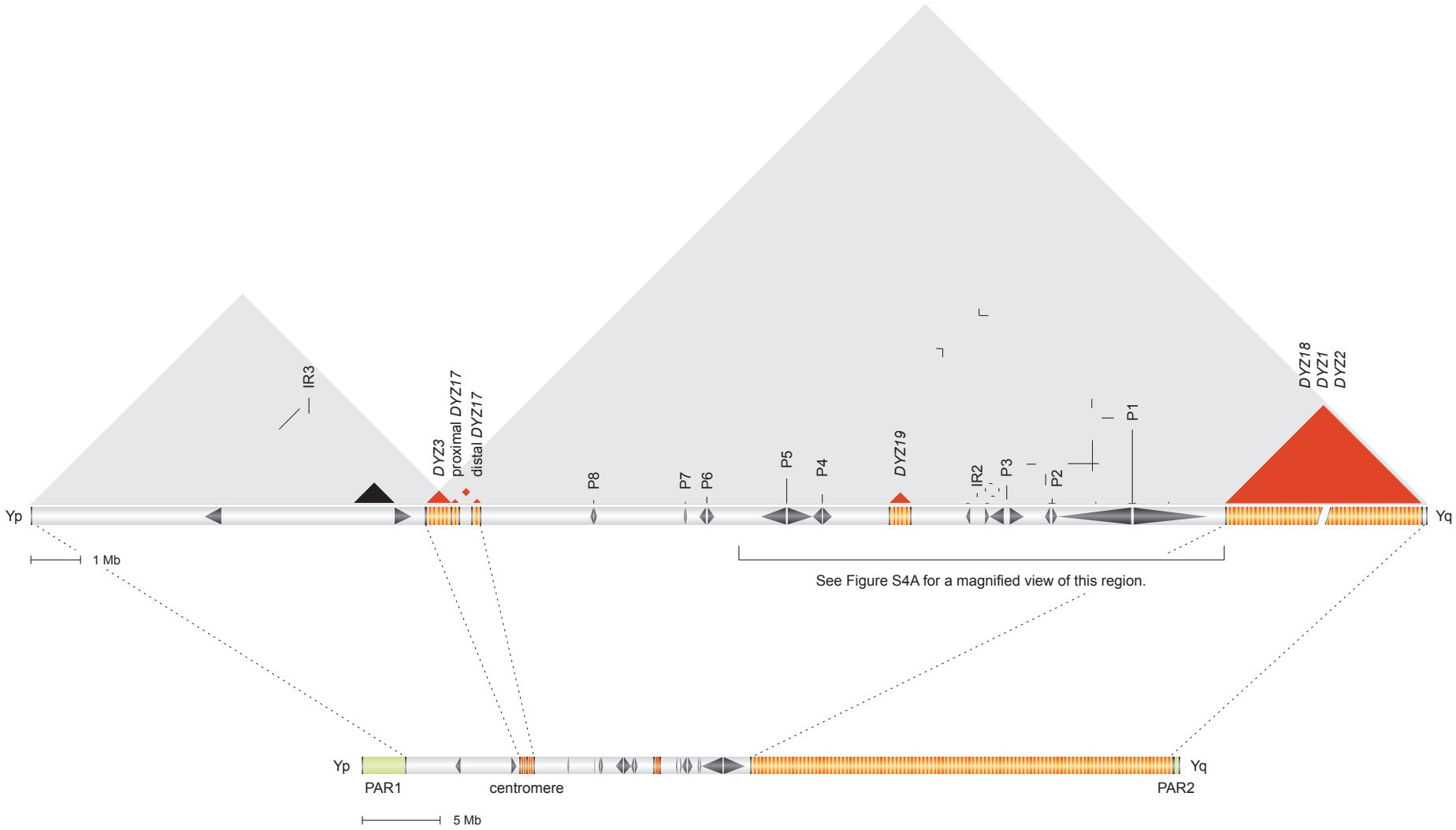
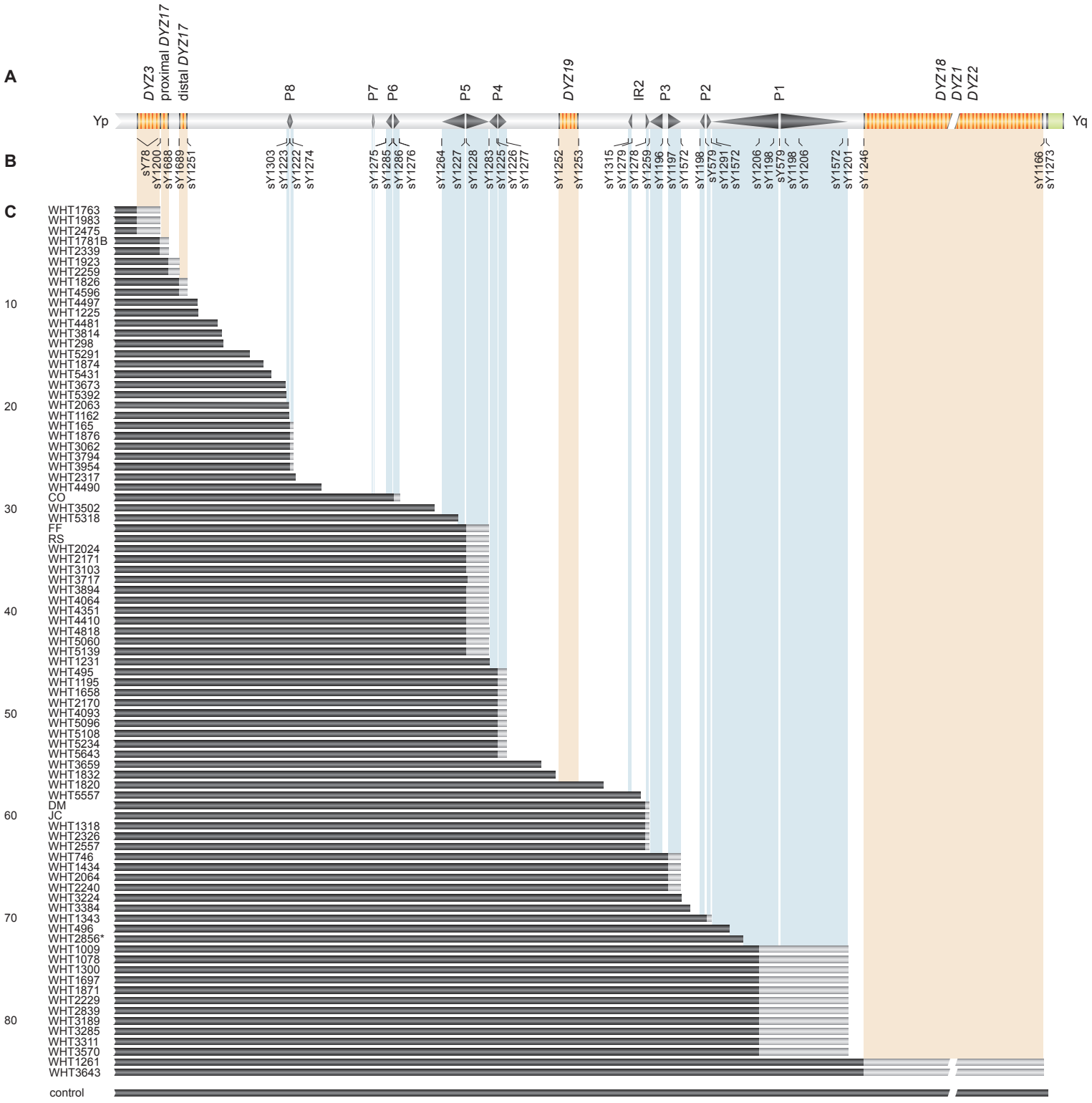


Figure S2. STS content of structurally anomalous Y chromosomes in 85 individuals with deletion breakpoints in the long arm (Yq) or centromere

(A) Expanded view of centromere and Yq, indicating locations of eight palindromes (P1 through P8), one inverted repeat (IR2), and five heterochromatic regions containing highly repetitive elements (*DYZ1-3* and *DYZ17-19*).

(B) STSs employed in fine mapping of deletion breakpoints. These STSs include boundary and spacer-flanking markers for each palindrome and inverted repeat, and markers around each of five blocks of heterochromatin.

(C) Results of testing genomic DNAs from 85 individuals, with identifiers indicated, for presence or absence of STSs. Solid black bars encompass STSs found to be present. Gray bars indicate breakpoint intervals that could not be further narrowed due to cross-amplification at other loci.



*The deletion in WHT2856 occurred on an inverted variant of palindromes P1 and P2 (see Repping et al., 2006).

Figure S3. Comprehensive results of testing for duplication, in idicYp chromosomes, of sequences immediately proximal to targeted palindromes

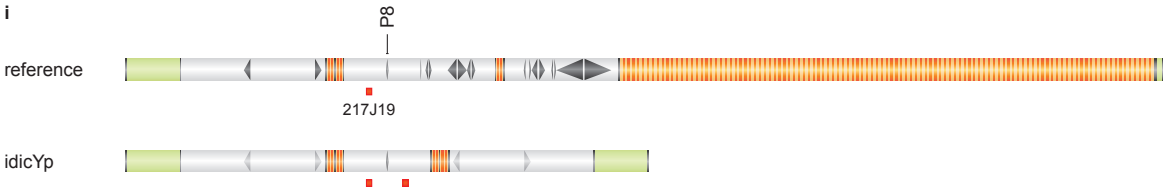
(A-F) For palindromes P8 (A), P6 (B), P5 (C), P4 (D), IR2 (E), and P3 (F) that, based on STS mapping, were hypothesized to have been targeted by ectopic homologous recombination in the formation of idicYp chromosomes, we designed specific FISH assays to determine, in individuals with idicYp chromosomes, the copy number of sequences located immediately proximal to targeted palindromes.

In each series of figures, we show: (i) schematics of a reference chromosome and of an idicYp formed by the proposed mechanism at each palindrome, and the locations of probes hybridized to interphase spreads; (ii) counting results tabulated for a control male and for each case tested with the probe indicated; (iii) counting results summarized graphically for each case; and (iv) representative FISH images for a control male and for an individual with an idicYp to demonstrate common FISH patterns.

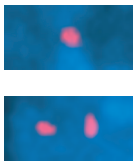
See Figure S4 for idicYp chromosomes formed at inverted repeats in P1. All FISH micrographs are displayed at the same magnification.

A P8

i



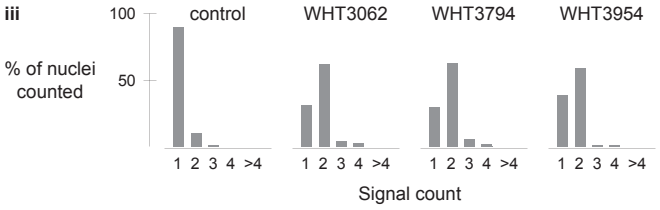
iv



ii 217J19

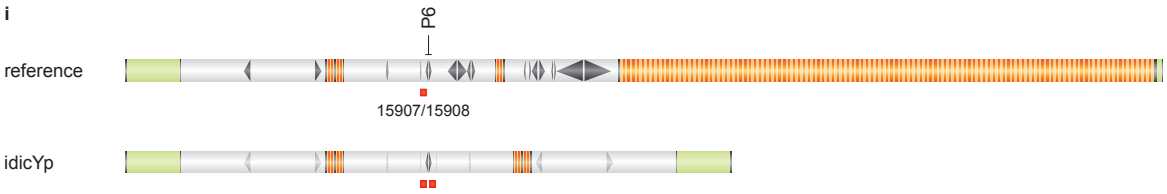
	Number of nuclei with signal count of					Inferred number of sites of hybridization
	1	2	3	4	>4	
control	179	21	0	0	0	1
WHT3062	63	124	8	5	0	2
WHT3794	60	125	11	4	0	2
WHT3954	78	118	2	2	0	2

iii

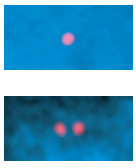


B P6

i



iv



ii 15907/15908

	Number of nuclei with signal count of					Inferred number of sites of hybridization
	1	2	3	4	>4	
control	117	32	1	0	0	1
CO	69	50	1	0	0	2

iii

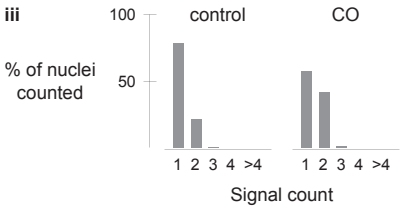
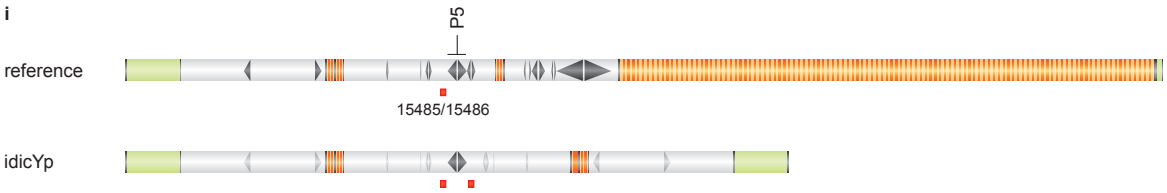


Figure S3 continued. Comprehensive results of testing for duplication, in idicYp chromosomes, of sequences immediately proximal to targeted palindromes

C P5

i

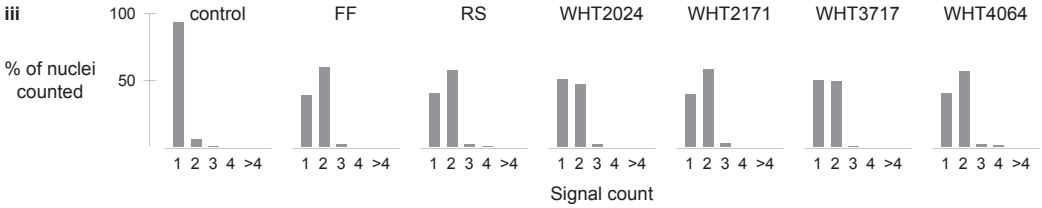


iv

ii 15485/15486

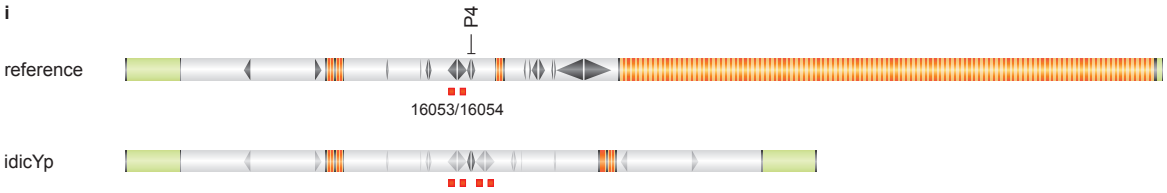
	Number of nuclei with signal count of 1	2	3	4	>4	Inferred number of sites of hybridization
control	187	12	1	0	0	1
FF	78	119	3	0	0	2
RS	81	115	4	0	0	2
WHT2024	102	94	4	0	0	2
WHT2171	79	116	5	0	0	2
WHT3717	100	99	1	0	0	2
WHT4064	81	113	4	2	0	2

iii

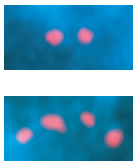


D P4

i



iv



ii 16053/16054

	Number of nuclei with signal count of 1	2	3	4	>4	Inferred number of sites of hybridization
control	103	97	0	0	0	2
WHT1658	14	60	72	54	0	4

iii

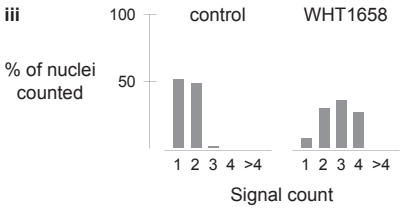
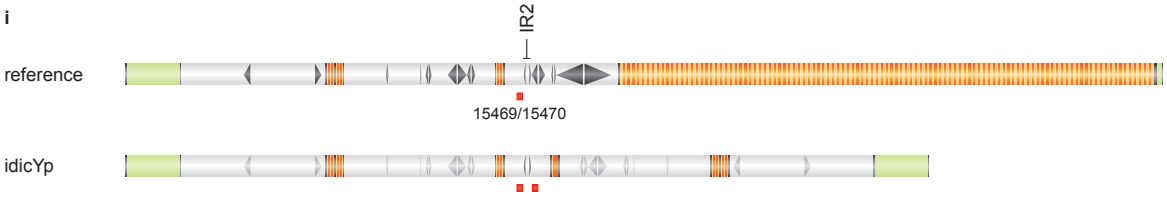


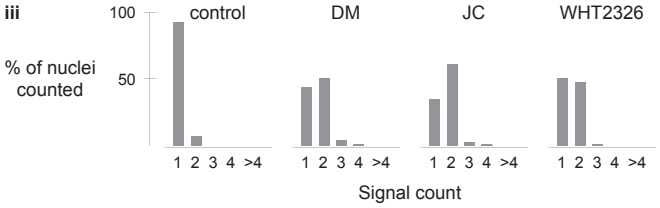
Figure S3 continued. Comprehensive results of testing for duplication, in idicYp chromosomes, of sequences immediately proximal to targeted palindromes

E IR2

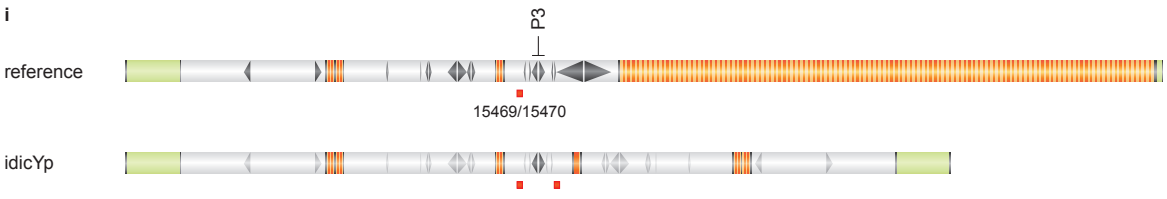


ii 15469/15470

	Number of nuclei with signal count of					Inferred number of sites of hybridization
	1	2	3	4	>4	
control	185	15	0	0	0	1
DM	88	102	8	2	0	2
JC	70	122	6	2	0	2
WHT2326	101	96	3	0	0	2



F P3



ii 15469/15470

	Number of nuclei with signal count of					Inferred number of sites of hybridization
	1	2	3	4	>4	
control	185	15	0	0	0	1
WHT1434	68	120	8	4	0	2
WHT2064	55	129	13	3	0	2
WHT2240	55	137	6	2	0	2

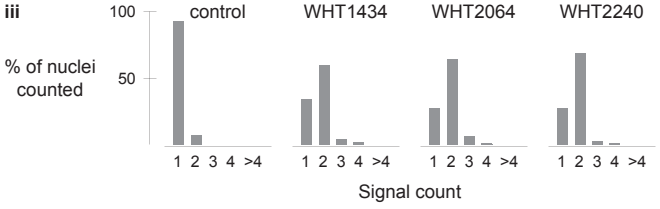


Figure S4. Comprehensive results of testing the copy number, in idicYp chromosomes with breakpoints in palindrome P1, of sequences located between P1 and P5

Large blocks of palindrome P1 are repeated in palindromes P2, P3, and P5. Like opposing arms of palindromes, these inverted repeats could serve as targets for the proposed model. In each case displaying an idicYp with a breakpoint in P1, the inverted repeats that were targeted in the formation of the idicYp can be determined by STS mapping and specific FISH assays.

(A) Triangular dot plot of the region of the MSY long arm spanned by palindromes P5 and P1, which features many amplicons in both inverted and direct orientation (plot adapted from Skaletsky et al., 2003). Color segmentation of the palindromes highlights the complex ampliconic structure of this region. In this plot, each dot represents a match of >98% within a window of 1000bp. Repeat elements have been masked. Euchromatic repeats are shown in black, heterochromatic repeat arrays in red. Inverted repeats including palindromes appear as vertical lines, direct repeats as horizontal lines.

(B) Ampliconic structure of the region spanned by P5 and P1 in the reference MSY and in idicYp chromosomes predicted to form by eight distinct ectopic homologous recombination events between inverted repeats in this region. Shown are (i) the reference sequence spanning P5 and P1, and the target sequences on sister chromatids and ampliconic structures of idicYp chromosomes predicted to form at the following inverted repeats: (ii) b3/b2 - proximal P1/P3; (iii) IR5 - proximal P1/P5; (iv) P1.2; (v) rg/gr - P1/P2; (vi) P1; (vii) P1.1; (viii) IR5 - distal P1/P5; and (ix) b4/b1 - distal P1/P3. Predicted idicYp chromosomes can be distinguished by the results of three STSs tested on genomic DNA - sY1291, sY1206, and sY1201 - and three FISH probes hybridized to interphase spreads - 1325K3, 18E8, and 100J21.

(C) Summary of predicted STS mapping and FISH results for each proposed idicYp shown in (B).

(D-F) Six individuals with idicYp chromosomes that, by mapping of STSs including sY1291, sY1206, and sY1201, displayed breakpoints in P1, were tested with three FISH probes to determine which inverted repeat had been targeted in each case. For each of three FISH probes 1325K3 (D), 18E8 (E), and 100J21 (F), we show: (i) counting results tabulated for a control male and for each of six cases tested; (ii) counting results summarized graphically for each case; and (iii) representative FISH images for a control and two individuals with idicYp chromosomes to demonstrate common FISH patterns. All FISH micrographs are displayed at the same magnification. For clarity, images in (E) and (F) were pseudocolored with Adobe Photoshop to match, for each probe, signal color with the color of the amplicon to which the probe hybridized. Images for controls and test cases were treated identically.

(G) Summary of observed STS mapping and FISH results in (D-F). By comparing observed results with predicted results in (C), we inferred which inverted repeat was targeted in the formation of each idicYp. Five of the six idicYp chromosomes had formed between opposing arms of P1, and one had formed between inverted repeats rg/gr in P1 and P2.

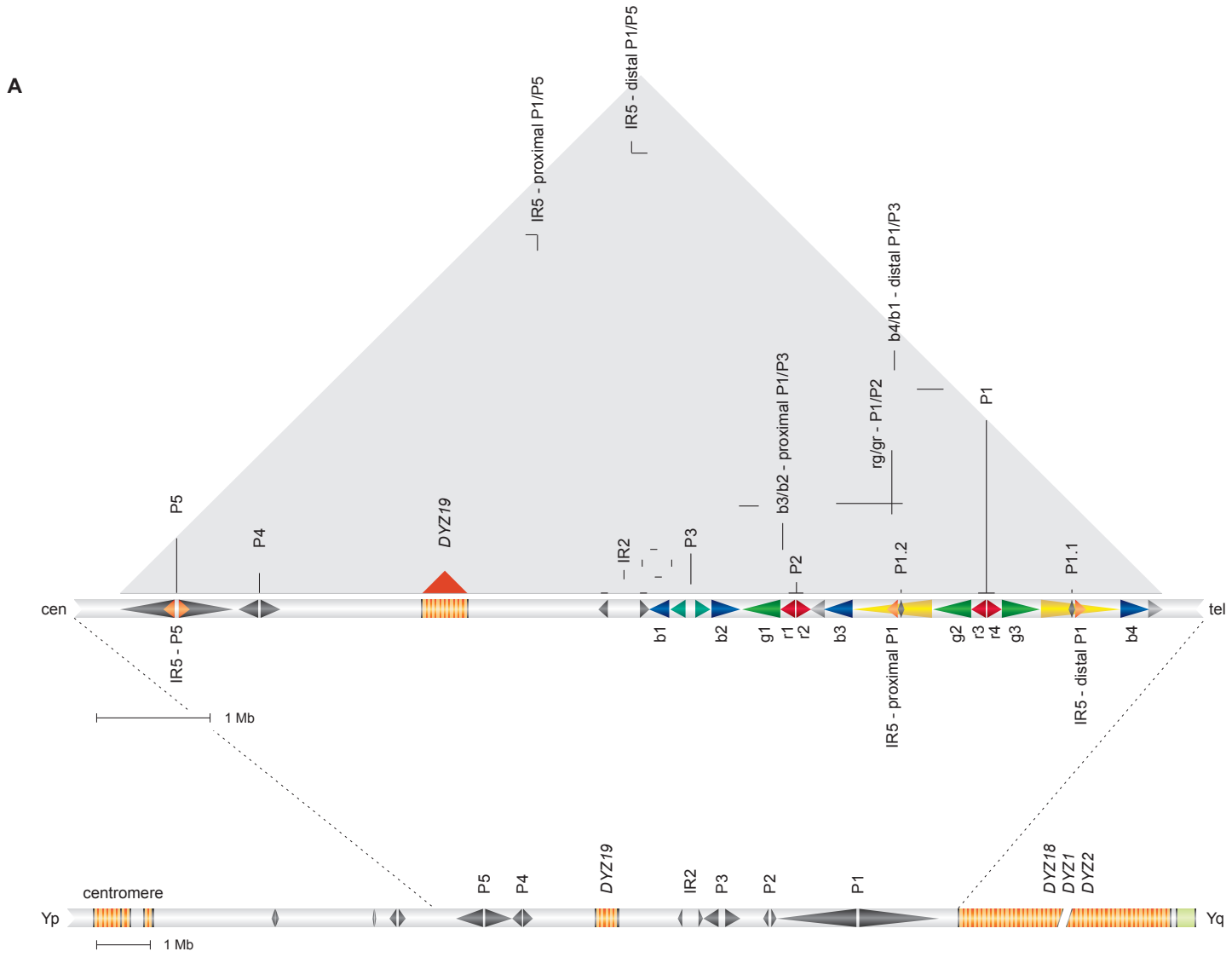


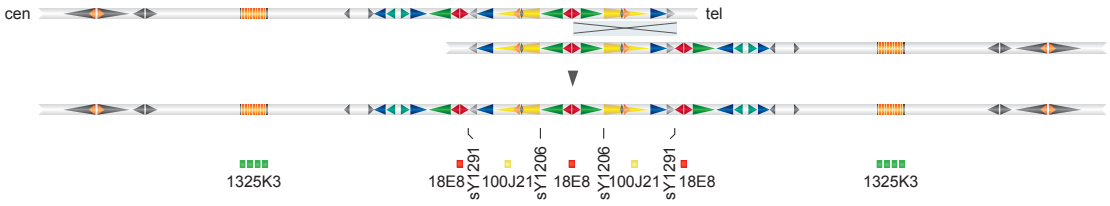
Figure S4 continued. Comprehensive results of testing the copy number, in idicYp chromosomes with breakpoints in palindrome P1, of sequences located between P1 and P5

B

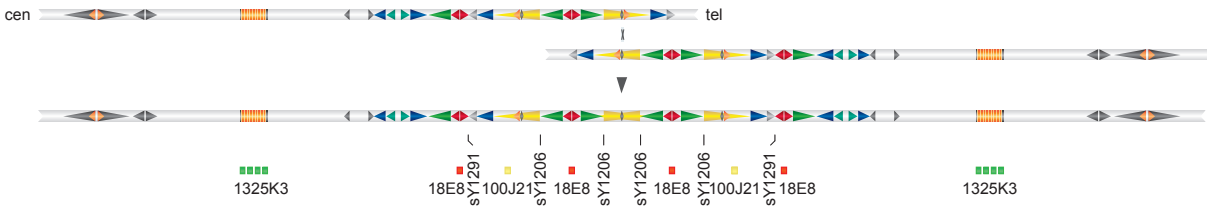
Figure S4 continued. Comprehensive results of testing the copy number, in idicYp chromosomes with breakpoints in palindrome P1, of sequences located between P1 and P5

B continued

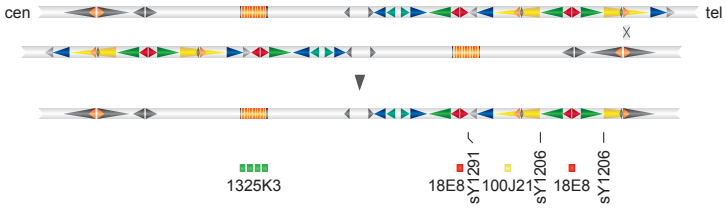
vi P1



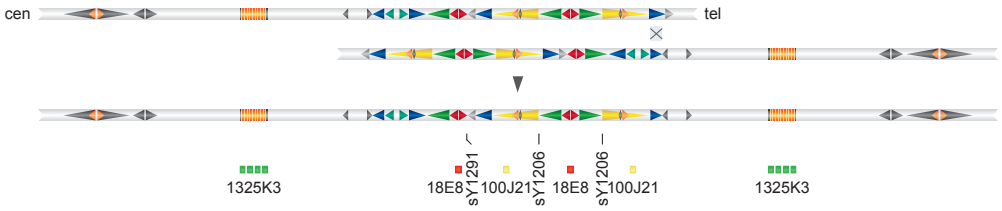
vii P1.1



viii IR5 - distal P1/P5



ix b4/b1 - distal P1/P3



C Summary of predicted STS mapping and FISH results

	Presence or absence of each STS			Number of copies present for each probe		
	sY1291	sY1206	sY1201	1325K3	18E8	100J21
reference	+	+	+	1	2	2
b3/b2 - proximal P1/P3	+	-	-	2	1	0
IR5 - proximal P1/P5	+	-	-	1	1	1
P1.2	+	-	-	2	2	2
rg/gr - P1/P2	+	+	-	2	2	1
P1	+	+	-	2	3	2
P1.1	+	+	-	2	4	2
IR5 - distal P1/P5	+	+	-	1	2	1
b4/b1 - distal P1/P3	+	+	-	2	2	2

Figure S4 continued. Comprehensive results of testing the copy number, in idicYp chromosomes with breakpoints in palindrome P1, of sequences located between P1 and P5

D 1325K3

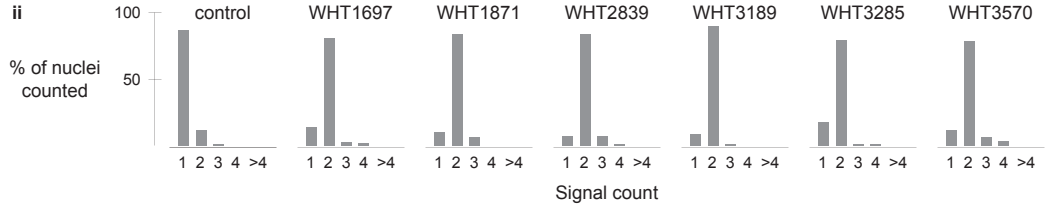
i

	Number of nuclei with signal count of 1	2	3	4	>4	Inferred number of sites of hybridization
control	173	24	3	0	0	1
WHT1697	28	162	6	4	0	2
WHT1871	20	167	13	0	0	2
WHT2839	15	168	14	3	0	2
WHT3189	18	179	3	0	0	2
WHT3285	35	159	3	3	0	2
WHT3570	23	157	13	7	0	2

iii

Note: Due to high levels of XO mosaicism in several individuals, nuclei with signal counts of zero were counted but excluded in the determination of the number of sites of hybridization.

ii



E 18E8

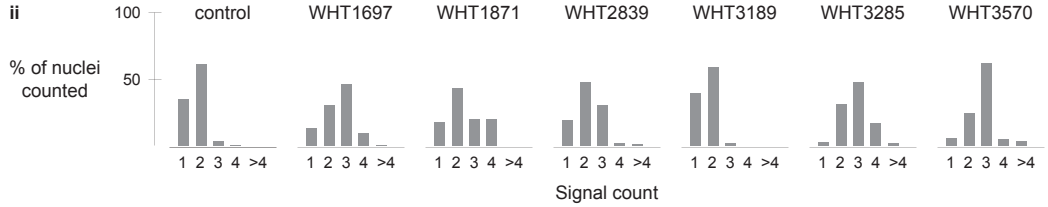
i

	Number of nuclei with signal count of 1	2	3	4	>4	Inferred number of sites of hybridization
control	68	118	6	1	0	2
WHT1697	21	48	74	15	1	3
WHT1871	10	24	11	11	0	3
WHT2839	45	112	72	4	2	3
WHT3189	87	132	4	0	0	2
WHT3285	6	62	94	33	3	3
WHT3570	8	38	92	7	5	3

iii

Note: Due to high levels of XO mosaicism in several individuals, nuclei with signal counts of zero were counted but excluded in the determination of the number of sites of hybridization.

ii



F 100J21

i

	Number of nuclei with signal count of 1	2	3	4	>4	Inferred number of sites of hybridization
control	45	146	8	1	0	2
WHT1697	70	115	3	0	0	2
WHT1871	35	63	1	1	0	2
WHT2839	51	89	5	0	0	2
WHT3189	136	14	0	0	0	1
WHT3285	63	125	6	2	0	2
WHT3570	51	138	10	1	0	2

iii

Note: Due to high levels of XO mosaicism in several individuals, nuclei with signal counts of zero were counted but excluded in the determination of the number of sites of hybridization.

ii

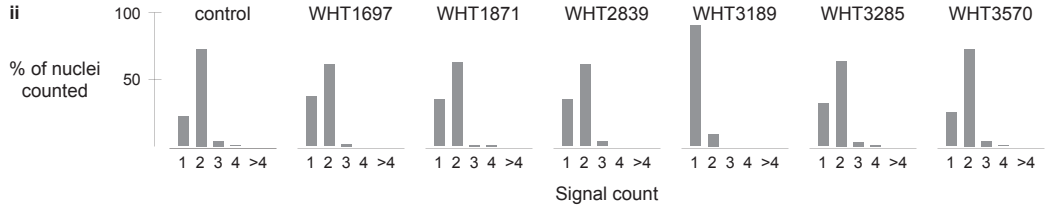


Figure S4 continued. Comprehensive results of testing the copy number, in idicYp chromosomes with breakpoints in palindrome P1, of sequences located between P1 and P5

G Summary of observed STS mapping and FISH results

	Presence or absence of each STS			Number of copies present for each probe			
	sY1291	sY1206	sY1201	1325K3	18E8	100J21	Inferred targeted repeat
control	+	+	+	1	2	2	reference
WHT1697	+	+	-	2	3	2	P1
WHT1871	+	+	-	2	3	2	P1
WHT2839	+	+	-	2	3	2	P1
WHT3189	+	+	-	2	2	1	rg/gr - P1/P2
WHT3285	+	+	-	2	3	2	P1
WHT3570	+	+	-	2	3	2	P1

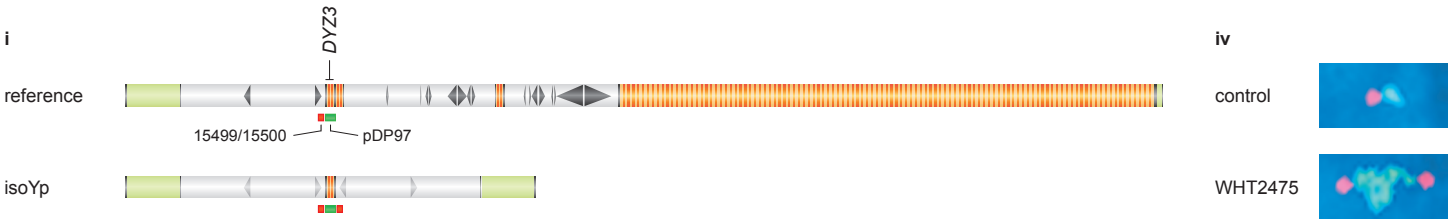
Figure S5. Comprehensive results of testing for duplication, in isoYp or idicYp chromosomes, of sequences immediately proximal to targeted heterochromatic repeats

(A-C) For heterochromatic repeats *DYZ3* (A), proximal *DYZ17* (B), and *DYZ18/DYZ1/DYZ2* (C) that, based on STS mapping, were hypothesized to have been targeted by ectopic homologous recombination in the formation of isoYp or idicYp chromosomes, we designed specific FISH assays to determine, in individuals with isoYp or idicYp chromosomes, the copy number of sequences located immediately proximal to targeted heterochromatic repeats.

In the series of figures (A and B), we show: (i) schematics of a reference chromosome and of an isoYp formed by the proposed mechanism at each heterochromatic repeat, and the locations of probes hybridized to interphase spreads; (ii) counting results tabulated for a control male and for each case tested with the probe indicated; (iii) counting results summarized graphically for each case; and (iv) representative FISH images for a control male and for an individual with an isoYp to demonstrate common FISH patterns. All images are displayed at the same magnification.

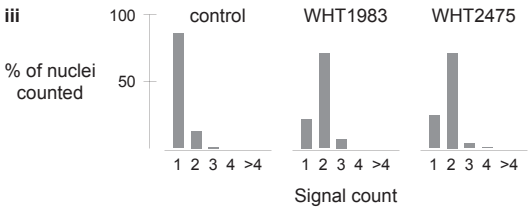
In the series of figures (C), we show: (i) schematics of a reference chromosome and of an idicYp formed by the proposed mechanism at *DYZ18/DYZ1/DYZ2*, and the locations of two probes hybridized to metaphase spreads; (ii) conclusions on the number of signals for probe 1136L22 for a control male and for each tested case; and (iii) representative FISH images for a control male and for an individual with an idicYp to demonstrate common FISH patterns. Images are at the same magnification.

A *DYZ3*

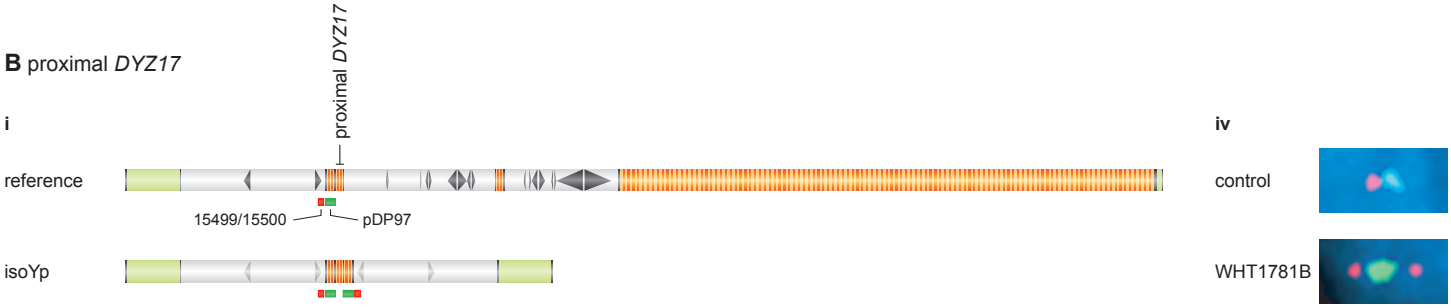


ii 15499/15500

	Number of nuclei with signal count of					Inferred number of sites of hybridization
	1	2	3	4	>4	
control	172	26	2	0	0	1
WHT1983	44	142	14	0	0	2
WHT2475	49	143	7	1	0	2



B proximal *DYZ17*



ii 15499/15500

	Number of nuclei with signal count of					Inferred number of sites of hybridization
	1	2	3	4	>4	
control	172	26	2	0	0	1
WHT1781B	125	73	2	0	0	2

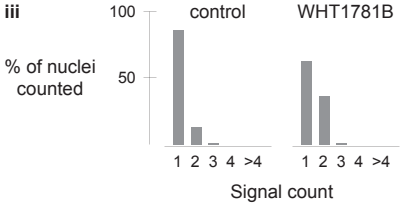
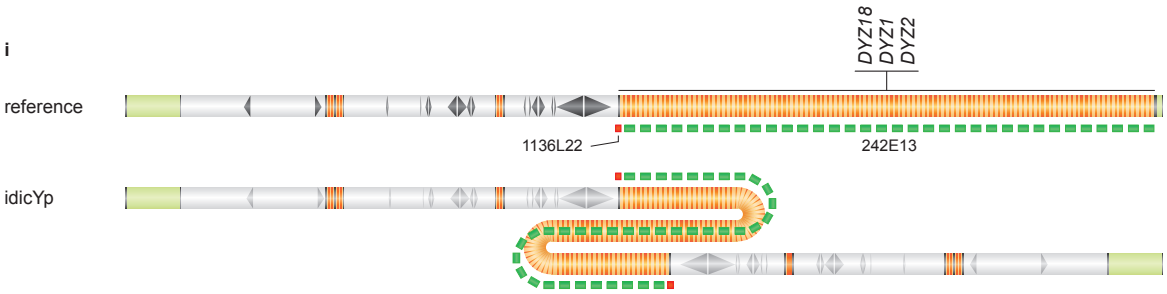


Figure S5 continued. Comprehensive results of testing for duplication, in isoYp or idicYp chromosomes, of sequences immediately proximal to targeted heterochromatic repeats

C *DYZ18/DYZ1/DYZ2*

i



ii 1136L22

	Inferred number of sites of hybridization
control	1
WHT1261	2
WHT3643	2

iii

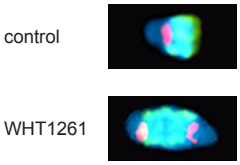


Figure S6. STS content of structurally anomalous Y chromosomes in two individuals with deletion breakpoints in the short arm (Yp)

(A) Schematic representation of Yp and centromeric region of Y chromosome, indicating locations of IR3 inverted repeat and male-determining gene *SRY*.

(B) STSs employed in fine mapping of deletion breakpoints in two individuals in whom sY14 was absent, and sY78 and sY1273 were present. These STSs include boundary and spacer-flanking markers for the IR3 inverted repeat, and a marker in Yp pericentromeric euchromatin.

(C) Results of testing genomic DNAs from two individuals for presence or absence of STSs. Solid black bars encompass STSs found to be present. Gray bars indicate breakpoint intervals within IR3's distal arm that could not be further narrowed due to cross-amplification of identical sequences in IR3's proximal arm.

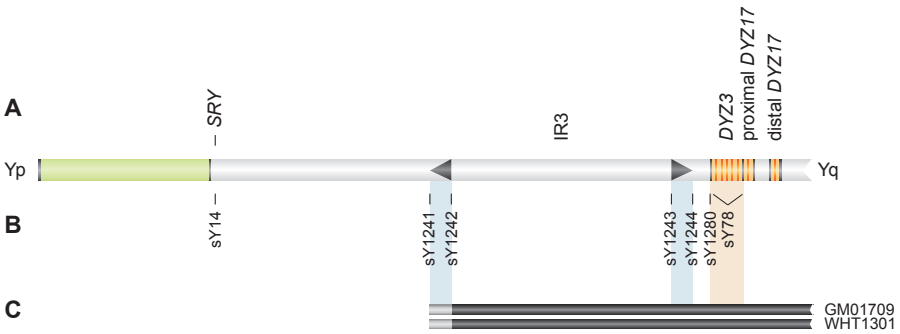
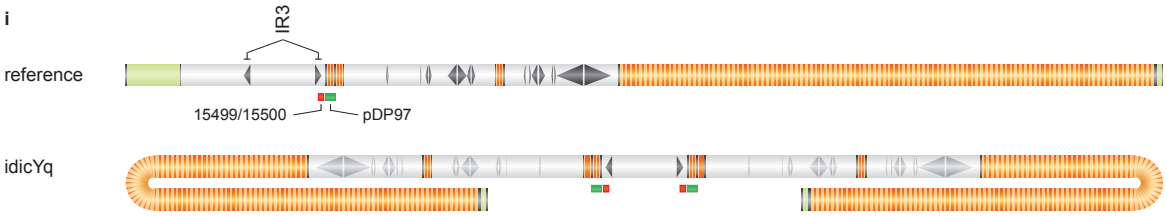
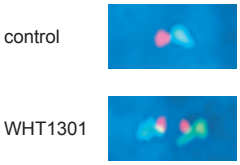


Figure S7. Comprehensive results of testing for duplication, in an idicYq, of sequences immediately proximal to Yp inverted repeat IR3

For inverted repeat IR3 that, based on STS mapping, was hypothesized to have been targeted by ectopic homologous recombination in the formation of idicYq chromosomes, we designed a specific FISH assay to determine, in an individual with an idicYq, the copy number of sequences located immediately proximal to IR3.

In the series of figures, we show: (i) schematics of a reference chromosome and of an idicYq formed by the proposed mechanism at IR3, and the locations of probes hybridized to interphase spreads; (ii) counting results tabulated for a control male and for the case tested with the probe indicated; (iii) counting results summarized graphically; and (iv) representative FISH images for a control male and for an individual with an idicYq to demonstrate common FISH patterns. Images are at the same magnification.





ii 15499/15500

	Number of nuclei with signal count of					Inferred number of sites of hybridization
	1	2	3	4	>4	
control	172	26	2	0	0	1
WHT1301	35	159	3	3	0	2

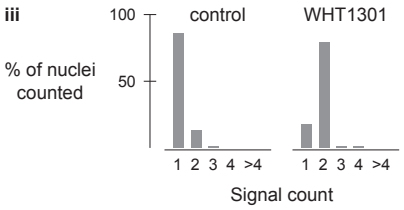
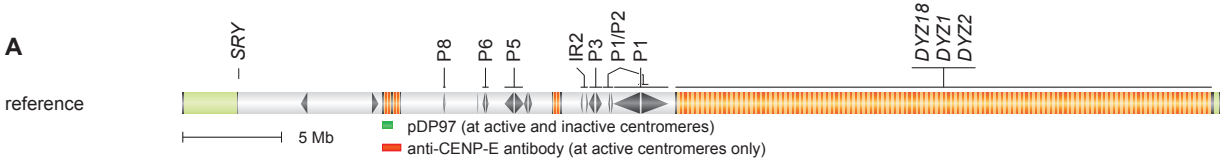
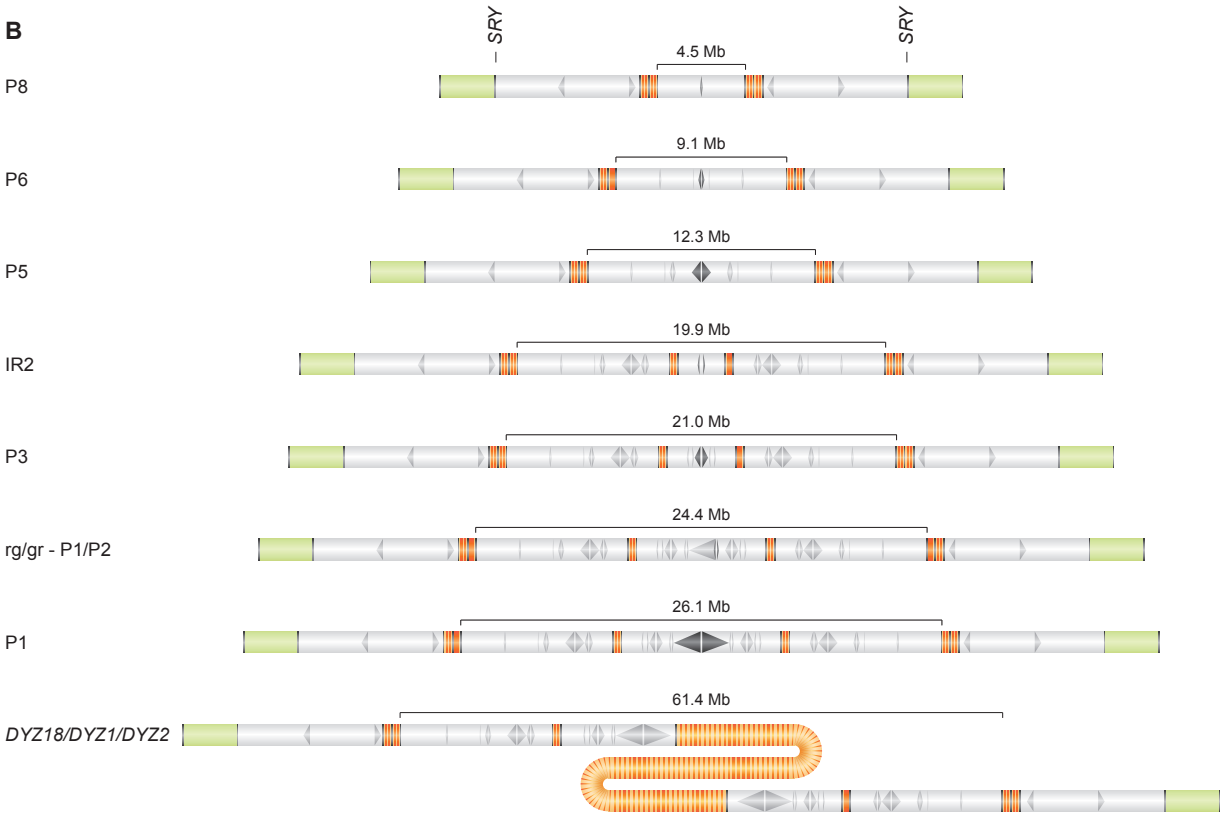


Figure S8. Results of testing centromere function in structurally dicentric (idicYp) chromosomes

(A) Schematic representation of the reference Y chromosome. FISH probe pDP97 (to centromeric alpha satellite [*DYZ3*] repeats), in green, identifies structural Y-chromosome centromeres. Anti-CENP-E antibody staining, in red, recognizes centromere protein CENP-E, which localizes exclusively to active centromeres.

(B) Summary of IF-FISH counting results for each of 13 cases tested. At left: Intercentromeric distances are shown above schematics of each type of idicYp tested. At right: Counting results of testing 13 cases for centromere activity.





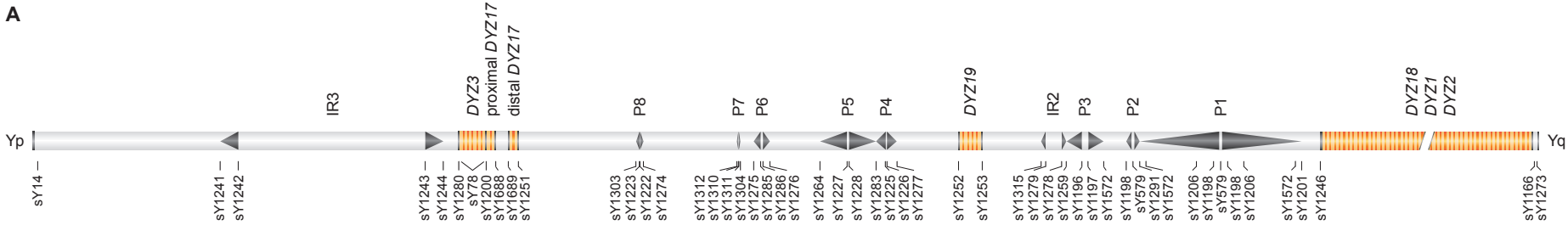
		Percent of structurally dicentric (idicYp) chromosomes that are	
	n	functionally dicentric	functionally monocentric
WHT3794	56	41%	59%
WHT3954	53	77%	23%
CO	61	64%	36%
FF	35	0%	100%
WHT3717	32	0%	100%
WHT2326	31	0%	100%
JC	30	0%	100%
WHT2240	32	0%	100%
WHT3189	32	0%	100%
WHT1697	31	0%	100%
WHT3285	32	0%	100%
WHT1261	20	0%	100%
WHT3643	32	0%	100%

Figure S9. Molecular signatures of 23 idicY and isoY chromosomes predicted to form by ectopic homologous recombination events involving MSY palindromes, inverted repeats, and highly repetitive heterochromatic regions

(A) Expected results, for each of 23 predicted idicY and isoY chromosomes formed at the targets indicated, of testing STSs that include distal Yp marker sY14 (*SRY*), centromeric marker sY78 (*DYZ3*), distal Yq marker sY1273, boundary and spacer-flanking markers for each of eight palindromes (P1-P8) and two inverted repeats (IR2 and IR3), and markers around each of five heterochromatic regions containing highly repetitive elements (*DYZ 1-3* and *DYZ17-19*).

(B) Predicted STS deletion maps below a triangular dot plot of the MSY reference sequence (see legend of Figure S1). Solid black bars encompass STSs predicted to be present. Gray bars indicate breakpoint intervals that would not be further narrowed due to cross-amplification at other loci.

A



	sY14	sY1241	sY1242	sY1243	sY1244	sY1280	sY78	sY1200	sY1688	sY1689	sY1251	sY1303	sY1223	sY1222	sY1274	sY1312	sY1310	sY1311	sY1304	sY1275	sY1285	sY1286	sY1276	sY1264	sY1227	sY1228	sY1283	sY1225	sY1226	sY1277	sY1252	sY1253	sY1315	sY1279	sY1278	sY1259	sY1196	sY1197	sY1572	sY1198	sY579	sY1291	sY1206	sY1201	sY1246	sY1166	sY1273
idicYp and isoYp chromosomes																																																
DYZ3	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
proximal *DYZ17*	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
distal *DYZ17*	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DYZ19	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
IR2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
b3/b2 - proximal P1/P3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
IR5 - proximal P1/P5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
P1.2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
rg/gr - P1/P2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
P1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
P1.1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
IR5 - distal P1/P5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
b4/b1 - distal P1/P3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
DYZ18/DYZ1/DYZ2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
idicYq and isoYq chromosomes																																															
IR3	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DYZ3	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Figure S9 continued. Molecular signatures of 23 idicY and isoY chromosomes predicted to form by ectopic homologous recombination events involving MSY palindromes, inverted repeats, and highly repetitive heterochromatic regions

B

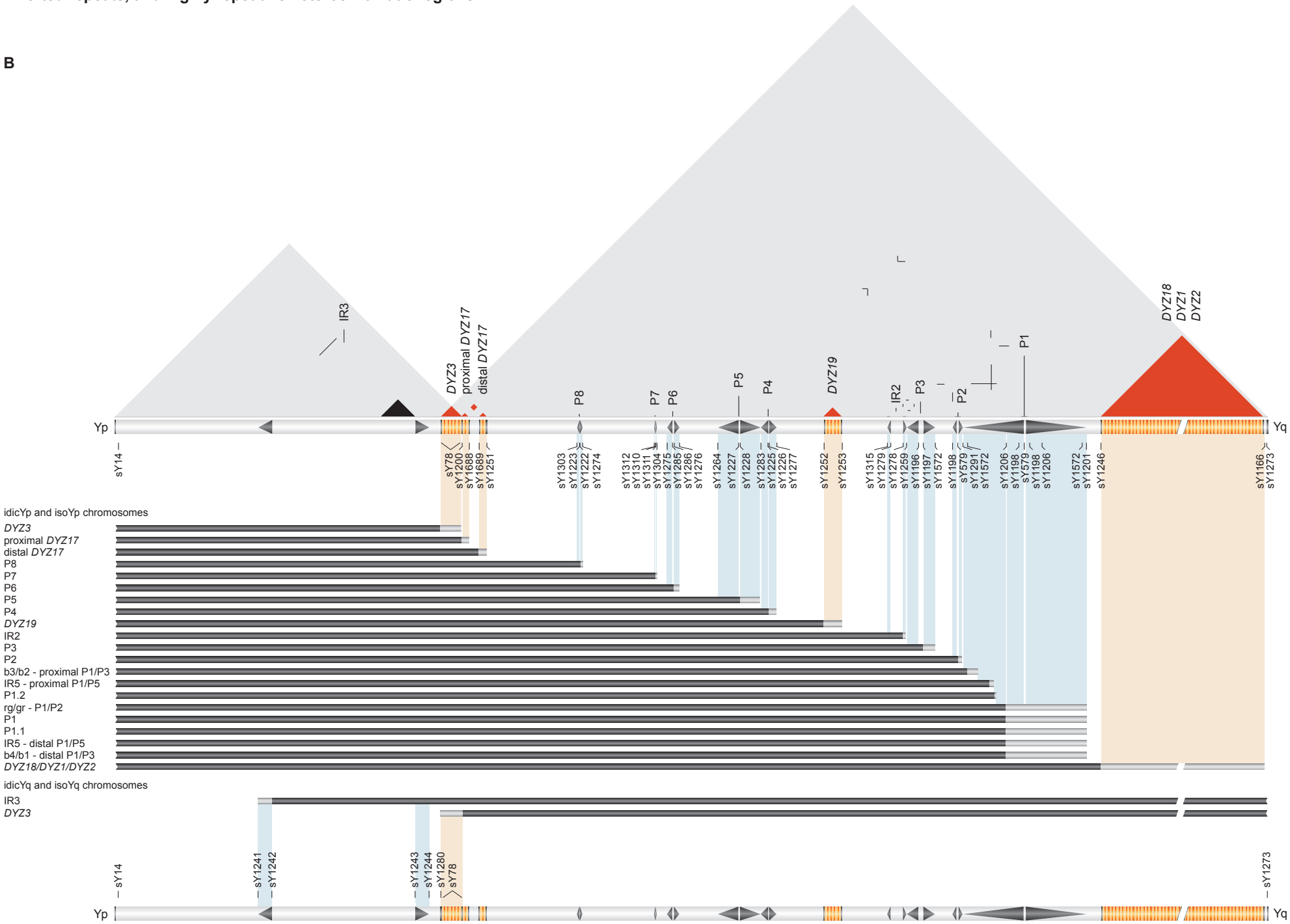


Table S1. Deletion breakpoint intervals of 85 individuals with breakpoints in the long arm (Yq) or centromere, and of two individuals with breakpoints in the short arm (Yp) of the MSY

In each table, individuals are ordered by breakpoint location in the Y chromosome. For each individual, the breakpoint interval is indicated by the most distal STS that was present and the most proximal STS that was absent.

Table S1A. Deletion breakpoint intervals of 49 individuals with breakpoints in distal arms of palindromes or inverted repeats on Yq

The 49 individuals with breakpoints in distal arms of palindromes or inverted repeats exhibit molecular signatures consistent with an idicYp formed by the proposed model. See Figures 2D and S2C for STS deletion mapping results, Table S4B for GenBank accession numbers of STSs.

Case identifier	Distal STS present	Proximal STS absent	Inferred location of breakpoint	References
WHT165	sY1222	sY1274	Distal arm of P8	
WHT1876	sY1222	sY1274	Distal arm of P8	Vollrath et al., 1992; Lahn et al., 1994
WHT3062	sY1222	sY1274	Distal arm of P8	
WHT3794	sY1222	sY1274	Distal arm of P8	
WHT3954	sY1222	sY1274	Distal arm of P8	
CO	sY1286	sY1276	Distal arm of P6	O'Reilly et al., 1992; Vollrath et al., 1992
FF	sY1228	sY1283	Distal arm of P5	O'Reilly et al., 1992; Vollrath et al., 1992
RS	sY1228	sY1283	Distal arm of P5	O'Reilly et al., 1992; Vollrath et al., 1992
WHT2024	sY1228	sY1283	Distal arm of P5	Vollrath et al., 1992
WHT2171	sY1228	sY1283	Distal arm of P5	Vollrath et al., 1992
WHT3103	sY1228	sY1283	Distal arm of P5	Silber et al., 1998
WHT3717	sY1228	sY1283	Distal arm of P5	
WHT3894	sY1228	sY1283	Distal arm of P5	
WHT4064	sY1228	sY1283	Distal arm of P5	
WHT4351	sY1228	sY1283	Distal arm of P5	
WHT4410	sY1228	sY1283	Distal arm of P5	
WHT4818	sY1228	sY1283	Distal arm of P5	
WHT5060	sY1228	sY1283	Distal arm of P5	
WHT5139	sY1228	sY1283	Distal arm of P5	
WHT495	sY1226	sY1277	Distal arm of P4	Vollrath et al., 1992
WHT1195	sY1226	sY1277	Distal arm of P4	Vollrath et al., 1992
WHT1658	sY1226	sY1277	Distal arm of P4	Vollrath et al., 1992
WHT2170	sY1226	sY1277	Distal arm of P4	Vollrath et al., 1992
WHT4093	sY1226	sY1277	Distal arm of P4	
WHT5096	sY1226	sY1277	Distal arm of P4	
WHT5108	sY1226	sY1277	Distal arm of P4	
WHT5234	sY1226	sY1277	Distal arm of P4	
WHT5643	sY1226	sY1277	Distal arm of P4	

Table S1A continued. Deletion breakpoint intervals of 49 individuals with breakpoints in distal arms of palindromes or inverted repeats on Yq

Case identifier	Distal STS present	Proximal STS absent	Inferred location of breakpoint	References
DM	sY1278	sY1259	Distal arm of IR2	O'Reilly et al., 1992; Vollrath et al., 1992; Reijo et al., 1995
JC	sY1278	sY1259	Distal arm of IR2	O'Reilly et al., 1992; Vollrath et al., 1992; Reijo et al., 1995
WHT1318	sY1278	sY1259	Distal arm of IR2	Cantrell et al., 1992; Vollrath et al., 1992; Reijo et al., 1995
WHT2326	sY1278	sY1259	Distal arm of IR2	
WHT2557	sY1278	sY1259	Distal arm of IR2	
WHT746	sY1197	sY1572	Distal arm of P3	Vollrath et al., 1992
WHT1434	sY1197	sY1572	Distal arm of P3	Vollrath et al., 1992; Reijo et al., 1995
WHT2064	sY1197	sY1572	Distal arm of P3	Vollrath et al., 1992; Reijo et al., 1995
WHT2240	sY1197	sY1572	Distal arm of P3	Vollrath et al., 1992; Reijo et al., 1995
WHT1343	sY579	sY1291	Distal arm of P2	
WHT1009	sY1206	sY1201	Distal arm of P1	
WHT1078	sY1206	sY1201	Distal arm of P1	Vollrath et al., 1992; Reijo et al., 1995
WHT1300	sY1206	sY1201	Distal arm of P1	
WHT1697	sY1206	sY1201	Distal arm of P1	
WHT1871	sY1206	sY1201	Distal arm of P1	
WHT2229	sY1206	sY1201	Distal arm of P1	Vollrath et al., 1992; Reijo et al., 1995
WHT2839	sY1206	sY1201	Distal arm of P1	
WHT3189	sY1206	sY1201	Distal arm of P1	
WHT3285	sY1206	sY1201	Distal arm of P1	
WHT3311	sY1206	sY1201	Distal arm of P1	
WHT3570	sY1206	sY1201	Distal arm of P1	

Table S1B. Deletion breakpoint intervals of six individuals with breakpoints in proximal arms of palindromes on Yq

In six cases with breakpoints in proximal arms of palindromes, breakpoints were fine-mapped with MSY Breakpoint Mapper, a database of Y-specific STSs (Lange et al., 2008). See Figures 2D and S2C for STS deletion mapping results, Table S4C for GenBank accession numbers of STSs.

Case identifier	Distal STS present	Proximal STS absent	Inferred location of breakpoint	References
WHT2063	sY595	sY1282	Proximal arm of P8	Vollrath et al., 1992
WHT1162	sY1774	sY1746	Proximal arm of P8	Goulmy et al., 1983; Vollrath et al., 1992
WHT5318	sY1235	sY828	Proximal arm of P5	
WHT1231	sY1741	sY1750	Proximal arm of P4	Vollrath et al., 1992
WHT496	sY1740	sY1742	Proximal arm of P1	Vollrath et al., 1992; Reijo et al., 1995
WHT2856[a]	sY1768	sY1770	Proximal arm of P1	

[a]The deletion in WHT2856 occurs on an inverted variant of palindromes P1 and P2 (see Repping et al., 2006).

Table S1C. Deletion breakpoint interval of one individual with a breakpoint in the spacer of Yq inverted repeat IR2

In one case with a breakpoint in the spacer of Yq inverted repeat, the breakpoint was fine-mapped with MSY Breakpoint Mapper, a database of Y-specific STSs (Lange et al., 2008). See Figures 2D and S2C for STS deletion mapping results, Table S4C for GenBank accession numbers of STSs.

Case identifier	Distal STS present	Proximal STS absent	Inferred location of breakpoint	References
WHT5557	sY3228	sY3223	In spacer of IR2	

Table S1D. Deletion breakpoint intervals of 20 individuals with breakpoints in pericentromeric euchromatin or in non-palindromic euchromatin on Yq
In 20 cases with breakpoints in pericentromeric euchromatin or non-palindromic euchromatin on Yq, breakpoints were fine-mapped with MSY Breakpoint Mapper, a database of Y-specific STSs (Lange et al., 2008). See Figures 2D and S2C for STS deletion mapping results, Table S4C for GenBank accession numbers of STSs.

Case identifier	Distal STS present	Proximal STS absent	Inferred location of breakpoint	References
WHT1923	sY1688	sY1689	Pericentromeric euchromatin	Vollrath et al., 1992
WHT2259	sY1688	sY1689	Pericentromeric euchromatin	
WHT4497	sY183	sY81	Between centromere and P8	
WHT1225	sY81	sY2303	Between centromere and P8	
WHT4481	sY629	sY630	Between centromere and P8	
WHT3814	sY1744	sY1743	Between centromere and P8	
WHT298	APXLP-5	sY2314	Between centromere and P8	
WHT5291	sY1184	UTY-28	Between centromere and P8	
WHT1874	sY1767	sY1769	Between centromere and P8	Vollrath et al., 1992
WHT5431	sY2372	sY89	Between centromere and P8	
WHT3673	sY1759	sY1762	Between centromere and P8	
WHT5392	sY1899	sY1900	Between centromere and P8	
WHT2317	sY2370	sY2382	Between P8 and P7	
WHT4490	sY1773	NLGN4Y-2	Between P8 and P7	
WHT3502	sY1753	sY1748	Between P6 and P5	
WHT3659	sY1751	sY1752	Between P4 and *DYZ19*	
WHT1832	sY1760	sY1755	Between P4 and *DYZ19*	Vollrath et al., 1992; Lahn et al., 1994
WHT1820	sY1763	sY1761	Between *DYZ19* and IR2	Vollrath et al., 1992
WHT3224	sY1572	sY1192	Between P3 and P2	
WHT3384	sY1756	sY1764	Between P3 and P2	

Table S1E. Deletion breakpoint intervals of nine individuals with breakpoints in one of four highly repetitive heterochromatic regions in the centromere or Yq and exhibiting molecular signatures consistent with an isoYp or idicYp formed by the proposed model
See Figures 2D and S2C for STS deletion mapping results, Table S4B for GenBank accession numbers of STSs.

Case identifier	Distal STS present	Proximal STS absent	Inferred location of breakpoint	References
WHT1763	sY78	sY1200	*DYZ3*	Vollrath et al., 1992
WHT1983	sY78	sY1200	*DYZ3*	Lahn et al., 1994
WHT2475	sY78	sY1200	*DYZ3*	Reijo et al., 1995; Reijo et al., 1996; Silber et al., 1998
WHT1781B	sY1200	sY1688	Proximal *DY17*	Vollrath et al., 1992
WHT2339	sY1200	sY1688	Proximal *DY17*	
WHT1826	sY1689	sY1251	Distal *DYZ17*	Vollrath et al., 1992
WHT4596	sY1689	sY1251	Distal *DYZ17*	
WHT1261	sY1246	sY1166	*DYZ18/DYZ1/DYZ2*	
WHT3643	sY1246	sY1166	*DYZ18/DYZ1/DYZ2*	

Table S1F. Deletion breakpoint intervals of two individuals with breakpoints in the Yp inverted repeat IR3 and exhibiting molecular signatures consistent with an idicYq formed by the proposed model

See Figures 5C and S6C for STS deletion mapping results, Table S4D for GenBank accession numbers of STSs.

Case identifier	Distal STS present	Proximal STS absent	Inferred location of breakpoint	References
GM01709	sY1242	sY1241	Distal arm of IR3	Aronson et al., 1983
WHT1301	sY1242	sY1241	Distal arm of IR3	Vollrath et al., 1992

Table S2. Characteristics of observed and predicted idicY and isoY chromosomes

Table S2A. IdicY and isoY chromosomes characterized in this study
As crossing over can occur in either the left or right arm of a palindrome, the target size is the sum of the two arms. Table S2B for references and additional data.

				Numbers of cases		
	Targeted repeat	Target size (kb)	IdicY or isoY size (Mb)	STS mapping consistent with our model	IdicY or isoY confirmed by SRY/cen FISH (observed/scorable)	Proposed mechanism validated by specific FISH (observed/tested)
idicYp chromosomes						
euchromatic breakpoints	P8	72	28.6	5	3/3	3/3
	P6	220	33.1	1	1/1	1/1
	P5	992	36.4	13	7/7	6/6
	P4	380	37.8	9	1/1	1/1
	IR2	124	43.9	5	3/3	3/3
	P3	566	45.0	4	3/3	3/3
	P2	244	46.9	1	not scorable	not tested
	P1	2,900	50.1	10	6/6	5/5
	P1/P2	1,116	48.5	1	1/1	1/1
			Total	49	25/25	23/23
heterochromatic breakpoints	*DYZ18/DYZ1/DYZ2*	31,700	85.4	2	2/2	2/2
			Total	2	2/2	2/2
isoYp chromosomes						
heterochromatic breakpoints	*DYZ3*	500	21.9	3	2/2	2/2
	proximal *DYZ17*	200	22.6	2	1/1	1/1
	distal *DYZ17*	200	23.9	2	not tested	not tested
			Total	7	3/3	3/3
idicYq chromosomes						
euchromatic breakpoints	IR3	596	101.4	2	1/1	1/1
			Total	2	1/1	1/1
			Grand total	**60**	**31/31**	**29/29**

Decision tree of analyzing 2,380 cases for presence of idicY and isoY chromosomes

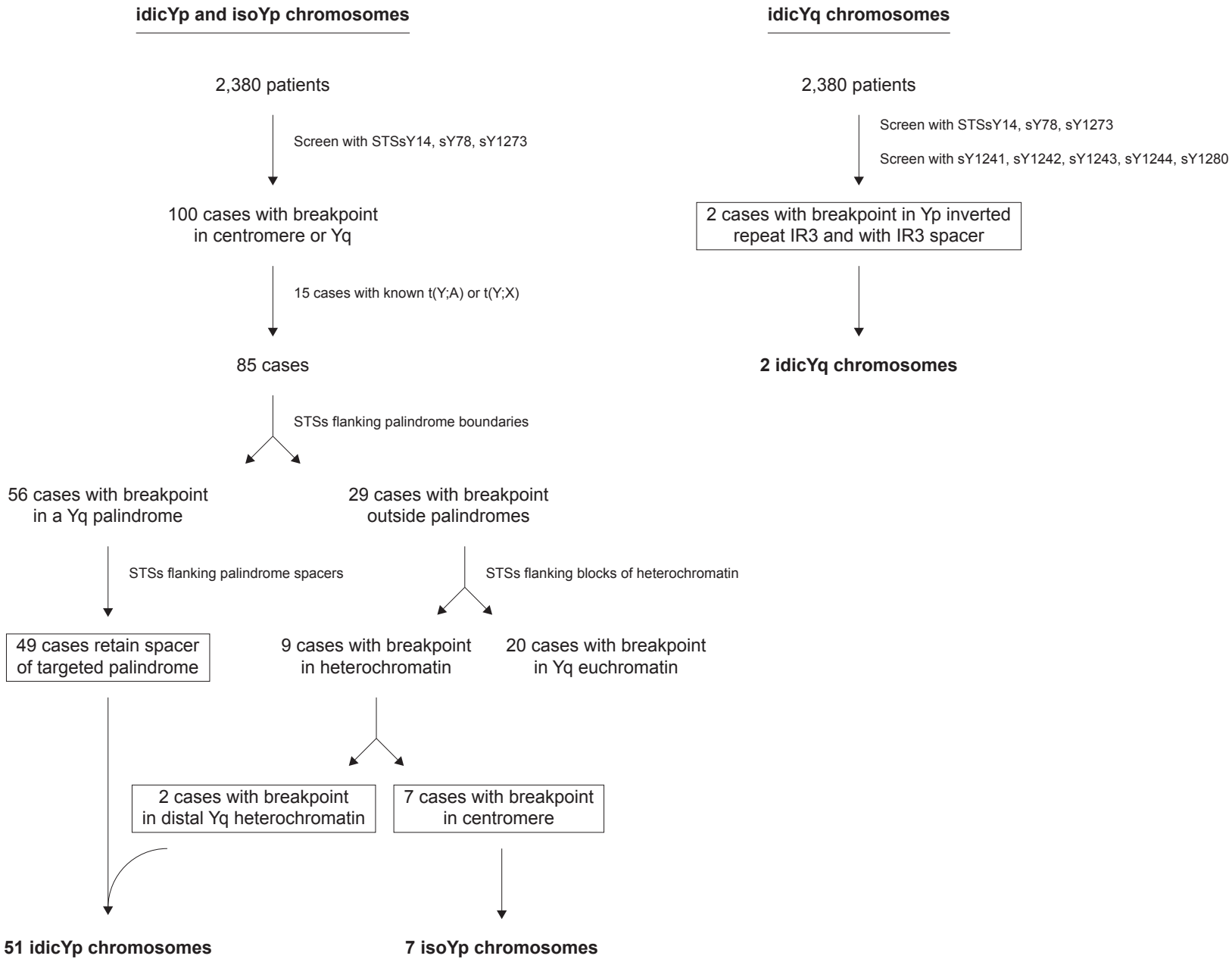


Table S2B. Target size, isochromosome size, and molecular signatures of 23 idicY and isoY chromosomes predicted to form by ectopic homologous recombination events involving MSY palindromes, inverted repeats, and highly repetitive heterochromatic regions

Target repeat sizes and isochromosome sizes are based on: euchromatic MSY sequence (Skaletsky et al., 2003) except average *TSPY* array, 673 kb (Repping et al., 2006); *DYZ3* array, 500 kb (Skaletsky et al., 2003); proximal *DYZ17* array, 200 kb, pericentromeric euchromatin, 449 kb, and distal *DYZ17* array, 200 kb (Kirsch et al., 2005); *DYZ19* array, 330 kb (our unpublished data); PAR1, 2.71, and PAR2, 335 kb (NCBI human genome assembly Build 36); estimated total Y chromosome, 59 Mb (Morton, 1991); *DYZ18/DYZ1/DYZ2* array, 31.7 Mb (calculated from referenced figures). For each isochromosome, the breakpoint interval is indicated by the most distal STS predicted to be present and the most proximal STS predicted to be absent. See Figures S9A and S9B for predicted STS deletion mapping results.

Targeted repeat	Single target size (kb)	Target size (kb)	IdicY or isoY size (Mb)	Distal STS present	Proximal STS absent
idicYp and isoYp chromosomes					
DYZ3	array	500	21.9	sY78	sY1200
proximal *DYZ17*	array	200	22.6	sY1200	sY1688
distal *DYZ17*	array	200	23.9	sY1689	sY1251
P8	36	72	28.6	sY1222	sY1274
P7	8.7	17.4	32.3	sY1311	sY1304
P6	110	220	33.1	sY1286	sY1276
P5	496	992	36.4	sY1228	sY1283
P4	190	380	37.8	sY1226	sY1277
DYZ19	array	330	40.9	sY1252	sY1253
IR2	62	124	43.9	sY1278	sY1259
P3	283	566	45.0	sY1197	sY1572
P2	122	244	46.9	sY579	sY1291
b3/b2 - proximal P1/P3	229	458	46.2	sY1291	sY1206
IR5 - proximal P1/P5	131	262	42.5	sY1291	sY1206
P1.2	9.9	18.8	48.7	sY1291	sY1206
rg/gr - P1/P2	558	1,116	48.5	sY1206	sY1201
P1	1,450	2,900	50.1	sY1206	sY1201
P1.1	9.9	18.8	51.5	sY1206	sY1201
IR5 - distal P1/P5	131	262	44.0	sY1206	sY1201
b4/b1 - distal P1/P3	168	336	48.6	sY1206	sY1201
DYZ18/DYZ1/DYZ2	array	31,700	85.4	sY1246	sY1166
idicYq and isoYq chromosomes					
IR3	298	596	101.4	sY1242	sY1241
DYZ3	array	500	96.1	sY78	sY1280

Table S3. Y chromosome gene content and copy number of 23 idicY and isoY chromosomes predicted to form by ectopic homologous recombination events involving MSY palindromes, inverted repeats, and highly repetitive heterochromatic regions

Based on euchromatic MSY sequence (Skaletsky et al., 2003) except average number of *TSPY* (Repping et al., 2006). See Figures 9A and 9B for additional information.

	Copy number per gene																											
Targeted repeat	PAR1 genes	*SRY*	*RPS4Y1*	*ZFY*	*TGIF2LY*	*PCDH11Y*	*TSPY*	*AMELY*	*TBL1Y*	*PRKY*	*USP9Y*	*DDX3Y*	*UTY*	*TMSB4Y*	*VCY*	*NLGN4Y*	*XKRY*	*CDY*	*HSFY*	*CYorf15A*	*CYorf15B*	*JARID1D*	*EIF1AY*	*RPS4Y2*	*RBMY1*	*PRY*	*BPY2*	*DAZ*
Reference	1	1	1	1	1	1	33	1	1	1	1	1	1	1	2	1	2	4	2	1	1	1	1	1	6	2	3	4
idicYp and isoYp chromosomes																												
DYZ3	2	2	2	2	2	2	66	2	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
proximal *DYZ17*	2	2	2	2	2	2	66	2	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
distal *DYZ17*	2	2	2	2	2	2	66	2	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P8	2	2	2	2	2	2	66	2	2	2	2	2	2	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0
P7	2	2	2	2	2	2	66	2	2	2	2	2	2	2	4	2	0	0	0	0	0	0	0	0	0	0	0	0
P6	2	2	2	2	2	2	66	2	2	2	2	2	2	2	4	2	0	0	0	0	0	0	0	0	0	0	0	0
P5	2	2	2	2	2	2	66	2	2	2	2	2	2	2	4	2	2	2	0	0	0	0	0	0	0	0	0	0
P4	2	2	2	2	2	2	66	2	2	2	2	2	2	2	4	2	4	4	2	0	0	0	0	0	0	0	0	0
DYZ19	2	2	2	2	2	2	66	2	2	2	2	2	2	2	4	2	4	4	4	2	2	2	0	0	0	0	0	0
IR2	2	2	2	2	2	2	66	2	2	2	2	2	2	2	4	2	4	4	4	2	2	2	2	2	4	0	0	0
P3	2	2	2	2	2	2	66	2	2	2	2	2	2	2	4	2	4	4	4	2	2	2	2	2	10	2	0	0
P2	2	2	2	2	2	2	66	2	2	2	2	2	2	2	4	2	4	4	4	2	2	2	2	2	12	4	2	2
b3/b2 - proximal P1/P3	2	2	2	2	2	2	66	2	2	2	2	2	2	2	4	2	4	4	4	2	2	2	2	2	12	4	1	2
IR5 - proximal P1/P5	2	2	2	2	2	2	66	2	2	2	2	2	2	2	4	2	3	4	2	1	1	1	1	1	6	2	1	2
P1.2	2	2	2	2	2	2	66	2	2	2	2	2	2	2	4	2	4	6	4	2	2	2	2	2	12	4	2	4
rg/gr - P1/P2	2	2	2	2	2	2	66	2	2	2	2	2	2	2	4	2	4	5	4	2	2	2	2	2	12	4	3	4
P1	2	2	2	2	2	2	66	2	2	2	2	2	2	2	4	2	4	6	4	2	2	2	2	2	12	4	4	6
P1.1	2	2	2	2	2	2	66	2	2	2	2	2	2	2	4	2	4	6	4	2	2	2	2	2	12	4	6	8
IR5 - distal P1/P5	2	2	2	2	2	2	66	2	2	2	2	2	2	2	4	2	3	4	2	1	1	1	1	1	6	2	3	4
b4/b1 - distal P1/P3	2	2	2	2	2	2	66	2	2	2	2	2	2	2	4	2	4	6	4	2	2	2	2	2	10	2	3	4
DYZ18/DYZ1/DYZ2	2	2	2	2	2	2	66	2	2	2	2	2	2	2	4	2	4	8	4	2	2	2	2	2	12	4	6	8
idicYq and isoYq chromosomes																												
IR3	0	0	0	0	0	0	33	1	1	1	2	2	2	2	4	2	4	8	2	2	2	2	2	2	12	4	6	8
DYZ3	0	0	0	0	0	0	0	0	0	0	2	2	2	2	4	2	4	8	2	2	2	2	2	2	12	4	6	8

Table S4. STSs used in the analysis of Y chromosome content
In each table, STSs are ordered by location in the Y chromosome, from distal Yp to centromere on the short arm (Yp), and centromere to distal Yq on the long arm (Yq).

Table S4A. Three STSs used in initial screening of 2,380 individuals for deletions in Yq
See Figure 2A for locations of STSs.

STS	GenBank Accession	Location in Y chromosome
sY14	G38356	Distal Yp gene *SRY*
sY78	G38359	*DYZ3* array in centromere
sY1273	G75500	Distal to *DYZ18/DYZ1/DYZ2* at boundary of PAR2

Table S4B. Landmark STSs used to determine Y chromosome content in individuals in whom distal Yp STS sY14 and centromeric STS sY78 were present, and distal Yq STS sY1273 was absent
See Figures 2C, S2B, and S4B for locations of STSs.

STS	GenBank Accession	Location in Y chromosome
sY78	G38359	*DYZ3* array in centromere
sY1200	G75479	Transition between centromeric *DYZ3* array and proximal *DYZ17* array
sY1688	BV448840	Transition between proximal *DYZ17* array and pericentromeric euchromatin
sY1689	BV448841	Transition between pericentromeric euchromatin and distal *DYZ17* array
sY1251	G75496	Transition between distal *DYZ17* and euchromatic Yq
sY1303	G75514	P8 proximal boundary
sY1223	G75485	P8 proximal flank of spacer
sY1222	G73595	P8 distal flank of spacer
sY1274	G75501	P8 distal boundary
sY1312	G73594	P7 proximal boundary
sY1310	G73592	P7 proximal flank of spacer
sY1311	G73593	P7 distal flank of spacer
sY1304	G73586	P7 distal boundary
sY1275	G75502	P6 proximal boundary
sY1285	G73584	P6 proximal flank of spacer
sY1286	G73585	P6 distal flank of spacer
sY1276	G75503	P6 distal boundary
sY1264	G72346	P5 proximal boundary
sY1227	G72343	P5 proximal flank of spacer
sY1228	G72344	P5 distal flank of spacer
sY1283	G75508	P5 distal boundary, P4 proximal boundary
sY1225	G73582	P4 proximal flank of spacer
sY1226	G73583	P4 distal flank of spacer
sY1277	BV703587	P4 distal boundary
sY1252	G75497	*DYZ19* proximal boundary
sY1253	G75498	*DYZ19* distal boundary
sY1315	G75515	IR2 proximal boundary
sY1279	G75505	IR2 proximal flank of spacer
sY1278	G75504	IR2 distal flank of spacer
sY1259	BV444812	IR2 distal boundary, P3 proximal boundary
sY1196	G67167	P3 proximal flank of spacer
sY1197	G67168	P3 distal flank of spacer
sY1572	BV444813	P3 distal boundary
sY1198	G67169	Boundary between green and red amplicons in P2 and P1
sY579	G63909	Spacer of P2 and P1
sY1291	G72340	P2 distal boundary, P1 proximal boundary
sY1206	G67171	Boundary between yellow and green amplicons in P1
sY1201	G67170	P1 distal boundary
sY1246	G75492	Proximal boundary between Yq euchromatin and *DYZ18/DYZ1/DYZ2*
sY1166	G66149	Y-specific sequence distal to *DYZ18/DYZ1/DYZ2*
sY1273	G75500	Distal to *DYZ18/DYZ1/DYZ2* at boundary of PAR2

Table S4C. STSs that define breakpoint intervals in individuals with breakpoints in proximal arms of palindromes, in the spacer of inverted repeat IR2, in pericentromeric euchromatin, or in non-palindromic euchromatin on Yq

See MSY Breakpoint Mapper (Lange et al., 2008) for locations of STSs.

STS	GenBank Accession	Location in Y chromosome
sY1688	BV448840	Transition between proximal *DYZ17* array and pericentromeric euchromatin
sY1689	BV448841	Transition between pericentromeric euchromatin and distal *DYZ17* array
sY183	G66624	Between centromere and P8
sY81	G66519	Between centromere and P8
sY2303	G66185	Between centromere and P8
sY629	G65852	Between centromere and P8
sY630	G49201	Between centromere and P8
sY1744	BV682275	Between centromere and P8
sY1743	BV682274	Between centromere and P8
APXLP-5	BV678979	Between centromere and P8
sY2314	G66193	Between centromere and P8
sY1184	G64731	Between centromere and P8
UTY-28	BV979197	Between centromere and P8
sY1767	BV682298	Between centromere and P8
sY1769	BV682300	Between centromere and P8
sY2372	G66221	Between centromere and P8
sY89	G66625	Between centromere and P8
sY1759	BV682290	Between centromere and P8
sY1762	BV682293	Between centromere and P8
sY1899	BV694008	Between centromere and P8
sY1900	BV694009	Between centromere and P8
sY595	G34985	Arms of P8
sY1282	BV681875	Arms of P8
sY1774	BV682305	Arms of P8
sY1746	BV682277	Arms of P8
sY2370	G66219	Between P8 and P7
sY2382	G66229	Between P8 and P7
sY1773	BV682304	Between P8 and P7
NLGN4Y-2	BV679041	Between P8 and P7
sY1753	BV682284	Between P7 and P6
sY1748	BV682279	Between P7 and P6
sY1235	BV210874	Arms of P5
sY828	BV703529	Arms of P5
sY1741	BV682272	Arms of P4
sY1750	BV682281	Arms of P4
sY1751	BV682282	Between P4 and *DYZ19*
sY1752	BV682283	Between P4 and *DYZ19*
sY1760	BV682291	Between P4 and *DYZ19*
sY1755	BV682286	Between P4 and *DYZ19*
sY1763	BV682294	Between *DYZ19* and IR2
sY1761	BV682292	Between *DYZ19* and IR2
sY3228	BV704093	In spacer of IR2
sY3223	BV704088	In spacer of IR2
sY1572	BV444813	P3 distal boundary

Table S4C continued. STSs that define breakpoint intervals in individuals with breakpoints in proximal arms of palindromes, in pericentromeric euchromatin, or in non-palindromic euchromatin on Yq

STS	GenBank Accession	Location in Y chromosome
sY1192	G67166	Unique sequence between P3 and green amplicon g1
sY1756	BV682287	Green amplicons
sY1764	BV682295	Green amplicons
sY1740	BV682271	Yellow amplicons
sY1742	BV682273	Yellow amplicons
sY1768	BV682299	Yellow amplicons
sY1770	BV682301	Yellow amplicons

Table S4D. Landmark STSs used to determine Y chromosome content in individuals in whom distal Yp STS sY14 was absent, and centromeric STS sY78 and distal Yq STS sY1273 were present
See Figures 5B and S6B for locations of STSs.

STS	GenBank Accession	Location in Y chromosome
sY14	G38356	Distal Yp in male-determining gene *SRY*
sY1241	G75487	IR3 distal boundary
sY1242	G75488	IR3 distal spacer flank
sY1243	G75489	IR3 proximal spacer flank
sY1244	G75490	IR3 proximal boundary
sY1280	G75506	Yp pericentromeric region
sY78	G38359	*DYZ3* array in centromere

Table S4E. STS used to amplify and sequence coding region of male-determining gene *SRY* in females carrying idicYp chromosomes

STS	GenBank Accession	Location in Y chromosome
SRY-1-6	BV679100	Distal Yp encompassing male-determining gene *SRY*

Table S5. Metaphase and interphase FISH assays of Y chromosome structure in individuals with breakpoints in the MSY

Table S5A. Metaphase FISH assays of Y chromosome structure
See Table S5B for information on probes.

Aim	Assay	Results
Detection of idicYp or isoYp chromosomes in individuals positive for sY14 and sY78, and negative for sY1273	Visualization of chromosome structure by co-hybridization of pDP1335 and pDP97	Figure 3
Detection of idicYq in an individual positive for sY78 and sY1273, and negative for sY14	Visualization of chromosome structure by co-hybridization of 242E11 and pDP97	Figure 5D

Table S5B. Probes used in metaphase FISH assays of Y chromosome structure
See Figures 3 and 5D for probe hybridization sites.

Probe	Hybridization site in reference Y chromosome	Source of DNA
pDP1335	*SRY* on distal Yp	Plasmid
pDP97	*DYZ3* elements in centromere	Plasmid
242E13	*DYZ1* elements in distal Yq heterochromatin	RPCI-11 BAC

Table S5C. Interphase and metaphase FISH assays of copy number of specific sequences in isochromosomes, to more precisely determine the sites of recombination leading to isochromosome formation
See Table S5D for information on probes.

Targeted repeat	Assay	Results
P8	Counting of 217J19 on interphase spreads	Figure S3A
P6	Counting of 15907/15908 on interphase spreads	Figure S3B
P5	Counting of 15485/15486 on interphase spreads	Figures 4B, 4C, and S3C
P4	Counting of 16053/16054 on interphase spreads	Figure S3D
IR2	Counting of 15469/15470 on interphase spreads	Figures 4B, 4D, and S3E
P3	Counting of 15469/15470 on interphase spreads	Figure S3F
P1	Counting of 1325K3 on interphase spreads	Figure S4D
P1	Counting of 18E8 on interphase spreads	Figure S4E
P1	Counting of 100J21 on interphase spreads	Figure S4F
DYZ3	Counting of 15499/15500 in co-hybridization with pDP97 on interphase spreads	Figure S5A
proximal *DYZ17*	Counting of 15499/15500 in co-hybridization with pDP97 on interphase spreads	Figure S5B
DYZ18/DYZ1/DYZ2	Visualization of copy number of 1136L22 in co-hybridization with 242E13 on metaphase spreads	Figures 4B, 4E, and S5C
IR3	Counting of 15499/15500 in co-hybridization with pDP97 on interphase spreads	Figures 5E and S7

Table S5D. Probes used in interphase and metaphase FISH assays of copy number of specific sequences in isochromosomes, to more precisely determine the sites of recombination leading to isochromosome formation
See Figures 4, 5E, S3, S4, S5, and S7 for probe hybridization sites.

Probe	Hybridization site in reference Y chromosome	Source of DNA
217J19	1 Mb proximal to P8	RPCI-11 BAC
15907/15908	2.5 kb proximal to P6	Long-range PCR[c]
15485/15486	44 kb proximal to P5	Long-range PCR[c]
16053/16054	9.7 kb and 477 kb proximal to P4[a]	Long-range PCR[c]
15469/15470	208 kb proximal to IR2, 581 kb proximal to P3	Long-range PCR[c]
1325K3	*DYZ19* elements in Yq	RPCI-11 BAC
18E8	Red amplicons in P1 and P2	Cosmid
100J21	Yellow amplicons in P1	RPCI-11 BAC
15499/15500	Proximal Yp between centromere and proximal arm of IR3[b]	Long-range PCR[c]
pDP97	*DYZ3* elements in centromere	Plasmid
1136L22	Spans proximal boundary between euchromatic Yq and *DYZ18/DYZ1/DYZ2* heterochromatin	RPCI-11 BAC
242E13	*DYZ1* elements *DYZ18/DYZ1/DYZ2* heterochromatin	RPCI-11 BAC

[a] In the arms of palindrome P5, 9.7 kb from the end of each arm
[b] 280 kb from *DYZ3* elements in centromere, 28 kb proximal to IR3
[c] See Table S5E for primer sequences and amplification protocol for synthesis of FISH probes by long-range PCR

Table S5E. Primers and amplification protocol for long-range PCR production of specific FISH probes

Left primer	Sequence	Right primer	Sequence	RPCI-11 BAC template	Product size (bp)
15907	GTGTGCATCAGGATTCATGGAGACAGAA	15908	TGTTTGGTTAGACACACCCTTTGCAAATTC	455E3	10,468
15485	GCTACACCCTTTGTGGAGAATTTCGGAC	15486	AGAAGGCTAATCTTCAAGGGTGGTGCAG	537C24	10,582
16053	ACACCACCTGAGGTCAGGACTTTGAGAC	16054	AGAAAATAGGATGTGTGCAGCCAACCAG	529I21	10,324
15469	CTTTTCCTGCCATTGCTTTTGGTGTTTT	15470	CAAGGGAGCCTTGATCAGCACTTTTCTT	209I11	10,446
15499	TTTCCGCACATTCTCCTAGCAAATCTCA	15500	GGCTGACAGAAGGGAGAAAATGAAAGGA	155J5	10,395

Long-range PCR was performed using Advantage 2 Taq polymerase (BD Biosciences) according to the manufacturer's instructions. Each primer was at 1 uM final concentration. For a 100 ul reaction, template DNA was either 20 uL of a 1/10 dilution of an overnight BAC innoculant or 50 ng of extracted BAC DNA. Amplification conditions were 95°C for 1 minute; 30 cycles of 95°C for 30 seconds, 68°C for 10 minutes; 68°C for 10 minutes.

Supplemental References

Aronson, M.M., Nichols, W.W., Mulivor, R.A., Greene, A.E., and Coriell, L.L. (1983). Chromosome maps of cell lines with specific monosomic or trisomic portions of the genome in the NIGMS Human Genetic Mutant Cell Repository. Cytogenet. Cell Genet. *36*, 652-658.

Cantrell, M.A., Bogan, J.S., Simpson, E., Bicknell, J.N., Goulmy, E., Chandler, P., Pagon, R.A., Walker, D.C., Thuline, H.C., Graham, J.M., Jr., et al. (1992). Deletion mapping of H-Y antigen to the long arm of the human Y chromosome. Genomics *13*, 1255-1260.

Goulmy, E., van Leeuwen, A., Blokland, E., Sachs, E.S., and Geraedts, J.P. (1983). The recognition of abnormal sex chromosome constitution by HLA-restricted anti-H-Y cytotoxic T cells and antibody. Immunogenetics *17*, 523-531.

Harrington, J.J., Van Bokkelen, G., Mays, R.W., Gustashaw, K., and Willard, H.F. (1997). Formation of de novo centromeres and construction of first-generation human artificial microchromosomes. Nat. Genet. *15*, 345-355.

Kirsch, S., Weiss, B., Miner, T.L., Waterston, R.H., Clark, R.A., Eichler, E.E., Munch, C., Schempp, W., and Rappold, G. (2005). Interchromosomal segmental duplications of the pericentromeric region on the human Y chromosome. Genome Res. *15*, 195-204.

Lahn, B.T., Ma, N., Breg, W.R., Stratton, R., Surti, U., and Page, D.C. (1994). Xq-Yq interchange resulting in supernormal X-linked gene expression in severely retarded males with 46,XYq- karyotype. Nat. Genet. *8*, 243-250.

Lange, J., Skaletsky, H., Bell, G.W., and Page, D.C. (2008). MSY Breakpoint Mapper, a database of sequence-tagged sites useful in defining naturally occurring deletions in the human Y chromosome. Nucleic Acids Res. *36*, D809-814.

Morton, N.E. (1991). Parameters of the human genome. Proc. Natl. Acad. Sci. USA *88*, 7474-7476.

O'Reilly, A.J., Affara, N.A., Simpson, E., Chandler, P., Goulmy, E., and Ferguson-Smith, M.A. (1992). A molecular deletion map of the Y chromosome long arm defining X and autosomal homologous regions and the localisation of the HYA locus to the proximal region of the Yq euchromatin. Hum. Mol. Genet. *1*, 379-385.

Reijo, R., Lee, T.Y., Salo, P., Alagappan, R., Brown, L.G., Rosenberg, M., Rozen, S., Jaffe, T., Straus, D., Hovatta, O., et al. (1995). Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. Nat. Genet. *10*, 383-393.

Reijo, R., Alagappan, R.K., Patrizio, P., and Page, D.C. (1996). Severe oligozoospermia resulting from deletions of azoospermia factor gene on Y chromosome. Lancet *347*, 1290-1293.

Repping, S., van Daalen, S.K., Brown, L.G., Korver, C.M., Lange, J., Marszalek, J.D., Pyntikova, T., van der Veen, F., Skaletsky, H., Page, D.C., et al. (2006). High mutation rates have driven extensive structural polymorphism among human Y chromosomes. Nat. Genet. *38*, 463-467.

Silber, S.J., Alagappan, R., Brown, L.G., and Page, D.C. (1998). Y chromosome deletions in azoospermic and severely oligozoospermic men undergoing intracytoplasmic sperm injection after testicular sperm extraction. Hum. Reprod. *13*, 3332-3337.

Skaletsky, H., Kuroda-Kawaguchi, T., Minx, P.J., Cordum, H.S., Hillier, L., Brown, L.G., Repping, S., Pyntikova, T., Ali, J., Bieri, T., et al. (2003). The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. Nature *423*, 825-837.

Vollrath, D., Foote, S., Hilton, A., Brown, L.G., Beer-Romero, P., Bogan, J.S., and Page, D.C. (1992). The human Y chromosome: a 43-interval map based on naturally occurring deletions. Science *258*, 52-59.