

Supplementary Tables

Alignments of CHORI-251 and PTB1 sequences (all alignments can be found at the following URL: <http://jura.wi.mit.edu/page>)

The sequences were aligned using ClustalW. The alignments were visually inspected for accuracy and manually adjusted. The alignment file (<http://jura.wi.mit.edu/page>) contains eight separate alignments. Alignments 1-6 are from the X-degenerate region and alignments 7 and 8 are from the non-X-degenerate, single-copy sequence (Supplementary Table 1). The assembly from the previous study¹ contains a small amount of sequence from the RPCI-43 library, so these regions were excluded (regions between alignments 1 and 2, between alignments 2 and 3, and between alignments 7 and 8). Also excluded from the alignment were all palindromes (P6, P7, P8, and CSP) as well as the pseudoautosomal region (PAR). We feel that inclusion of these sequences does not give the most accurate estimate of Y chromosome nucleotide divergence. However, inclusion of these sequences does not dramatically affect the estimate of overall percent divergence. Under no set of circumstances does the value approach the level of 0.0422% calculated by Kuroki *et al.*² (Supplementary Tables 2 and 3).

Supplementary Table 1. CHORI-251 – PTB1 Alignments 1-8

Alignment	Sequence	CHORI-251		PTB1	
		Start BAC	End BAC	Start BAC	End BAC
1	X-degenerate	375D06	223P03	620F03	253H05
RPCI-43 sequence					
2	X-degenerate	228G02	228G02	183L08	548J12
RPCI-43 sequence					
3 ¹	X-degenerate	230I19	417F24	548J12	005K16
P8					
4	X-degenerate	267E02	450H03	520M19	100K06
P7					
5	X-degenerate	072D14	557M20	087E03	085B05
P6					
6	X-degenerate	299B14	490L21	224I01	461C11
Gap					
7	Non-X-degen., single-copy	346F02	559G23	547B05	519P18
RPCI-43 sequence					
8	Non-X-degen., single-copy	014P15	267E22	519P18	015J14

¹ Alignment 3 spans a region in the CHORI-251 sequence that contains a gap of approximately 14,000 bp.

Supplementary Table 2. Nucleotide divergence between CHORI-251 and PTB1 in all sequence classes – single-copy, palindrome, and pseudoautosomal region (PAR)

Sequence in alignment	Sites	Substitutions	Percent divergence
X-degenerate	7642771	169	0.0022
Non X-degenerate, single copy	822637	9	0.0011
P6 + P7 + P8(partial) ¹	860384	86	0.0099
CSP	392481	11	0.0028
PAR	132131	176	0.1332

¹Both the CHORI-251 and PTB1 sequences contain gaps in the middle of palindrome P8.

Supplementary Table 3. Nucleotide divergence between CHORI-251 and PTB1, with and without palindromes and pseudoautosomal region (PAR) in alignments

Sequence in alignment	Percent divergence
X-degenerate + non-X-degen., single copy	0.0021
With P6 + P7 + P8(partial) ¹	0.0028
With CSP	0.0021
With PAR	0.0041
All sequence	0.0046

¹Both the CHORI-251 and PTB1 sequences contain gaps in the middle of palindrome P8.

1. Hughes, J. F. et al. Conservation of Y-linked genes during human evolution revealed by comparative sequencing in chimpanzee. *Nature* **437**, 100-3 (2005).
2. Kuroki, Y. et al. Comparative analysis of chimpanzee and human Y chromosomes unveils complex evolutionary pathway. *Nat Genet* (2006).