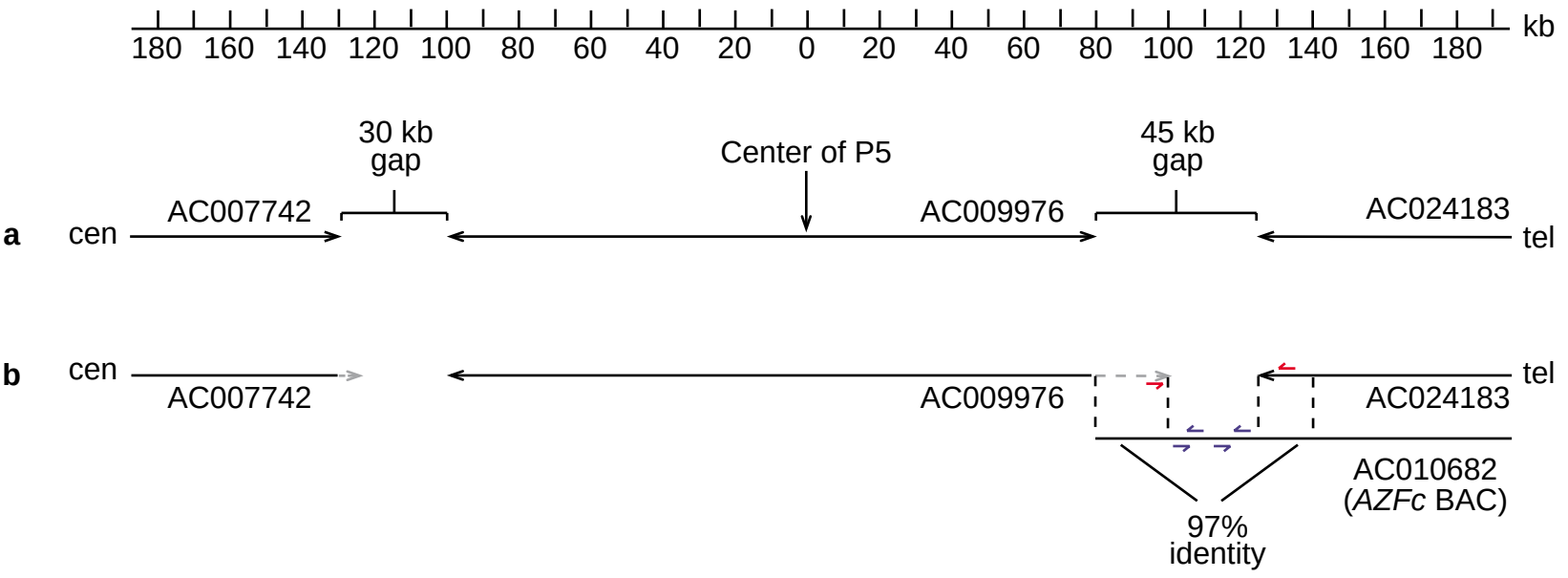
Supplementary Figure 11

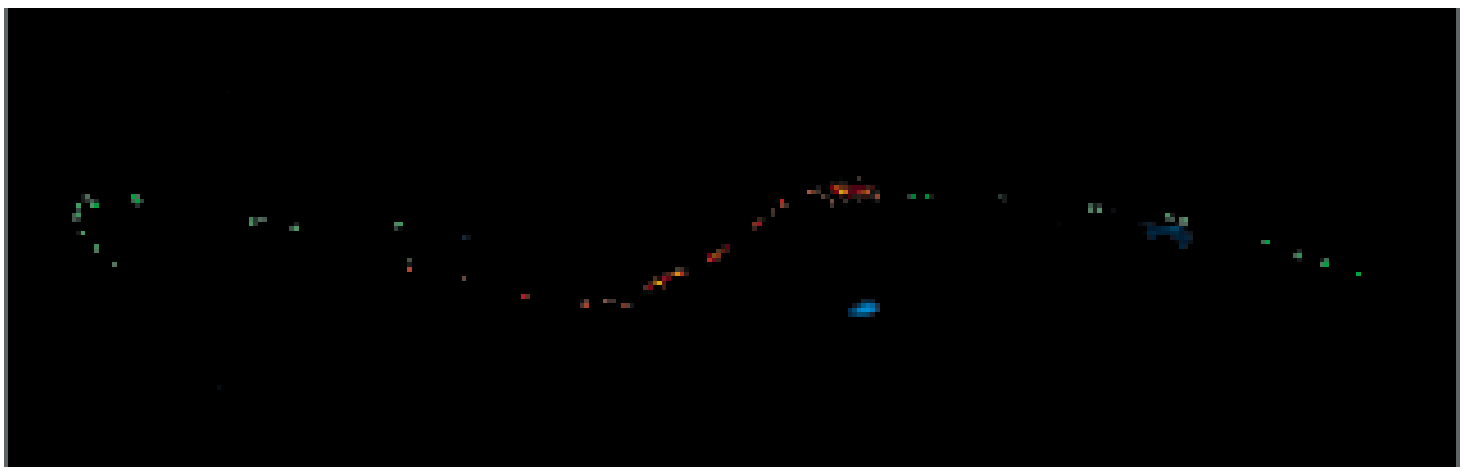
Closing map gaps in MSY palindrome P5



a, Clone map of the central region of palindrome P5, including the spacer and inner palindrome arms, showing two gaps. These gaps occur in essentially identical sequence in the two arms of P5. The scale at top gives positions relative to the center of P5.

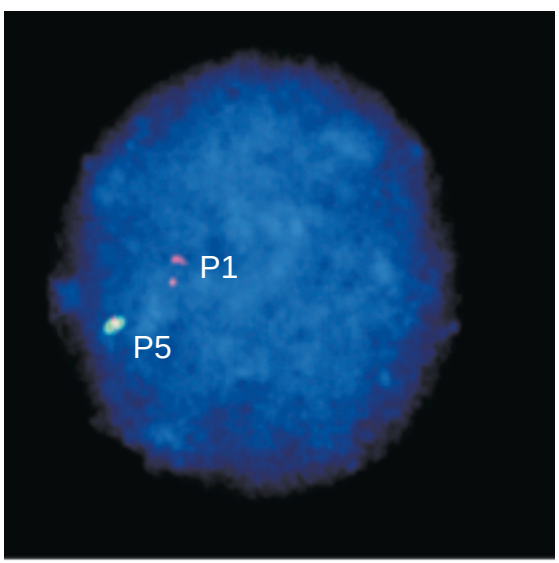
b, Obtaining sequence from the P5 clone gaps. Chromosomal FISH had provided evidence for the symmetry of the inner parts of the arms of P5, which contain the gaps (see panels c and d, below), and all available evidence indicates that the entire sequences of the arms of P5 are essentially identical (including the gaps). Therefore we closed these gaps by sequencing three overlapping long-range PCR products, each of which contains DNA coamplified from sequence representing both gaps. Because the orientation of AC009976 is arbitrary (and probably polymorphic) we designed one primer (red) from its proximal (sic) end. An additional primer (red) was designed from the proximal end of AC024183, and four other primers (blue) were designed from 97%-identical sequence from BAC AC010682, which represents the AZFc region, several Mb distant. To avoid amplifying homologous sequences from AZFc, as a template for PCR we used DNA from Y chromosomes deleted for this region [patients WHT3706, WHT3722; Kuroda-Kawaguchi T, *et al*. *Nat. Genet*. **29**, 279 (2001)].

c



c, Representative fibre FISH with probes detecting the center of P5 (red, RP11-144F9, similar to AC009976) and the inner parts of the arms pf P5 (green, AC024183).

d



d, Representative interphase nucleus probed with fluorescently labeled BACs as in c. At this resolution the red signal from RP11-144F9 is superimposed on the green signal from AC024183, producing a yellow dot. Probe RP11-144F9 (red) also hybridizes to the 97% identical sequence in AZFc, where this sequence occurs in one copy in each of the two arms of palindrome P1 (in and near the mini-palindromes P1.1 and P1.2; see Figure 2a and Supplementary Figure 2a)