A) D13Z1/D21Z1

GTGATGTGTGTACCCAGC(T/C)AAAGGAGTTGAACATTTCTATTTGATAGAGCA
GTTTGGAAACACTCTTTTTTGTTGGAAAAATGCA(A/G)GTGGATATTtggatagctttgag
gatttcgtt

only in 13  →  only in 21

B) Ladder

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D13Z1 LNA  114 bp
D21Z1 LNA
D13Z1 clamp  104 bp

mouse  hamster  human  negative
**Figure S4. Specific nucleotide substitutions in α-repeats allow for locked nucleic acid, PCR-based discrimination between centromeres 13 and 21.** Nucleotide substitutions present in the homologue arrays D13Z1/D21Z1 from centromere 13 or 21, respectively, can be used to accurately detect the abundance of repeats in each of these almost identical centromeres. PCR assays were developed to specifically detect either centromere 13 or 21. A) The centromere arrays D13Z1/D21Z1 present in chromosomes 13 and 21 are almost 100% identical, except for two single nucleotide substitutions present in either centromere 13 or 21 (letters in italics). Primers modified with locked nucleic acids (LNAs) were designed to target the sequences underlined, with LNA modification at the nucleotide substitution and one base before and after it. For the centromere 13 (D13Z1)-specific variant, the PCR reaction contains a forward primer with LNA modification; the reverse primer used was not modified (bottom sequence in lowercase letters). B) Specific detection of Chromosome 13 D13Z1 or Chromosome 21 D21Z1 using modified LNA primers. DNA isolated from human/rodent hybrids containing a single human chromosome was used to assess whether the LNA-modified primers can indeed differentiate between centromeres 13 and 21. DNA from mouse or parental hamster cells is included to control for cross-species hybridization of repeats, along with human DNA isolated from peripheral blood lymphocytes that served as a positive control. Water was used as a further negative control. The PCR assay for the centromere 13 repeat D13Z1 consisted of an LNA forward primer and an unmodified reverse primer that binds both centromere 13 and 21 repeats (see also Fig. S5a). The PCR assay for centromere 21 D21Z1 contains both forward and reverse LNA primers that bind D21Z1 substitutions and a clamp for D13Z1, which is the same forward primer used to detect D13Z1, but this time phosphorylated to inhibit D13Z1 amplification (see also Fig. S5b). The D21Z1 assay detects substitutions in both centromere 13 and 21, but
substantially reduces the non-specific amplification of D13Z1. The gel displays the amplification products at 16 PCR cycles.