Palindromic sequences that can adopt hairpin and cruciform structures are a potent source of chromosomal breakage and rearrangements, and play a significant role in the pathogenesis of diseases. In humans, palindromic sequences have been found at chromosomal breakpoints of non-recurrent and recurrent translocations that can cause Emanuel syndrome. Palindrome-mediated large deletions, interchromosomal insertions and translocations cause several types of εγδβ thalassemia, X-linked congenital hypertrichosis syndrome and hereditary renal cell carcinoma. Also, palindromes are implicated in the amplification of regions with genes that promote tumorigenesis in colon and breast cancer; medulloblastoma and lymphoma. Despite the critical impact of palindromes on genome maintenance and diseases, how these repeats cause chromosome breakage and rearrangements in eukaryotic cells is largely unknown. Using yeast Saccharomyces cerevisiae as a model system, we uncovered three distinct mechanisms for DSB formation at palindromic loci, one of them involves previously uncharacterized nuclease. We are using a unique set of structurally different palindromic sequences that allows easy and sensitive physical monitoring of DSBs. We also built a novel conditional quasi-palindrome system that allows us to follow de novo DSB formation during the cell cycle. Overall, we anticipate that these studies will uncover new mechanisms responsible for chromosomal fragility in yeast cells, which are highly relevant for our understanding of the detrimental effects of these repeats in humans.