*Supplementary Material*

**Suppementary Figures**



**Supplementary Figure 1.** Sequence alignment of StoIGSTR\_188 and StoTR06\_159. Two complete StoIGSTR\_188 from the 45S rDNA IGS region and the StTR159 consensus are shown. A total of 29 bp from three regions (nt 4–12, 41–50, and 82–91) were deleted in StTR159. The FISH targets of StoTR06\_159\_OP1 and StoTR06\_159\_OP2 are shown in yellow and blue highlights, respectively. The green highlight shows the overlapping regions between targets of StoTR06\_159\_OP1 and StoTR06\_159\_OP2 but because each probe is strand-specific, they do not compete for target DNA.



**Supplementary Figure 2.** FISH of 45S rDNA coding sequence and St\_IGS\_DR463. PLOPs from 18S rDNA and St\_IGS\_DR463 colocalized at the NOR site in chromosome 3S. No extra-NOR signal was observed for St\_IGS\_DR463. Bar = 10 µm.



**Supplementary Figure 3.** Sequence alignment of StoTR03\_178 and StoTR05\_180.A) Sequence alignment of StoTR03\_178 and StoTR05\_180 consensus sequences showed 71%. Highlighted nucleotides are polymorphic sites. Colored background arrows are target regions of PLOPs listed in Supplementary Table 1: blue for < 80% identity with non-target sequence and green for StoTR05\_180\_OP2 which has > 82%. Boxed sequences correspond to CENP-B box-like sequences. Boxed red arrows show the 7 bp palindromic dyad symmetry unique to StoTR03\_178.

**Supplementary Table 1.** List of the primers and PLOPs used for FISHa

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Name** | **Oligo Name** | **Primer/PLOP sequences (5′—3′)** | **Length****(bp)** | **Site in consensus** | **Modification** |
| StoTR01\_86 | StoTR01\_86\_OP1 | TTAATCAGTTTTCGCCGATGAGTGTTTCG | 29 | 44..72 | 5′-FAM |
| StoTR01\_86\_OP2 | CATCAGTTTTCGCCAATGAGTGTTTCG | 27 | 4..30 |
| StoTR03\_178 | StoTR03\_178\_OP1 | CCGGAATATGTTAAGACATGATCCACGCT | 29 | 145..173 | 5′-Cy5 |
| StoTR03\_178\_OP2 | ATCTCAGAAACCTTCACGAATTACGAGGC | 29 | 14..42 |
| StoTR03\_178\_OP3 | CCGGAGTGGTTTTGATGCTCCAATTGGA | 28 | 98..125 |
| StoTR04\_55 | StoTR04\_55\_OP | GCGAAAACTGATTAAAAAAAGAAAAATGAATATCAAG | 37 | 2..38 | 5′-AMCA |
| StoTR05\_180 | StoTR05\_180\_OP1 | GATTTAATGCTCGAATGGGGCTCGTGATC | 29 | 62..90 | 5′-Texas Red |
| StoTR05\_180\_OP2 | GTTGTTGCACAAGTGAGTCAAACCGATC | 28 | 5..32 |
| StoTR05\_180\_OP3 | TGTTTAGACATGACTTGACACACCTTCCA | 29 | 94..122 |
| StoTR05\_180\_OP4 | TGAGTTCTTTTGAGATTCAATCGCGATTT | 29 | 136..164 |
| StoTR06\_159 | StoTR06\_159\_OP1 | TGCATATGCTGGGTCAAAATGAAGCCTAT | 29 | 36..64 | 5′-Cy3 |
| StoTR06\_159\_OP2 | AGGCTTCCTTGTGTCATAGGCTTCATTTT | 29 | 21..49 |
| StoIGS\_463 | StoIGS\_463\_PLOP1 | AAACCAATATATATTCTATTTTTCGTGATT | 30 | 17..46 | 5′-FAM |
| StoIGS\_463\_PLOP2 | CAAATGATTGATAAGCCTTTAATTTTATTA | 30 | 60..89 |
| StoIGS\_463\_PLOP3 | GAAATTTTGGGGTTAAGCTTATATATTTTT | 30 | 256..258 |

aFor 45S rDNA, 5S rDNA, and telomere repeats, see Waminal et al. (2018).

**Supplementary Table 2.** Summary of distribution of the *Senna* repeats in the 13 *Senna tora* chromosomes.

|  |  |
| --- | --- |
| **Chr. No.** | **Chromosome features** |
| 1 | Intercalary colocalization of the the StoTR02\_7\_tel and StoTR04\_55 on the long arm (L); paracentromeric colocalization of the DAPI bands, StoTR04\_55 and StoTR01\_86 on small arm (S); centromeric colocalization of the the StoTR02\_7\_tel and all StTRs except StoTR01\_86, the StoTR02\_7\_tel on the both termini regions. |
| 2 | Sto\_45S\_CDS colocalization of the major signals of StoTR06\_159 on NOR; the intercalary colocalization of StoTR04\_55, StoTR06\_159 on 2S; paracentromeric colocalization of the StoTR02\_7\_tel, StoTR04\_55 and StoTR05\_180 on 2S; centromeric colocalization of the StoTR02\_7\_tel, StoTR04\_55, StoTR06\_159, StoTR03\_178, StoTR05\_180; centromeric colocalization of the telomeric repeats and all StTRs except StoTR01\_86; telomeric repeats on the both termini regions |
| 3 | Subtelomeric colocalization of the the StoTR02\_7\_tel and StoTR04\_55 on 3S; interstitial colocalization of the DAPI band and StoTR01\_86 on 3S; paracentromeric colocalization of StoTR03\_178 on 3S; centromeric colocalization of StoTR02\_7\_tel, Sto\_5S, StoTR03\_178 and StoTR05\_180. |
| 4 | Interstitial colocalization of the DAPI bands and StoTR01\_86 on 4S; paracentromeric StoTR03\_178 on 2S; paracentromeric colocalization of the StoTR02\_7\_tel and StoTR04\_55; centromeric colocalization of the StoTR02\_7\_tel, StoTR04\_55, StoTR03\_178 and StoTR05\_180. |
| 5 | Interstitial colocalization of the the StoTR02\_7\_tel and StoTR04\_55; Interstitial StoTR05\_180 on 5S; paracentromeric colocalization of StoTR04\_55 and StoTR01\_86 on 2S; centromeric colocalization of the StoTR02\_7\_tel, StoTR04\_55, StoTR03\_178, StoTR05\_180. |
| 6 | Interstitial colocalization of the StoTR02\_7\_tel, StoTR04\_55, StoTR01\_86 and StoTR05\_180 on 6L; centromeric colocalization of the StoTR02\_7\_tel, StoTR04\_55, StoTR03\_178 and StoTR05\_180. |
| 7 | Interstitial colocalization of StoTR04\_55, StoTR03\_178, StoTR05\_180 on 7L; paracentromeric colocalization of StoTR04\_55 and StoTR01\_86; centromeric colocalization of the StoTR02\_7\_tel, StoTR04\_55, StoTR01\_86, StoTR03\_178 and StoTR05\_180. |
| 8 | Interstitial StoTR04\_55 and StoTR05\_180on 8L; intercalary colocalization of the telomeric repeats and StoTR04\_55; paracentromeric colocalization of StoTR04\_55 and StoTR01\_86; centromeric colocalization of the StoTR02\_7\_tel, StoTR04\_55, StoTR03\_178 and StoTR05\_180. |
| 9 | Centromeric colocalization of the StoTR02\_7\_tel, StoTR04\_55, StoTR06\_159, StoTR03\_178 and StoTR05\_180.  |
| 10 | Interstitial colocalization of the StoTR02\_7\_tel, StoTR04\_55, StoTR06\_159; paracentromeric colocalization of DAPI band and StoTR01\_86; centromeric colocalization of the StoTR02\_7\_tel, StoTR04\_55, StoTR03\_178 and StoTR05\_180.  |
| 11 | Paracentromeric StoTR04\_55 on 10L; weak intercalary StoTR01\_86 on 10S; centromeric colocalization of the StoTR02\_7\_tel, StoTR04\_55, StoTR01\_86 StoTR03\_178 and StoTR05\_180.  |
| 12 | Subtelocentric Sto\_5S on 12S; pericentromeric colocalization of the StoTR02\_7\_tel, StoTR04\_55 and StoTR03\_178.  |
| 13 | Minor interstitial StoTR05\_180 on 12L; intercalary colocalization of the StoTR02\_7\_tel and StoTR04\_55; paracentromeric colocalization of the DAPI band and StoTR01\_86; centromeric colocalization of the StoTR02\_7\_tel, StoTR04\_55, StoTR01\_86 StoTR03\_178 and StoTR05\_180.  |