

# Programmed genome rearrangements in *Oxytricha* produce transcriptionally active extrachromosomal circular DNA

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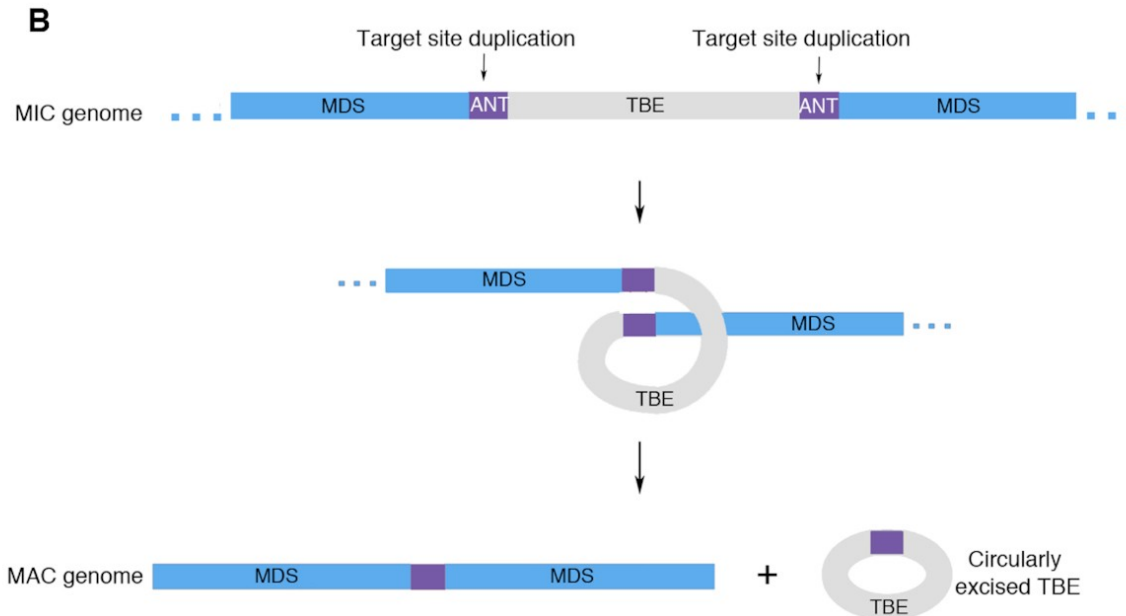
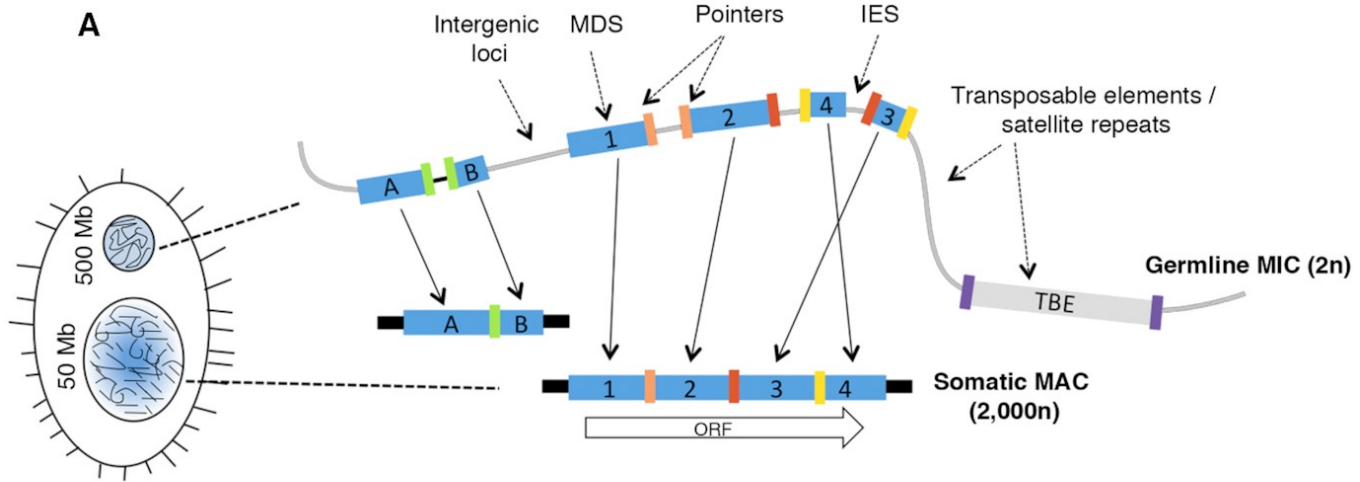
Presenter: Abdulmelik Mohammed

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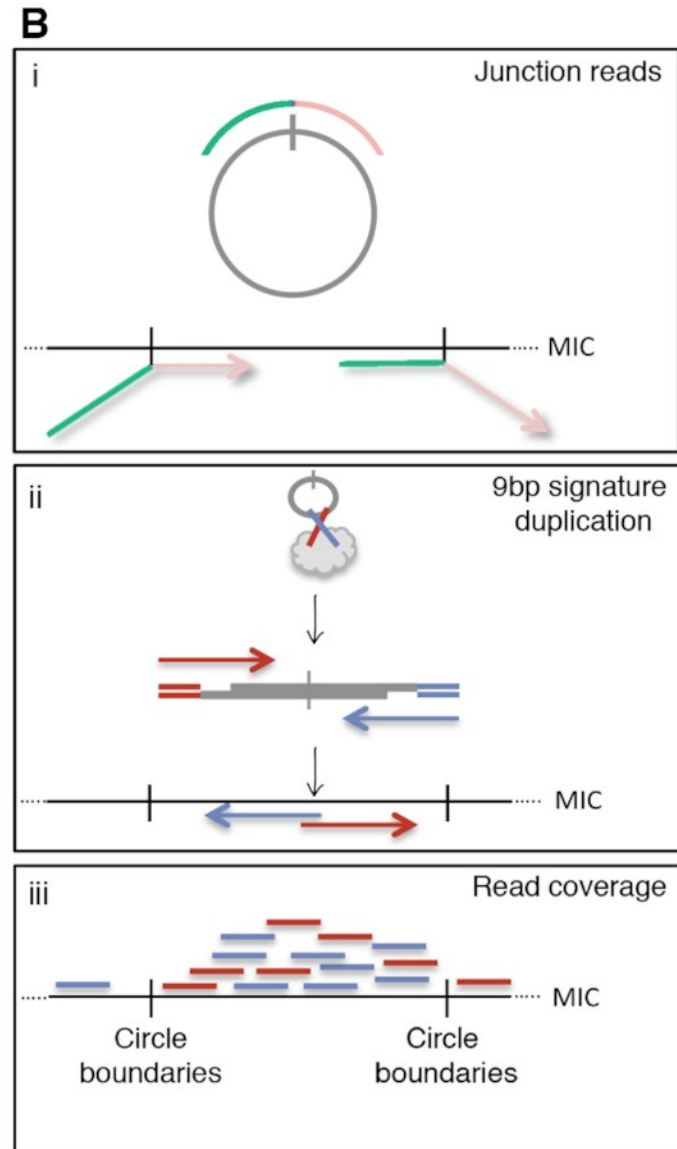
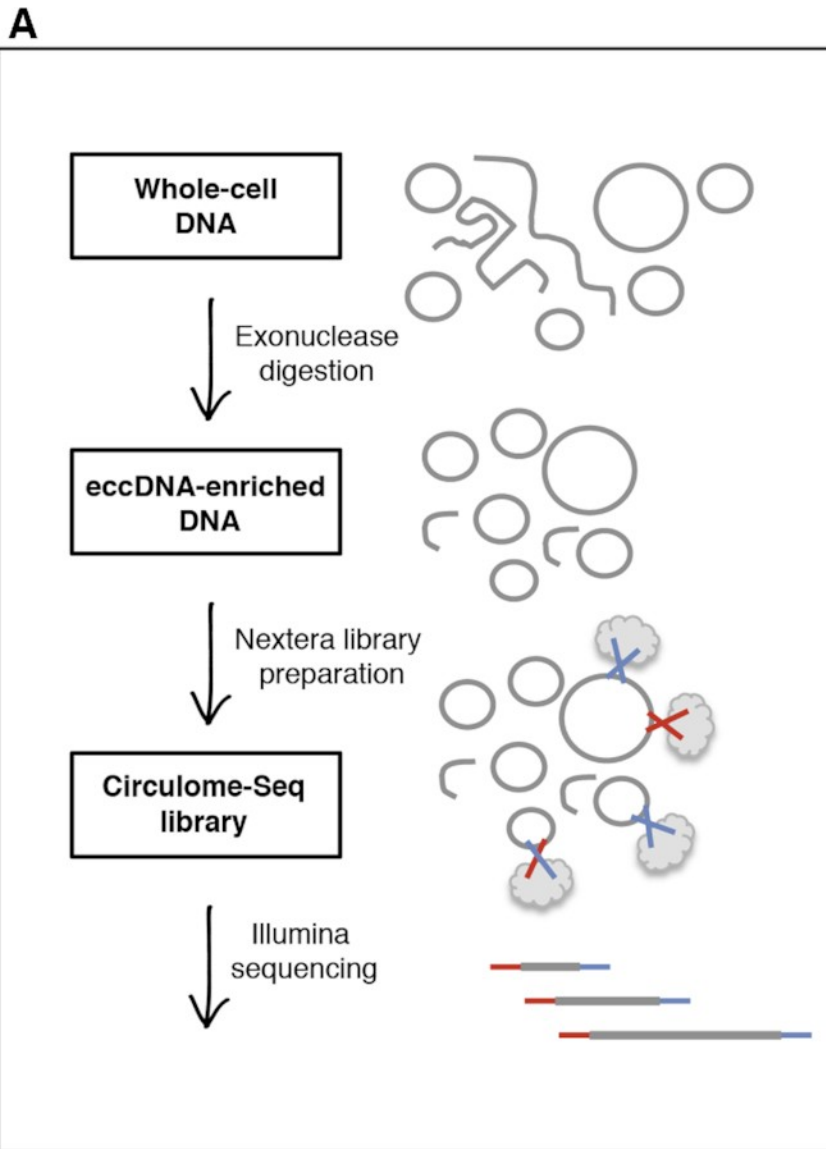
# Goals

- characterize eccDNA genome-wide during DNA rearrangement in *Oxytricha*
    - circularized TBE (Telomere Bearing Element) sequences
    - non-repetitive MIC-limited sequences
  - investigate transcription of eccDNAs during genome rearrangement
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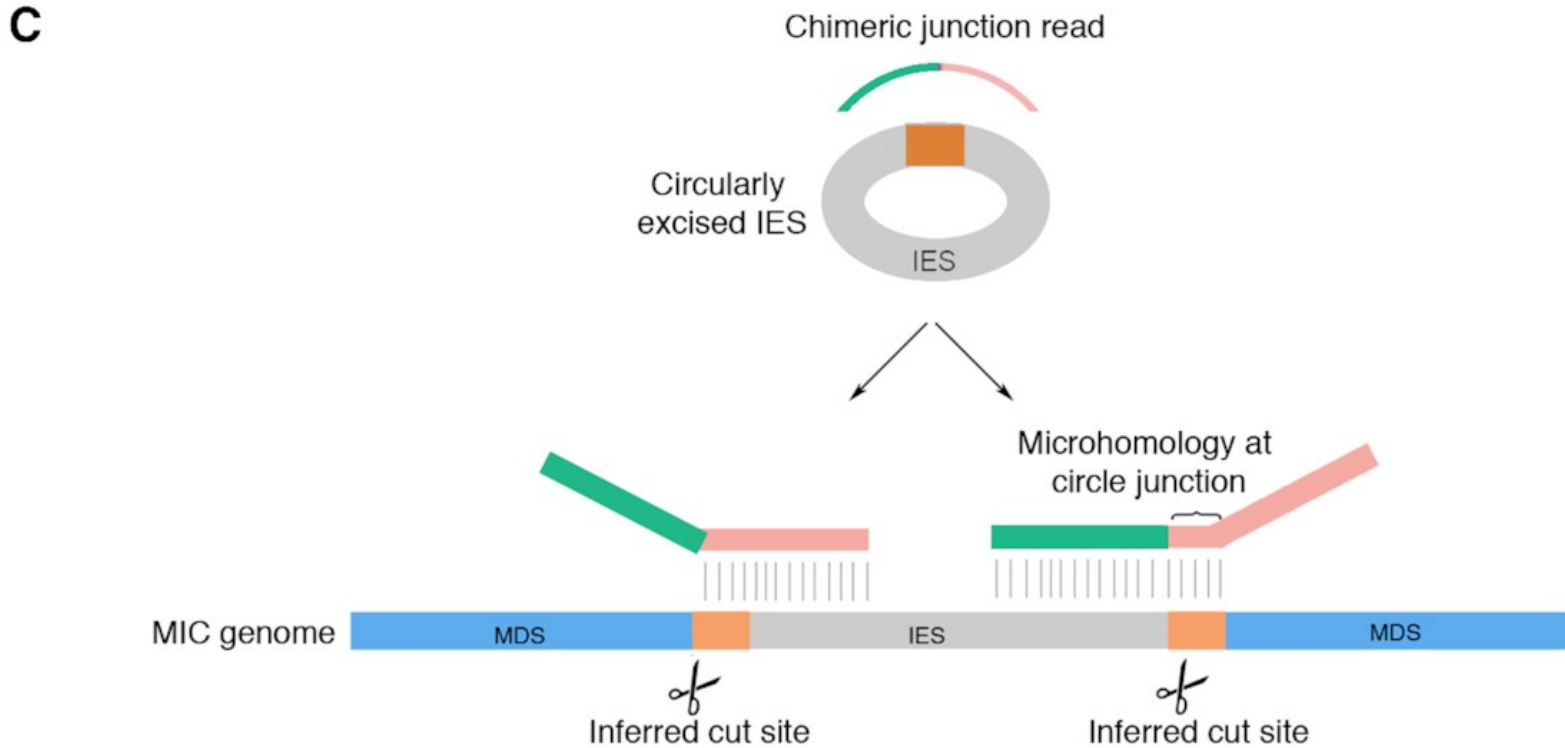
# A model for circular TBE elimination during rearrangement



# Experimental and bioinformatic pipeline to identify eccDNA in *Oxytricha* genome-wide



# Hypothetical pathway for the excision of an IES via recombination at pointers

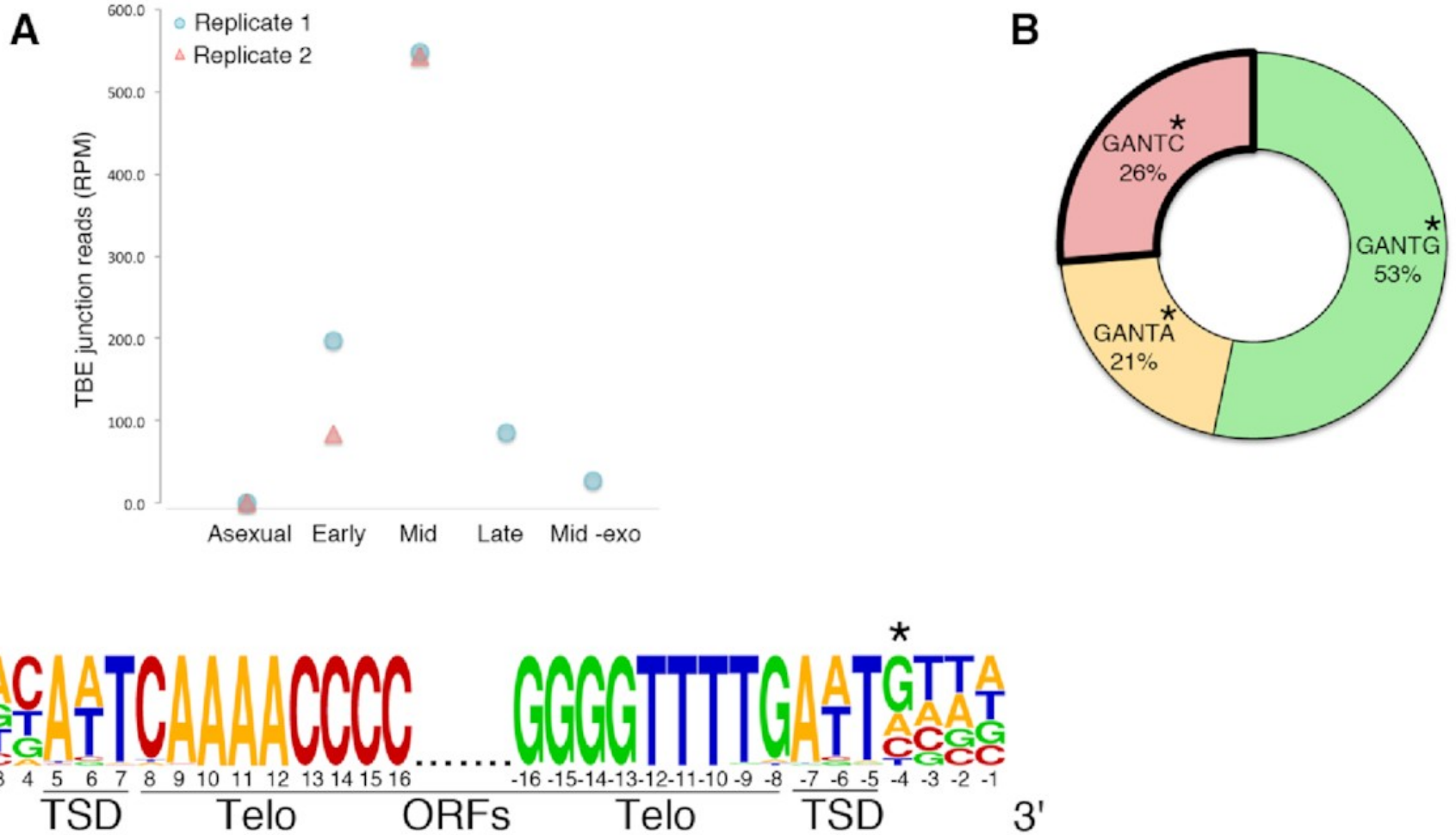


# Experimental timepoints

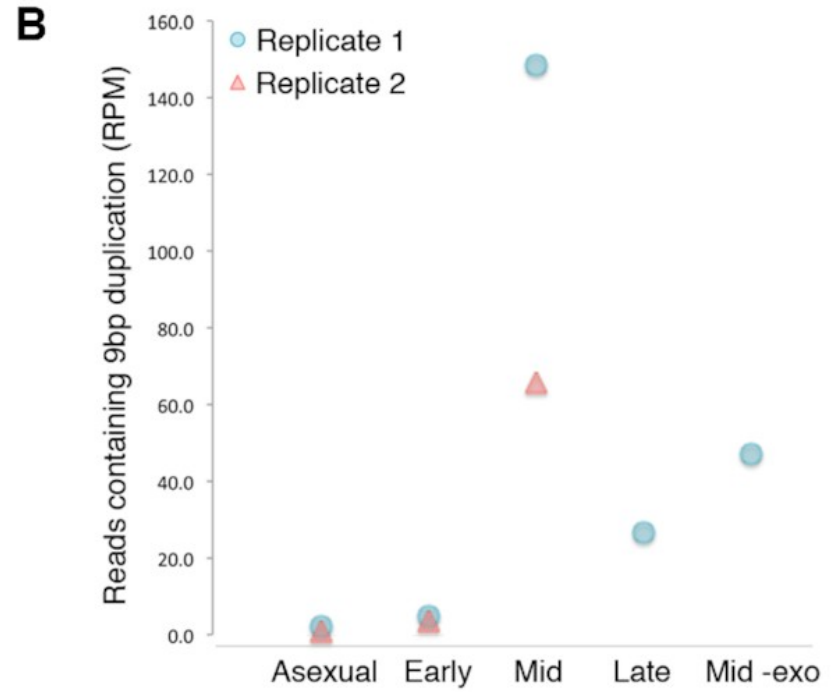
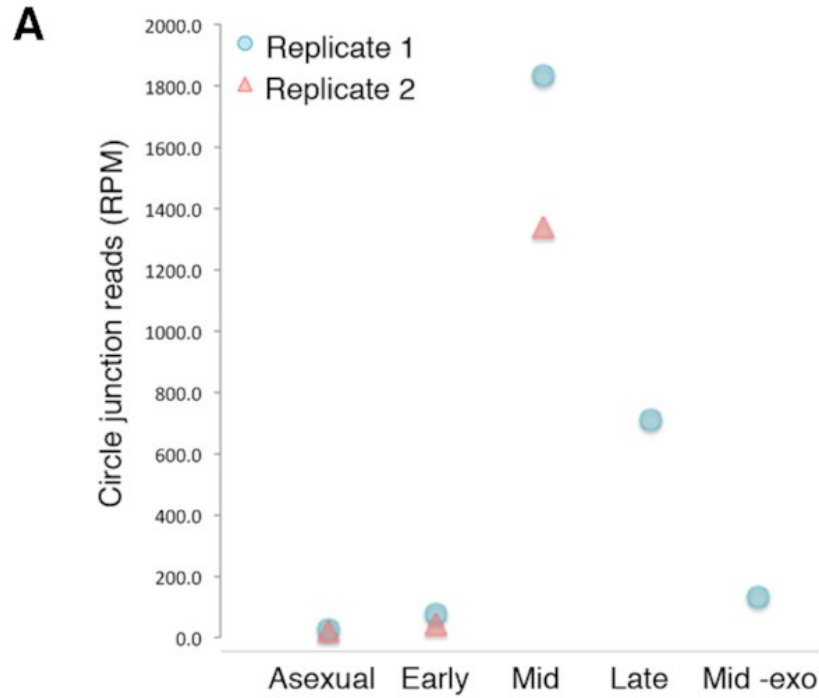
Cells of compatible mating types mixed



# Circular DNA enrichment coupled to deep-sequencing reveals circularly excised TBEs



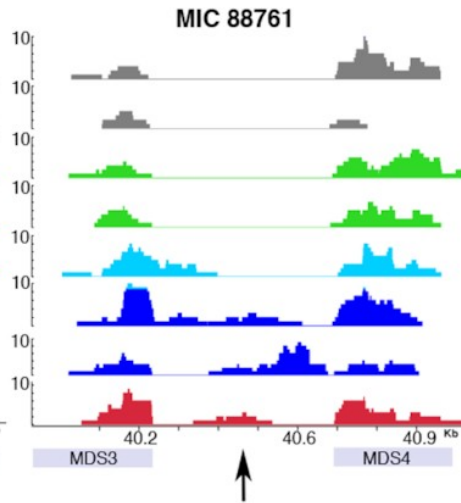
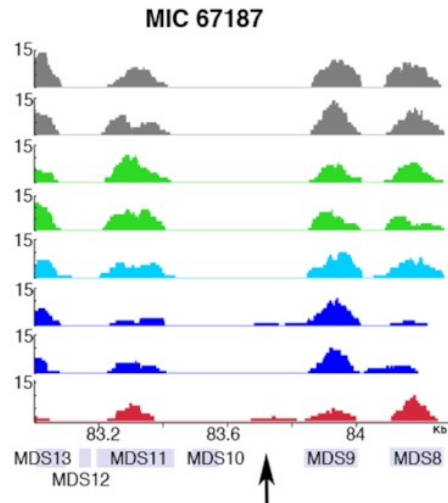
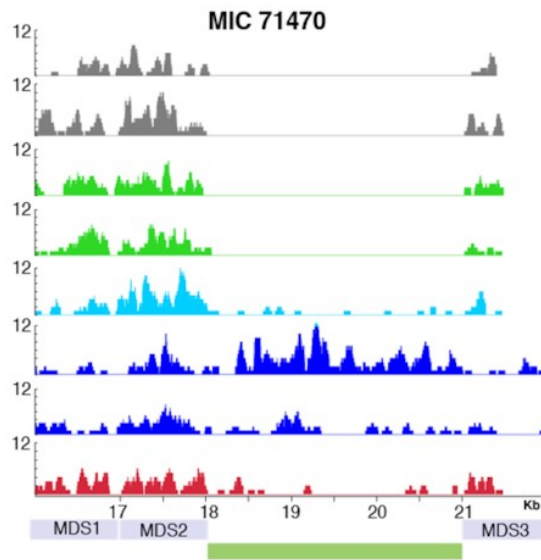
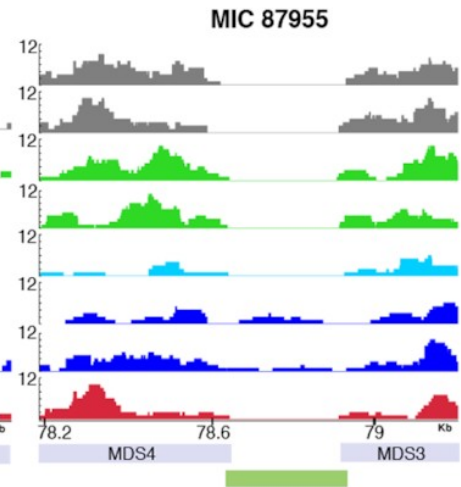
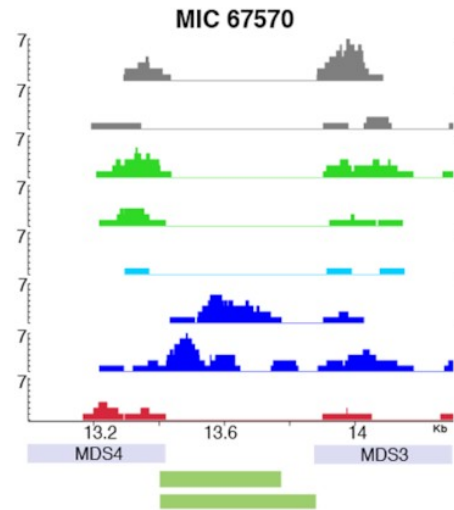
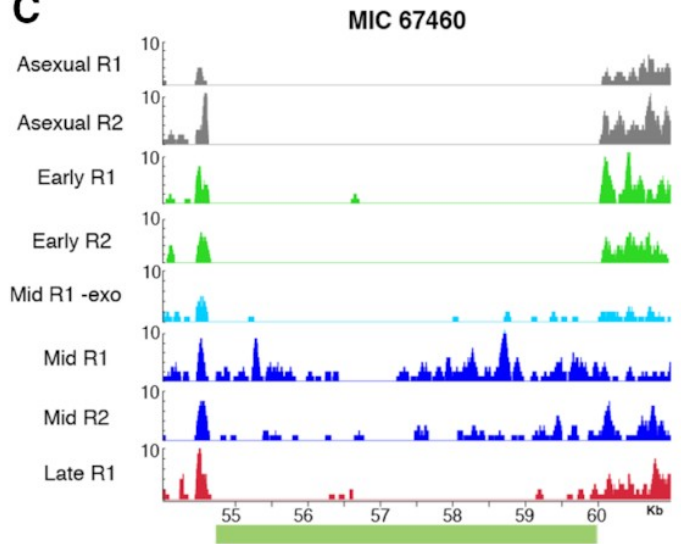
# Circulome-seq reveals circularly excised non-repetitive MIC-limited loci





# Example genome tracks showing mapped reads that were enriched for circular DNA

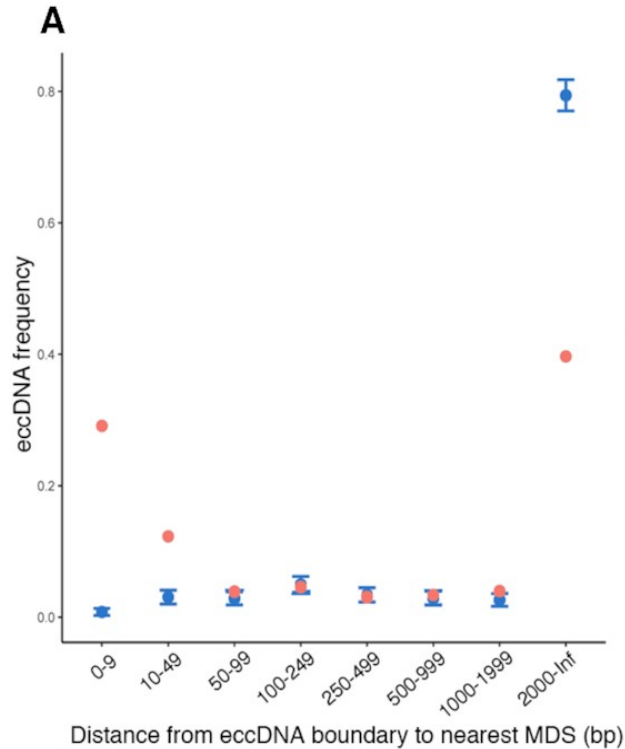
**C**



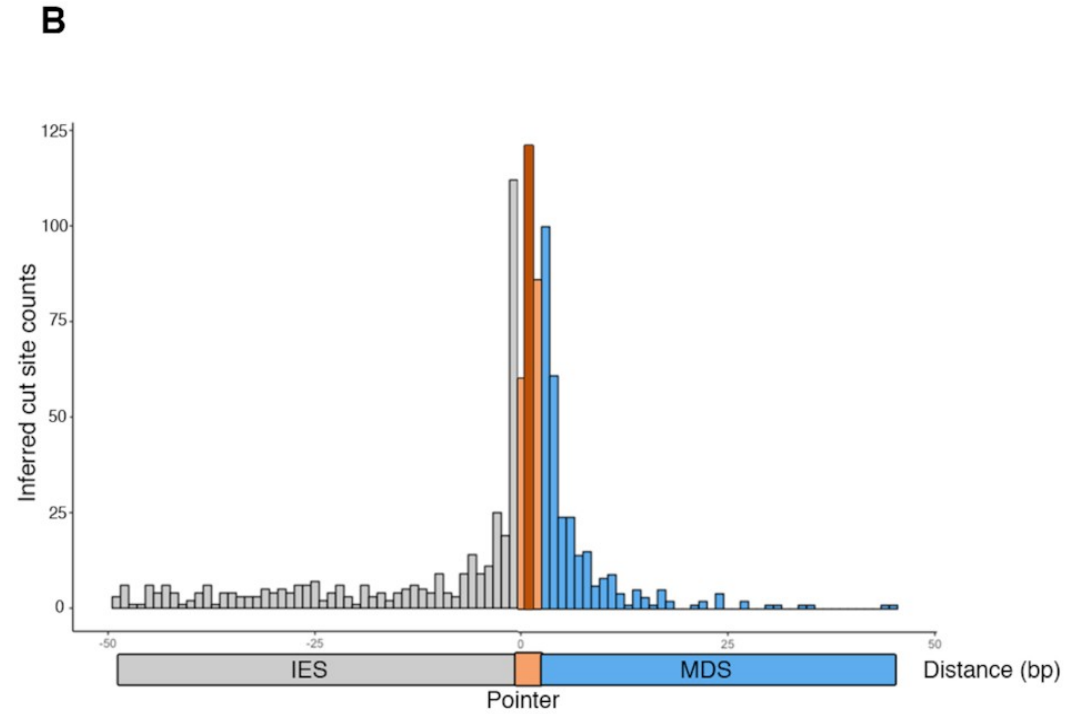
# Circulome-seq read statistics

| Time point | Replicate ID | No. of mapped reads | No. of TBE junction reads | Normalized TBE junction read counts (RPM) | No. of circle junction reads | Unique circle isoform counts | Normalized non-repetitive circle junction read counts (RPM) | No. of 9bp duplication reads | Normalized 9bp duplication read counts (RPM) |
|------------|--------------|---------------------|---------------------------|---|------------------------------|------------------------------|---|------------------------------|--|
| Asexual    | 1            | 1 271 072           | 0                         | 0.0                                       | 33                           | 27                           | 26.0  | 3                            | 2.4  |
|            | 2            | 1 091 793           | 0                         | 0.0                                       | 23                           | 16                           | 21.1  | 1                            | 0.9  |
| Early      | 1            | 993 575             | 197                       | 198.3                                     | 74                           | 71                           | 74.5  | 5                            | 5.0  |
|            | 2            | 1 498 785           | 125                       | 83.4                                      | 61                           | 48                           | 40.7  | 5                            | 3.3  |
| Mid        | 1            | 1 185 659           | 649                       | 547.4                                     | 2176                         | 2039                         | 1835.3  | 176                          | 148.4  |
|            | 2            | 1 095 501           | 594                       | 542.2                                     | 1464                         | 1362                         | 1336.4  | 72                           | 65.7   |
| Late       | 1            | 1 085 662           | 93                        | 85.7                                      | 771                          | 715                          | 710.2   | 29                           | 26.7   |
| Mid -exo   | 1            | 1 543 270           | 42                        | 27.2                                      | 199                          | 182                          | 128.9   | 73                           | 47.3   |

# Characteristics of high confidence rearrangement-specific non-repetitive eccDNA

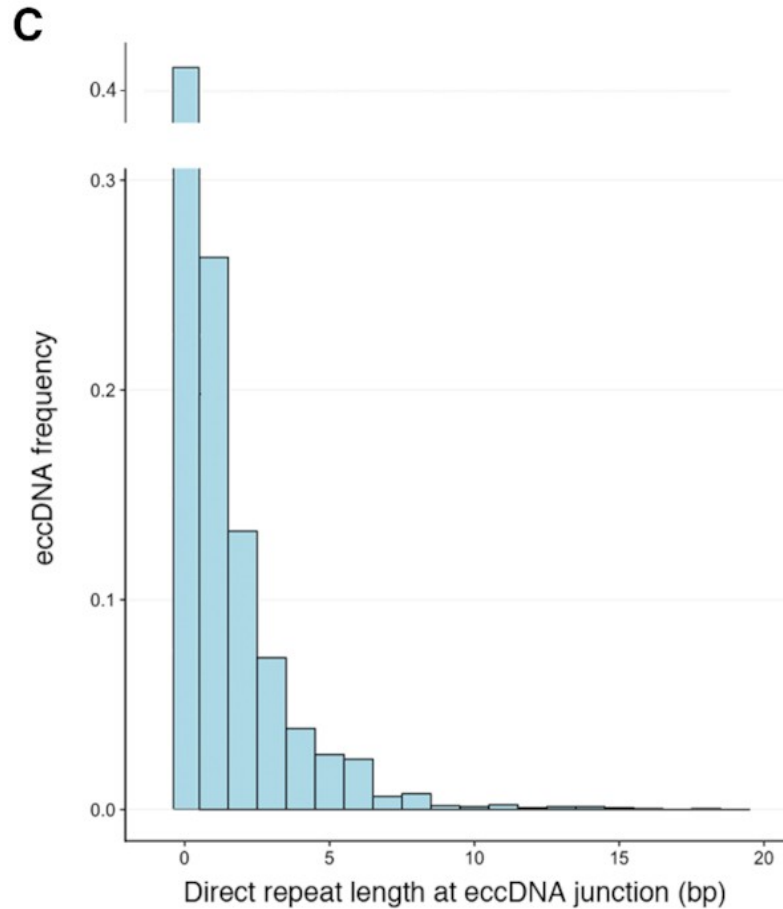


Data for the 2432 high confidence eccDNA identified in mid rearrangement



The locations of 933 cut sites inferred from high confidence eccDNA that are within 50 bp of an MDS boundary that also have high confidence pointer annotations

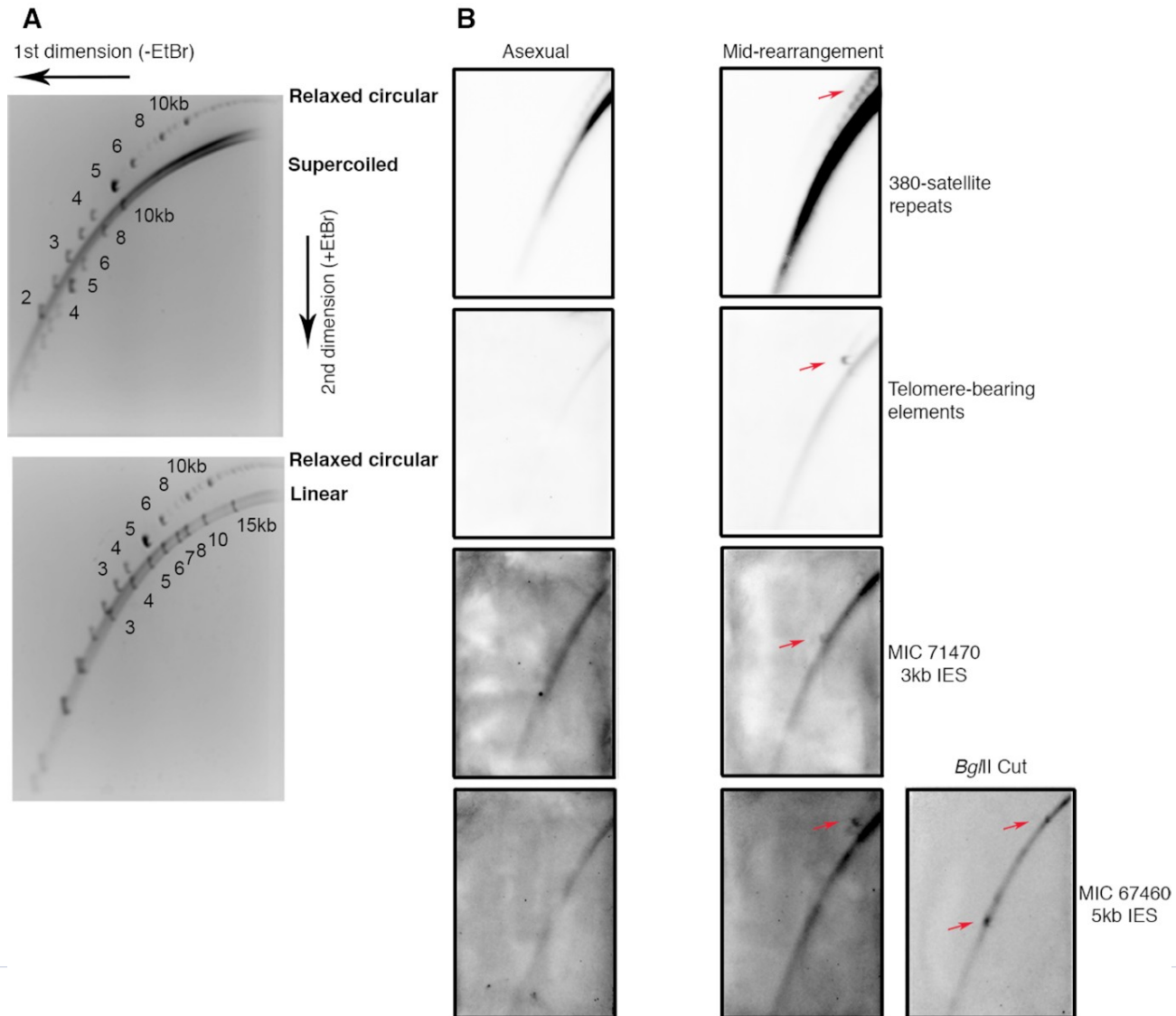
The length of the direct repeats flanking circularly excised loci for the 2432 high confidence eccDNA



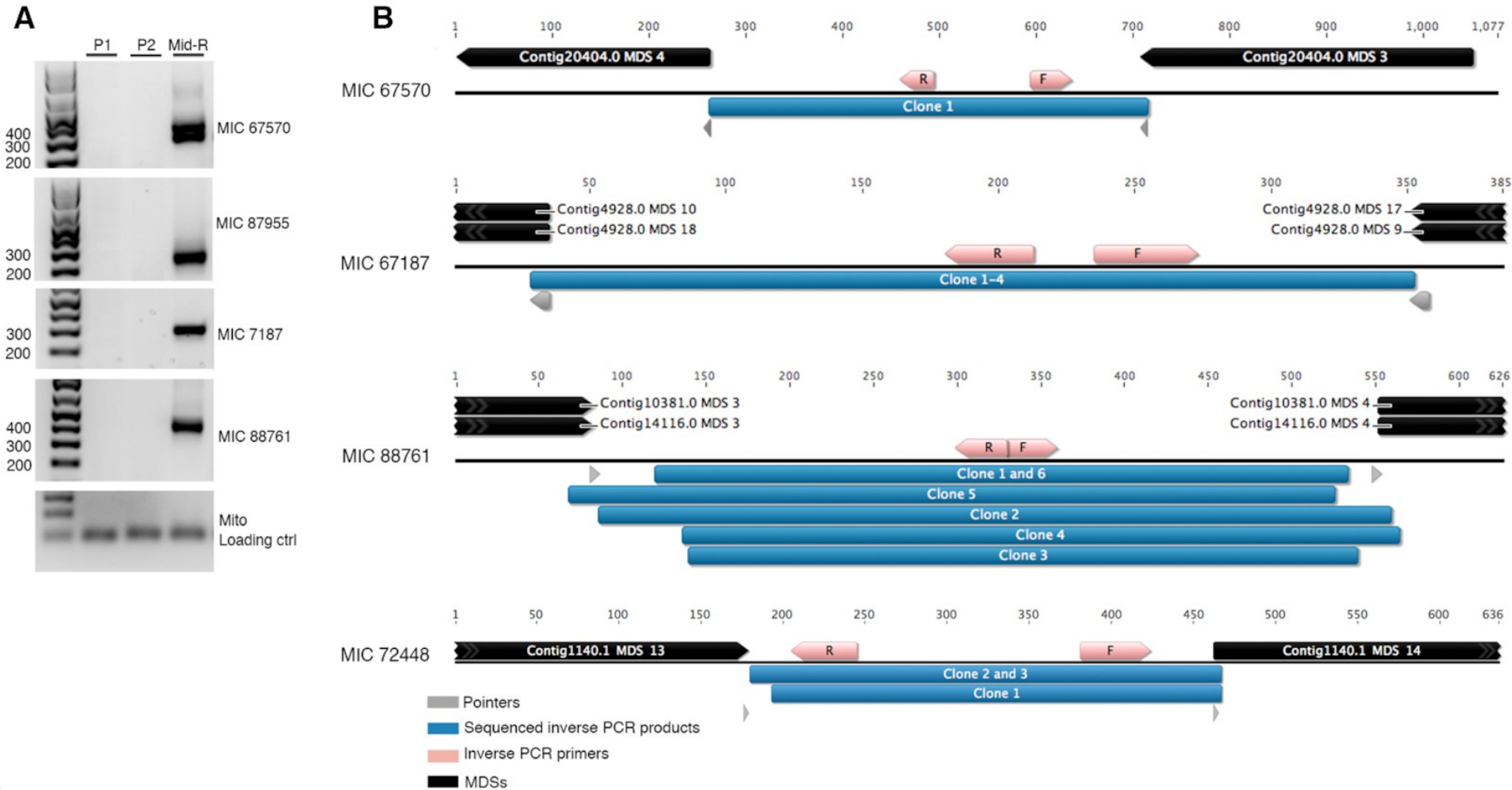
## Categories of non-repetitive MIC-limited loci giving rise to eccDNA

|               | <b>No. of MAC contigs</b> | <b>No. of eliminated sites in the MIC</b> | <b>No. of eliminated sites containing eccDNA</b> | <b>Percentage of eliminated sites containing eccDNA</b> |
|---------------|---------------------------|---|--|---|
| Non-scrambled | 15 680                    | 119 613                                   | 555  | 0.46  |
| Scrambled     | 2818                      | 10 151                                    | 4  | 0.04  |
| Intergenic    |                           | 16 846                                    | 17   | 0.10  |

# Validating the circular topology of eccDNA using 2D agarose gel

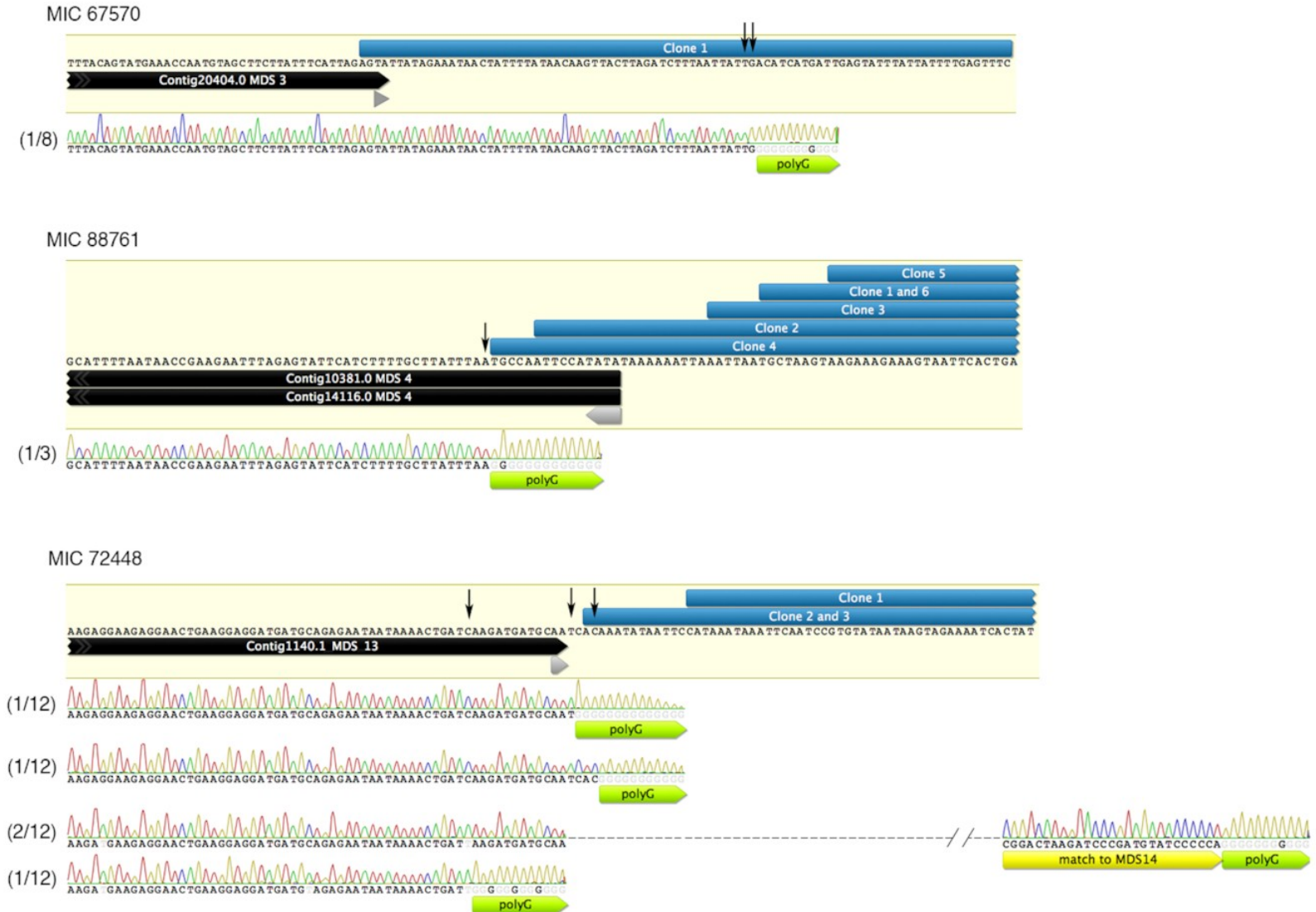


# Inverse PCR validation of rearrangement-specific eccDNA in non-repetitive MIC-limited loci



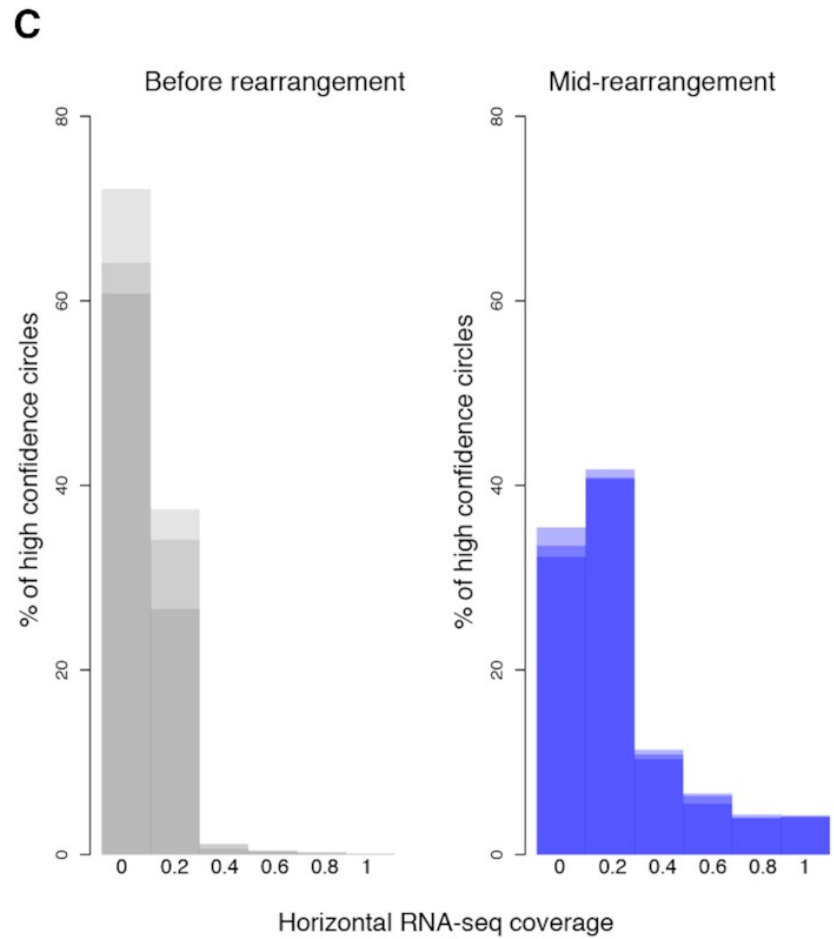
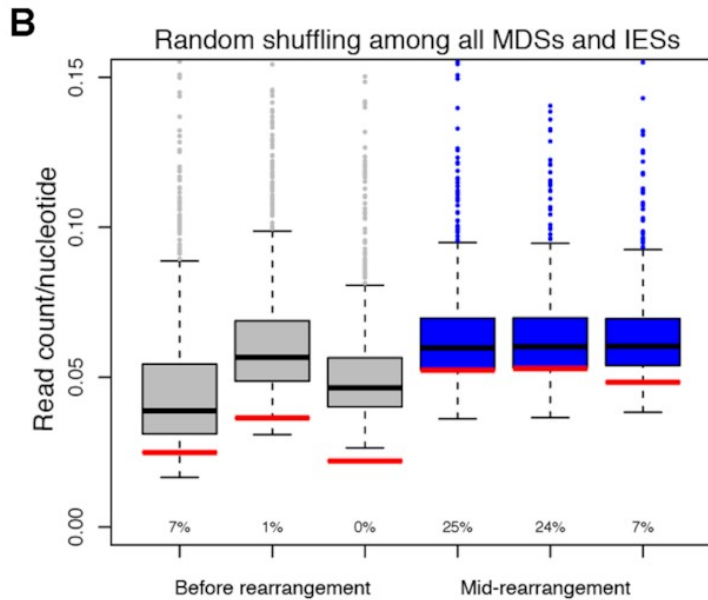
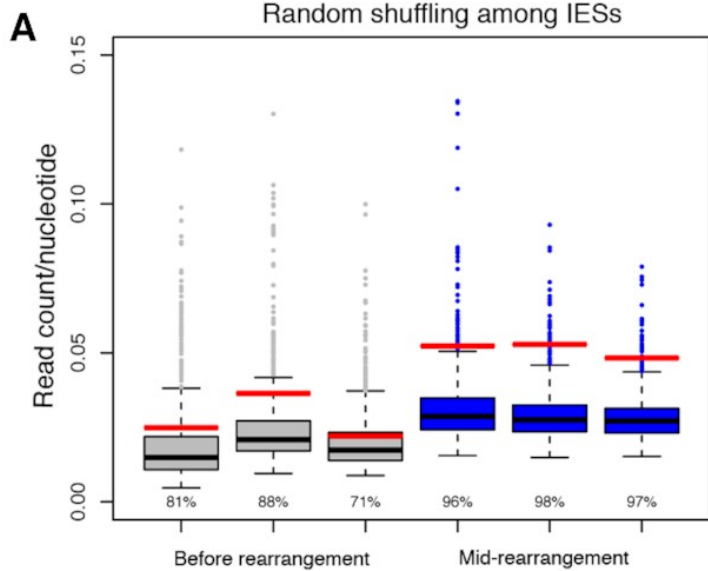
Terminal transferase treatment of genomic DNA during mid-rearrangement suggests variable 3' DNA breaks at MDS boundaries for three loci

C



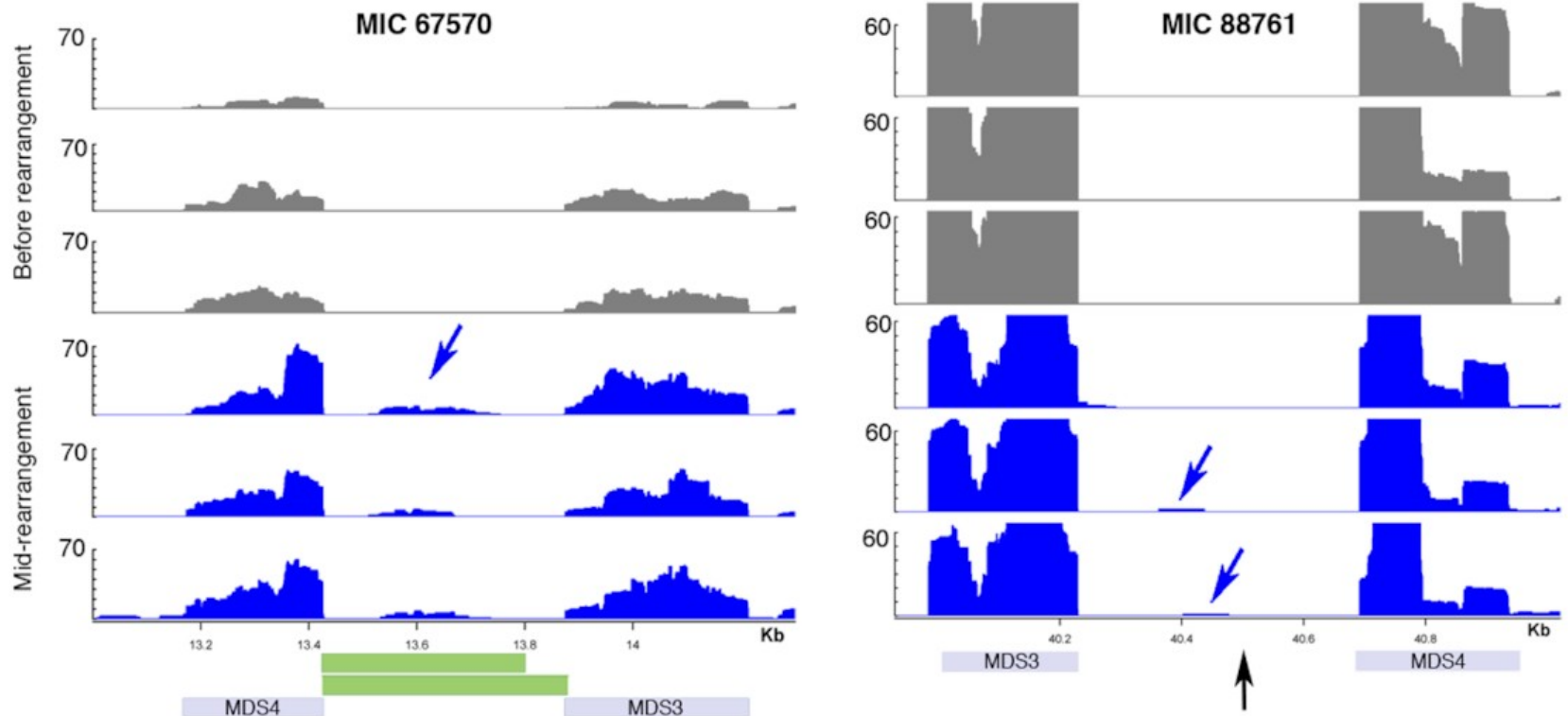


# Rearrangement-specific transcription from high confidence eccDNA ...



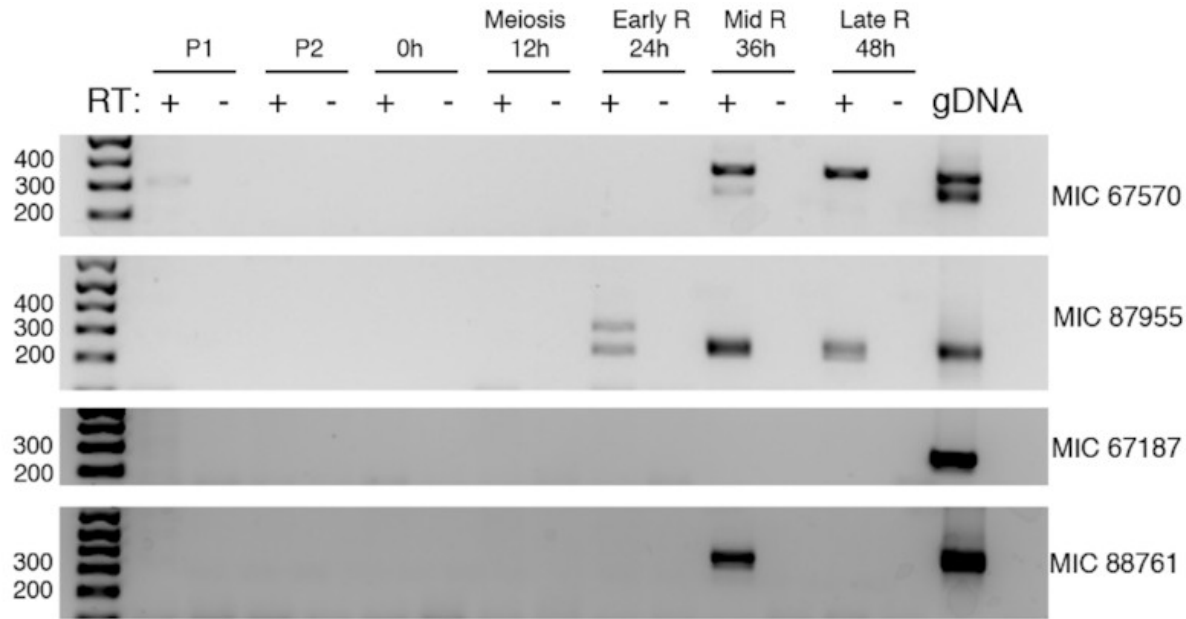
Genome tracks for two validated eccDNA showing RNA-seq read coverage in the region that circularizes

D

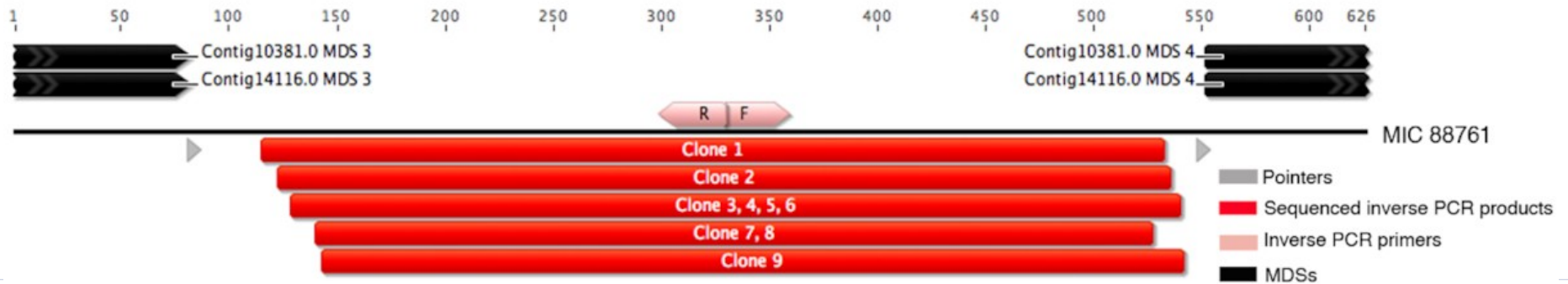


# Inverse RT-PCR suggests transcription across eccDNA junctions

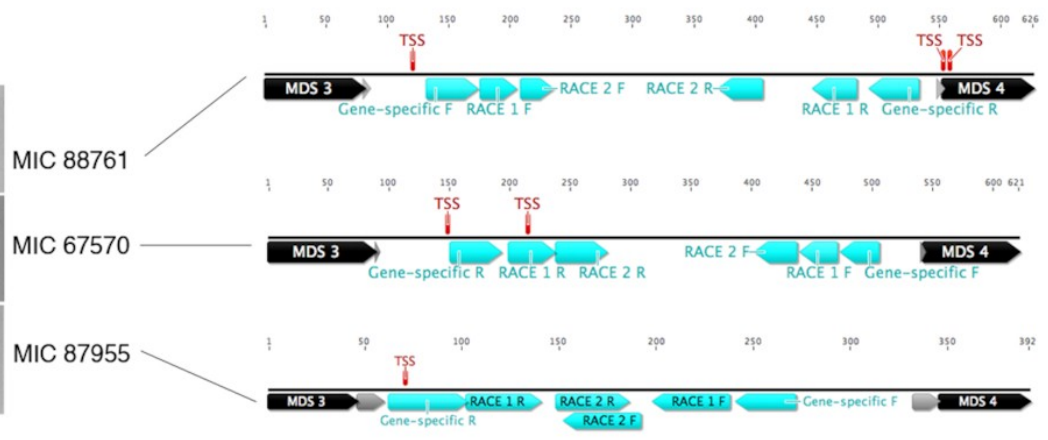
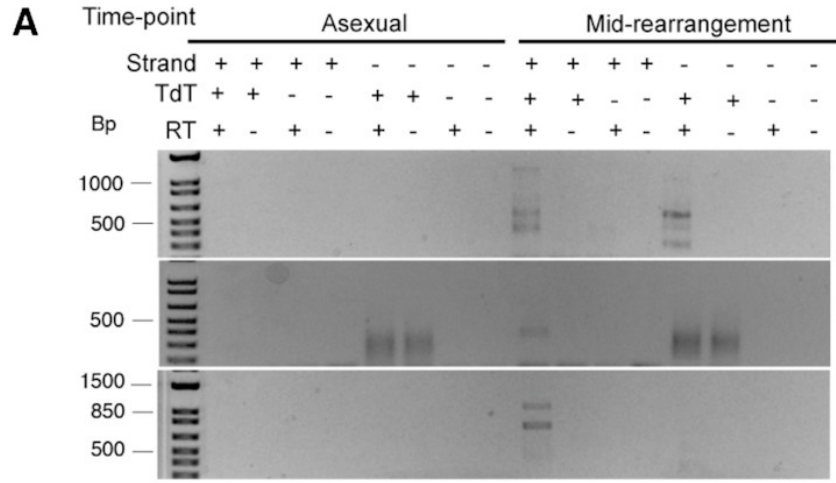
**A**



**B**

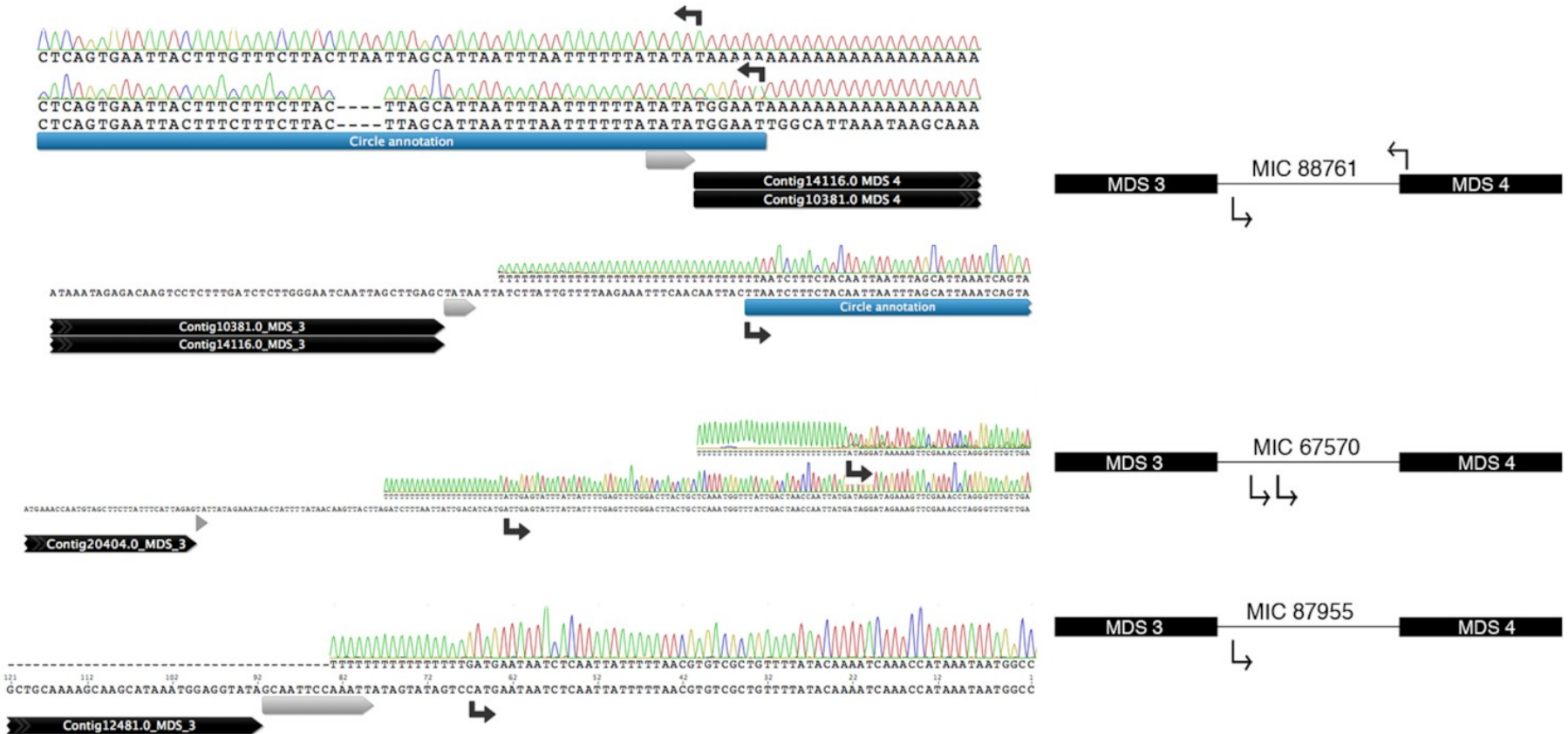


# 5'- RACE reveals TSS sites within eccDNA ...



# 5'- RACE reveals TSS sites within eccDNA

B



# Conclusion

- circularly eliminated TBE transposons
    - precise excision of TBEs
    - two other junction motifs, GANTA and GANTG, in addition to GANTC motif
  - circles in non-repetitive MIC-limited loci
    - circularization occurs at variable and imprecise junctions
    - 40% of circles map far away from MDS boundaries
    - evidence for low levels of circularization of scrambled IESs
    - the set of high confidence eccDNA is significantly enriched in non-scrambled IESs
    - 17 cases of circularization of intergenic loci between MDSs that map to different MAC chromosomes
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