Recombination and Replication of Plasmid-Like Derivatives of a Short Section of the Mitochondrial Chromosome of Neurospora crassa

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ABSTRACT

The 21-kbp mitochondrial chromosome of the stp-ruv strain of Neurospora crassa undergoes regional amplification yielding plasmid-like supercoiled circles varying in size from subunit length to very high multimers. A comparison of the base sequence of the five plasmids studied, with the region of the chromosome from which they were derived, indicated that the amplified chromosomal segments were determined by a recombination-excision process near or within two structurally distinctive regions. One of these, consisting of nearly uninterrupted strings of Cs and Gs straddling tandem PstI site direct repeats, could form an extended hairpin loop with only a few mismatches. It was found at or near the 5′ exchange point of all of the plasmids. An extended 35-bp sequence containing 17-base sequence dependence for intramolecular recombination appears to have occurred in regions where single strand breaks are likely to occur or be stabilized, namely in regions capable of forming reasonably stable hairpin loops and/or regions with many extended direct repeats (Ripley 1982). The extraordinary feature of the recombinational events generating the nested set of Neurospora mitochondrial plasmids is that the 5′ excision region common to all of the plasmids is almost identical to a sequence about 10 kbp downstream that was found (Devries et al. 1986) to be the site of recombination generating the 8 μ circle in the stopper mutant E35. The sequence, an extended, nearly uninterrupted string of Cs followed by a nearly homogeneous string of Gs straddling tandemly repeated PstI sites, is one of a class of sequences distributed widely throughout the Neurospora mitochondrial chromosome but particularly within the section of the chromosome devoted to the protein synthetic machinery, that is likely to be a site for hairpin loop formation (Yin et al. 1982). The replication pattern of the several chromosomes of the original stopper mutant suggested the existence of at least two replication initiation sequences (ori sequences) in the normal mitochondrial chromosome

The mitochondrial genome of stp-ruv was shown to consist primarily of two circular chromosomes (21 and 36 kbp) instead of the single 62 kbp of the wild-type or the population of recombinant derivatives of the stopper (stp) mutant from which it was derived (Gross, Mary and Levine 1989). In addition to supporting replication of the two genetically complementary circular chromosomes, stp-ruv, upon subculture, yielded secondary derivatives in which highly amplified short sections of the 21-kbp circle were found in supercoiled circles varying from subunit length to very high multimers. We show here that amplified subunits form a nested set of “plasmids” resembling those found by Turker, Domenico and Cummings (1987a, b) in longevity mutants of a senescent strain of Podospora anserina.

The high level of amplification and the ease of enrichment for plasmids with different subunit size, offered the opportunity to study the mechanism and sequence dependence for intramolecular recombination and autonomous replication of the section of the Neurospora mitochondrial chromosome from which the plasmids were derived. The sequences obtained indicate a strong general similarity between the recombinational hotspots (plasmid excision-junction sites) in the Neurospora mitochondrial chromosome and those found involved in recombination in yeast and Podospora mitochondrial DNA. In most instances recombination appears to have occurred in regions where single strand breaks are likely to occur or be stabilized, namely in regions capable of forming reasonably stable hairpin loops and/or regions with many extended direct repeats (Ripley 1982). The extraordinary feature of the recombinational events generating the nested set of Neurospora mitochondrial plasmids is that the 5′ excision region common to all of the plasmids is almost identical to a sequence about 10 kbp downstream that was found (Devries et al. 1986) to be the site of recombination generating the 8 μ circle in the stopper mutant E35. The sequence, an extended, nearly uninterrupted string of Cs followed by a nearly homogeneous string of Gs straddling tandemly repeated PstI sites, is one of a class of sequences distributed widely throughout the Neurospora mitochondrial chromosome but particularly within the section of the chromosome devoted to the protein synthetic machinery, that is likely to be a site for hairpin loop formation (Yin et al. 1982).

The replication pattern of the several chromosomes of the original stopper mutant suggested the existence of at least two replication initiation sequences (ori sequences) in the normal mitochondrial chromosome

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(Gross, Hsieh and Levine 1984) which are likely to be involved in replication initiation of the two chromosomes of stp-ruv (Gross, Mary and Levine 1989). The isolation of a set of highly amplified plasmids derived from a common region of the 21-kbp chromosome of stp-ruv seemed to afford the opportunity to identify at least one of the ori sequences. We report here that the plasmids derived from the 21-kbp chromosome of Neurospora contain an AT rich 14-bp sequence that is nearly identical to a sequence retained by all members of a similar set of Podospora plasmids (Turrer, Domenico and Cummings 1987a, b) and that both sequences are nearly identical to a sequence presumed to be near the replication origin of the Drosophila yakuba mitochondrial chromosome (Goddard, Fauron and Wolstenholme 1982). An analysis of the GenBank sequence database revealed a remarkable association of the sequence with the plus strand of organelle DNA.

MATERIALS AND METHODS

All of the strains used in this study were derived from stp-ruv, which in turn was derived from the stopper (stp) mutant IAR155[II]2,107A as described in Gross, Hsieh and Levine (1984) and in Gross, Mary and Levine (1989). Plasmid producing derivatives stp-ruv 315 and 172 were obtained after growth of the original stp-ruv down the length of growth tubes as described by Ryan, Beadle and Tatum (1943). The 286/291 plasmid producing derivative arose spontaneously as described previously (Gross, Mary and Levine 1989) while stp-ruv 101 was derived from stp-ruv 286/291 after one passage down the length of a growth tube. Conidia of each of the strains were preserved dry in silica gel and samples used as the inoculum source. The spontaneous as described previously (Gross, Mary and Levine 1989) while stp-ruv 101 was derived from stp-ruv 286/291 after one passage down the length of a growth tube. Conidia of each of the strains were preserved dry in silica gel and samples used as the inoculum source. The stp-ruv derivatives are named according to the sequence length of the majority class of plasmid produced. In most cases a single class was in the overwhelming majority. In the case of stp-ruv 286/291, the size difference of the two plasmid subunits was too small to allow estimation of the relative amount of each in mitochondrial (mt) DNA by conventional electrophoretic methods. However, about an equal number of each were found in the small sample of primary pSCC31 clones derived from supercoiled DNA. It is not known whether mixed multimers are derived from the two.

The isolation of mitochondria, the purification and separation of supercoiled circular DNA from linear and nicked circular DNA by CsCl density gradients has been described previously (Gross, Hsieh and Levine 1984). Hybridization at normal stringency was in 45 to 50% formamide at 42° as described by SOUTHERN (1975) using 32P-labeled nick translated DNA. Hybridizations at low stringency were in 10% formamide and post hybridization washes were not allowed to exceed 37°.

Plasmid subunits were cloned into the HindIII site of pBR322 or pSCC31 (Cheng and Modrich 1983) and, after resolation, cloned into M13 or, alternatively, cloned directly into the phage. In each case, at least six M13 clones (three in each orientation) were sequenced using dGTP and dITP by the Sequenase procedure as directed by the kit manufacturer (U.S. Biochemical Corp.). Resolution of the strings of 11 and 12 Cs of pNM315 and 172 was accomplished by repeated mixed electrophoretic analyses after sequencing in M13 and by the procedure of Maxam and Gilbert (1980). The HindIII, 19a, sequence was obtained in only one direction after cloning in pSCC31 but the part of the sequence relevant to these studies and presented here was consistent with all of the plasmid sequencing data.

RESULTS

The chromosomal composition of stp-ruv mitochondria as well as the chromosomal population of the stopper (stp) mutant from which it was derived and the normal chromosome of wild-type II mitochondria are presented in Gross, Mary and Levine (1989) and in Gross, Hsieh and Levine (1984). The region of the chromosome from which the plasmids are derived is illustrated in Figure 1 which also presents the HindIII restriction patterns and hybridizations which establish the identity of each of the plasmids. The ladder-like electrophoretic pattern of uncut supercoiled circular DNAs from each of the strains and several of the cross hybridizations of cloned plasmid subunits with the intact polymeric series of plasmid derivatives are presented in Figure 2.

Despite the absence of imposed selection and purification procedures, the electrophoretograms and hybridizations presented in Figures 1 and 2 indicate that the cultures are reasonably homomorphic with respect to plasmid production by the time they are recognized as supporting plasmid growth. Nevertheless, HindIII restriction fragments that differed in size from the major plasmid subunits were found in the mtDNA preparations from each of the strains. The background plasmid composition varied greatly from strain to strain and from culture to culture. For example, the minor 5.3-kbp element found in the blot of the electrophoretogram of stp-ruv 286/291 presented in Gross, Mary and Levine (1989) is not detectable in the electrophoretogram of Figure 1 despite the fact that the cultures analyzed were started with the same inoculum of silica gel dried conidia. The mt chromosome populations of two of the derivatives, stp-ruv 172 and stp-ruv 315 clearly differ from that of their stp-ruv parent while those of stp-ruv 286/291 and stp-ruv 101 are quite similar to each other and their parent (except for a 4.0 HindIII fragment which is occasionally found in stp-ruv 101 mt DNA). The differences are largely confined to the large HindIII fragments and therefore likely to be derived from HindIII, 1. The mt population of stp-ruv 172 is clearly more heterogeneous than the others. Preliminary experiments indicate the presence of several recombinant types in which the 1.0-kbp deletion of the parental strain has been extended to include HindIII, 13 (see Figure 1, Gross, Mary and Levine 1989). We mention this here because the chromosomal composition of the mitochondria of the strain of origin is important in determining whether or not two plasmids with similar sequence anomalies are likely to have dependent or independent derivations.
This is especially true for psNM172 and psNM315 both of which appear to have arisen in mitochondria with shortened versions of the HindIII, 1–3, junction fragment of the 36-kbp chromosome. The sequence homology of the plasmids to sections of the mt chromosome and to each other is illustrated by the electrophoretograms and hybridizations of Figures 1 and 2. All of the cross hybridizations of the plasmids and the strains of derivation were done and found consistent with those included in the two figures presented here as well as in Figure 4 of Gross, Mary and Levine (1989) for psNM286. The hybridizations of Figure 1 indicate that psNM172, psNM315 and psNM291 as previously shown for psNM286 (Gross, Mary and Levine 1989) hybridize to HindIII, 7a and 19a. The same was found to be true for psNM101 but, as indicated in Figure 1, can only be clearly ascertained against a high background, at much lower stringency. Figure 2 shows that each of the plasmid subunits hybridizes with the ladder of supercoiled polymeric molecules in uncut covalently closed DNA isolated from each of the different plasmid producing stp-ruv derivatives. Again, hybridizations of psNM101 DNA with itself, as well as DNA from the other strains, were inconsistent unless performed at low stringency. This, plus the appearance of an extra band of intermediate density to normal linear and supercoiled circular molecules in ethidium bromide, CsCl density gradients, suggested that the plasmid subunit was likely to be very AT rich. Indeed, the DNA used in the electrophoretogram and blot of stp-ruv 101, was from the intermediate density band. The nucleotide sequence in the vicinity of the HindIII, 7a–19a, junction is presented in Figure 3 along with the sequences at the splice junctions of each of the plasmid subunits. Indeed, the plasmids comprise a nested set of derivatives of a very AT-rich region in the vicinity of the HindIII, 7a–19a junction. The region is bounded by two sequences which appear to be preferred sites for excision. The 5’ excision sites of all of the plasmids except psNM101 are in the vicinity of the directly repeated PstI sites located between extended sequences of mostly Cs and mostly Gs. This sequence is almost identical to the sequence found by DeVries et al. (1986) to be involved in the formation, by recombination, of the 8µ circular chromosome of mutant E35 mitochondria. The preferred 3’ site of excision appears to be near the very prominent 35-bp sequence containing 17-bp direct repeats starting at position 195. The most startling fact emerging from the sequence analysis, which may indeed reflect the kind of structures involved in the recombination process, is the introduction of sequence alterations, mostly the insertion of G and C residues in the vicinity of the 5′ excision site. (As indicated in the figure and discussed below, the apparent transposition of an A from position 65 to 63 is more likely to result from a secondary recombination event than mutation.) There is no direct way to determine the precise
position of the insertion of the additional G of psNM172 and psNM315 within the string of Gs between -70 and -75. However, one can guess at its likely insertion point from a consideration of the likelihood of its base pairing with a C, arising from a T (-99) to C transition as illustrated in panel III of Figure 3.

The insertion of an extra G in psNM172 and 315 follows directly from the sequence analysis. The common origin of the T to C transition in the two plasmids is much less certain. There is a string of 11 Cs 5’ to the tandem PstI repeats of psNM315 while the string is 12 Cs in psNM172. It is possible to generate an 11 C sequence by recombination between the Cs at 216 and -96 of the normal sequence. However, a string of 12 Cs cannot be generated the same way. It was pointed out previously, on the basis of the similarity of the electrophoretic patterns of stp-ruv 172 and stp-ruv 315 mt chromosomal DNA, that psNM172 and psNM315 are likely to have been derived from a common source. The G insert common to both supports this and suggests that the T-C transition of both has a common origin. According to this view psNM172, since it is the shorter of the two, was derived either from psNM315 or a precursor to both. This view is encouraged further by the sequence at the 5’ splice site of psNM172 which could have arisen as a primary recombination event concomitant with an A transposition from 65 to 68 but appears likely to have arisen as a secondary recombination event between the As at 62 and 207 in a plasmid-like psNM315. It is as yet impossible to rule out recombination or deletion rather than mutagenesis as the source of the apparent T to C transition of psNM172. It should be emphasized, however, that no base changes have been observed other than those in the vicinity of a recombination site.

psNM101 was isolated after passage of stp-ruv 286/291 down the length of a growth tube in an attempt to select for shorter plasmids which might be expected to initiate replication more frequently (equivalent to the replicating “demons” of SPIEGELMAN et al. 1968). Since the exchange points for psNM101 are wholly within the 286-bp sequence, it could have been derived from either of the two plasmids in the parental strain. The 5′ breakpoint is in an interval containing three AATT direct repeats immediately 3′ to the G-
Mitochondrial Plasmid-Like Elements

A search of GenBank using the Wordsearch program strongly supports the view that TATATAAGGAGT is an important structural component at least one class of organelle specific origins of replication. Thirteen sequences were retrieved from the nearly 20,000 sequences of the database as of October 4, 1988. All 13 sequences, including that of Podospora, were, like Neurospora, of the plus strand. The organism of origin, the sequence, position and base substitutions at positions 9 and 10 as well as pertinent references are listed in Table 1 which also includes the Drosophilia entry which apparently was not submitted for inclusion in the database. The table summarizes entries from 14 different sequences in 10 different organisms only 3 of which are sequences of nuclear genes. The majority of the entries are from mitochondrial and chloroplast sequences. An extrachromosomal, macronuclear gene of Tetrahymena is of some interest that the sequence was not found in the yeast mt chromosome where AT-rich sequences are not all rare. It is of some interest that the sequence was not found in the yeast mt chromosome where AT-rich sequences have been associated with regions presumed to serve as ori sequences for highly amplified rho particles (Bernardi 1982). In 13 out of the 15 entries (including...
### TABLE 1

GenBank<sup>®</sup> search for TATATAGAXXTATA

<table>
<thead>
<tr>
<th>Organism</th>
<th>Organelle</th>
<th>Position and strand</th>
<th>XX</th>
<th>Sequence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus nidulans</td>
<td>Mitochondrion</td>
<td>527-540</td>
<td>AA</td>
<td>tRNAs Arg and Asp and ATPase subunit 6</td>
<td>GRISI et al. (1982)?</td>
</tr>
<tr>
<td>A. nidulans</td>
<td>Mitochondrion</td>
<td>+</td>
<td></td>
<td></td>
<td>KOECHEL and KUENTZEL (1981)</td>
</tr>
<tr>
<td>Drosophila yakuba&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Mitochondrion</td>
<td>+ 49-62</td>
<td>TA</td>
<td>AT-rich replication region</td>
<td>GODDARD, FAURON and WOLSTEHOLME (1982)</td>
</tr>
<tr>
<td>Homo sapiens</td>
<td>Nucleus</td>
<td>3084-3097</td>
<td>TA</td>
<td>Upstream to c-sis proto oncogene</td>
<td>VAN DEN OUWELAND et al. (1987)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marchantia polymorpha</td>
<td>Chloroplast</td>
<td>96465-96478</td>
<td>GA</td>
<td>Complete genome</td>
<td>UMESONO et al. (1984)</td>
</tr>
<tr>
<td>M. polymorpha</td>
<td>Chloroplast</td>
<td>+ 5356-5369</td>
<td>AA</td>
<td>Complete genome</td>
<td>UMESONO et al. (1984)</td>
</tr>
<tr>
<td>Pea</td>
<td>Chloroplast</td>
<td>3080-3093</td>
<td>TA</td>
<td>Photosystem II 44-kD polypeptide</td>
<td>BOOKJANS et al. (1986)</td>
</tr>
<tr>
<td>Podospora anserina</td>
<td>Mitochondrion</td>
<td>+ 385-598</td>
<td>TC</td>
<td>Plasmids</td>
<td>TURKER, DOMENICO and CUMMINGS (1987a)</td>
</tr>
<tr>
<td>Soybean (Glycine max)</td>
<td>Chloroplast</td>
<td>+ 28-41</td>
<td>AT</td>
<td>psbA gene, trnH IR1</td>
<td>SPIELMAN and STUTZ (1983)</td>
</tr>
<tr>
<td>Tetrahymena thermophila</td>
<td>Macronucleus</td>
<td>+ 505-518</td>
<td>TA</td>
<td>tRNA genes (extrachromosomal)</td>
<td>YAO, ZHU and YAO (1985)</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>Nucleus</td>
<td>165-178</td>
<td>TT</td>
<td>cAMP kinase (TPK3)</td>
<td>TODA et al. (1987)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Genetics Computer Group Sequence Analysis Software Package (DEVEREUX, HAEBERLI and SMITHIES 1984).

<sup>b</sup> Not in database.

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**DISCUSSION**

The region of the Neurospora mt chromosome from which the nested set of "plasmids" was derived is functionally homologous to the region of *Podospora anserina*’s chromosome from which a similar set was obtained by TURKER, DOMENICO and CUMMINGS.
This similarity raises three physiological issues regarding the relationship of DNA sequence to structure and function of the mt chromosome. The sequence analysis proved relevant to two of these problems but has only increased our curiosity about the third, perhaps most interesting of the three, the functional utility for regional recombination and amplification. The data obtained, however, do permit drawing some specific conclusions about the relation between sequence and nonhomologous intramolecular recombination of the mt chromosome and allow the identification of a sequence that may be a common component to one class of replication origins of extranuclear DNA.

Sequence analysis indicates that all of the plasmids, except psNM101 arose by an intramolecular recombination event within or adjacent to the string of Cs 5' to the tandemly repeated Pst sites. As indicated in Figure 3, the string of Cs could form a stable hairpin loop by base pairing with the complimentary predominantly G region 3' to the repeated Pst sites. A nearly identical pattern of Cs and Gs on opposite sides of directly repeated Pst sites was described by Devries et al. (1986) at the splice site of the 8μ circle of mutant E35. Yin et al. (1982) pointed out that directly repeated Pst sites within regions that can form extended hairpin loops are distributed widely throughout the chromosome, especially in the section defined by EcoRI, 1 region of the mt chromosome are sites for intramolecular reciprocal recombination. The products of the exchange, in this instance, are the two nonoverlapping derivative circles of the normal chromosome that are minor components of chromosome populations in wild-type mitochondria but constitute the major fraction of the chromosomes present during the growth phase of the stopper mutant IAr155(II) 2,107A (Gross, Hsieh and Levine 1984). It is clear from the rational set of multimers found for each of the plasmids that some form of homologous, intramolecular recombination must be involved in resolving multimeric circular forms during or soon after replication no matter what the mechanism of replication. It seems, then, that the primary plasmid sequence is generated by an illegitimate recombination process but once formed the resolution of the replicating form depends fairly strictly on sequence homology.

The question of the role of the consensus sequence: TATATAGAXATATA as an origin of replication is a tantalizing one. It is retained by all of the Neurospora plasmids and a nearly identical version of it is retained by the corresponding set of plasmids in the related ascomycete, P. anserina. The proposal that the sequence is at least part of an initiation recognition sequence gains credibility from the discovery that it is part of the very AT-rich region in the presumed replication initiation region of D. yakuba (Goddard, Fauron and Wolstenholme 1982). The correlation of the location of the sequence to the plus strand of organelle DNA and its retention in so diverse a phylogenetic set of organisms, including diptera, higher plants, fungi and protozoa suggests some intrinsic structural importance to the sequence and a dependence on transcription. The distribution of ori-like sequences of Table 1, however, does not suggest a preferential site of residence for ori sequences. In fact, the proximity of the ori sequences of Podospora and
Neurospora to the rRNA sequence is not at all equivalent. The sequence is several kbp from the start of the rRNA sequence in Podospora and falls within a region quite dense with tRNA sequences (Turker, Domenico and Cummings 1987a, b). In Neurospora the sequence is about 35 bp downstream from the start of the large rRNA sequence (Garriga, Bertrand and Lambowitz, 1984) and no promoter-like sequences are found between it and the ori sequence (A. M. Lambowitz, personal communication). It appears then, that while Neurospora mt DNA replication may depend on a tRNA derived primer, in general, any nascent mRNA promoter might do.  

It is important to reiterate that there must be at least two potential replication origins in the Neurospora chromosome to support the independent replication of the two circular chromosomes of stp-ruv (Gross, Mary and Levine 1989) and the multiple chromosome set of the stopper mutant from which it was derived (Gross, Hsieh and Levine 1984). While the 14-bp sequence proposed as the ori sequence of the nested set might be the primary replication origin for the 21-kbp circle, the data presented do not establish it as such. Mannella, Goewert and Lambowitz (1979) presented some evidence suggesting the involvement of two other regions in the vicinity of EcoRI, 4-6 and 3-10 in the replication process, both of which fall within the 36 kbp chromosomal region. It is of some special interest to determine whether the replication of the normal, full length circular chromosome, is initiated from a single or multiple sites.

Recombination and replication of segments of the mitochondrial chromosome have now been found to occur in a variety of plants and fungi (Palmer and Shields 1984; Turker, Domenico and Cummings 1987b; Gross, Hsieh and Levine 1984) in both normal and mutant derivatives. In the fungi, at least, recombinational subdivision of the mitochondrial chromosome is commonly associated with clearly detrimental phenotypes. But, as in the case of the rho mutants of yeast, senescent mutants of Podospora and stopper mutants of Neurospora, these mutations are at some advantage and become fixed as part of the mitochondrial genotype. Two questions arise: What is the advantage of maintaining a recombination mechanism that is potentially dangerous to cell survival and how does the normal mitochondrial genome keep replication of its parts from getting out of hand? The propensity for the generation of amplified subregions of the chromosome by legitimate and illegitimate recombination appears to have a strong structural basis. Yin et al. (1982) have pointed out the many tandem Pst site repeats punctuating intervals between the tRNA sequences and other coding sequences as well, many of which, like the one described here, are in regions capable of intrastrand hairpin loop formation.

A sequence almost identical to the one described here has been found to be the site of recombination for the production of the 8 µ circle of Devries et al. (1986). The retention of these sequences in such great numbers suggests a role in some kind of regulatory system involving sectional transcription as implied previously by Wright, Horrum and Cummings (1982) for P. anserina. Accordingly, if recombination initiated at one of the hairpin loops happens to include an ori sequence, replication might proceed as an inexcusable consequence of the replication and transcriptional control mechanisms of the mitochondrion, independently of any selective physiological advantage that might accrue. It is clear that there is much yet to be learned from the stopper and senescent mutants about the replication and function of the mitochondrial chromosome.

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LITERATURE CITED


Grisi, E., T. A. Brown, R. B. Waring, C. Scazzocchio and R. W. Davies, 1982 Nucleotide sequence of a region of the

S. R. Gross et al.
Mitochondrial Plasmid-Like Elements

Spiekel, M., and E. Stutz, 1985 Nucleotide sequence of soybean chloroplast DNA regions which contain the psbA and trnH genes and cover the ends of the large single copy region and one end of the inverted repeats. Nucleic Acids Res. 11: 7157–7167.

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