Homologous recombination was shown to enable the expansion of CTG·CAG repeat sequences. Other prior investigations revealed the involvement of replication and DNA repair in these genetic instabilities. Here we used a genetic assay to measure the frequency of homologous intermolecular recombination between two CTG·CAG tracts. When compared with non-repeating sequences of similar lengths, long (CTG·CAG)n repeats apparently recombine with an ~60-fold higher frequency. Sequence polymorphisms that interrupt the homogeneity of the CTG·CAG repeat tracts reduce the apparent recombination frequency as compared with the pure uninterrupted repeats. The orientation of the repeats relative to the origin of replication strongly influenced the apparent frequency of recombination. This suggests the involvement of DNA replication in the recombination process of triplet repeats. We propose that DNA polymerases stall within the CTG·CAG repeat tracts causing nicks or double-strand breaks that stimulate homologous recombination. The recombination process is RecA-dependent.

Micro- and minisatellite instability has been associated with human genetic diseases (1–4). Approximately 14 hereditary neurological diseases are caused by the genetic instabilities of triplet repeat sequences (TRS) in or near relevant genes (reviewed in Ref. 1). Long tracts of repeating CTG·CAG sequences are responsible for myotonic dystrophy and several other diseases. Normal individuals have 5–37 repeats in the myotonic dystrophy protein kinase gene, whereas the mutations (expansions) can be in the range of 50–3000 repeats. Thus, an explosive allele length change during intergenerational transmission can occur in this autosomal dominant disease, thus causing an increase in the severity of the disease and a decrease of the age at onset (anticipation).

The mechanisms of genetic instabilities have been widely investigated in the past 6 years (1). DNA replication (5–8) and repair including methyl-directed mismatch repair (9–11), nucleotide excision repair (12), DNA polymerase III exonuclease-lytic proofreading (13), and double-strand break repair (14) have been implicated. Repetitive sequences promote homologous recombination in prokaryotic as well as eukaryotic systems, presumably by virtue of forming unusual DNA secondary structures (15–22). In fact, homologous recombination has been implicated in the instability of repetitive sequences (23–26). Similar findings have also been made with CTG·CAG repeats using a two-plasmid system in Escherichia coli (27, 28). Multiple fold expansions, deletions, and the exchange of point mutations between tracts were found in this system; these events were dependent on the presence of TRS tracts on both plasmids, CTG·CAG repeat lengths longer than 30, and a functional recA gene.

Previously (29), it was proposed that CTG·CAG tracts might function as recombination hot spots in the bovine genome. More recently, Young et al. (30) surveyed the genome of Saccharomyces cerevisiae and suggested that these repeats could be recombination hot spots based on the distribution of triplet repeats therein. However, no experimental evidence exists regarding the capacity of triplet repeat sequences to influence the apparent frequency of homologous recombination.

Here we present the first genetic assay for the determination of the apparent frequency of intermolecular homologous recombination between CTG·CAG tracts. We have identified several factors that influence the recombination frequency. In our companion paper (31), we have furthermore established a genetic assay for monitoring the apparent recombination frequency of CTG·CAG repeats tracts in an intramolecular system.

**EXPERIMENTAL PROCEDURES**

Cloning of the (CTG·CAG)n and Non-repetitive Sequences into pBR322 and pFW25—The (CTG·CAG)n repeats were obtained from pRW4026, pRW3297, pRW3246, and pRW3248 that contain (CTG·CAG)67, (CTG·CAG)98, (CTG·CAG)175, respectively, and were cloned into the HincII site of the polylinker of pUC19 for transformation. Although the (CTG·CAG)67 and (CTG·CAG)98 tracts are uninterrupted (perfectly repeating) triplets, the (CTG·CAG)98 and (CTG·CAG)175 sequences contain two G to A interruptions at repeats 28 and 59 or 28 and 69, respectively (5). All of these repeated tracts have 19 and 41 bp of non-repetitive human flanking sequences 5’ and 3’, respectively, to the (CTG·CAG)n repeats (5, 33). The plasmids were maintained in E. coli HB101 (Invitrogen) (mcrB, mnr, hsdS20 (rK, mB), recA1, supE44, ara14, galK2, lacY1, proA2, rpsL20, (SmR)), xyl5, leuB6, mtl-1).

The CTG·CAG-containing sequences were subcloned into pBR322 and pFW25 (34) as follows. Fragments containing (CTG·CAG)n were prepared by digesting the pUC19NotI derivatives with either NotI or EcoRI/HindIII (New England Biolabs, Inc.). The DNA was then blunt-ended by filling in the cohesive ends with 1 unit of the Klenow fragment.
of *E. coli* polymerase I (U. S. Biological Corp.) and dNTPs, electro-phoresed on a 7% polyacrylamide gel in TAE (40 mM Tris acetate, 1 mM EDTA, pH 8) buffer, and the band containing the triplic repeat fragment excised. The DNA was then eluted from the excised band and purified by phenol extraction (35). The pBR322 and pFW25 vectors were electro-processed into *E. coli* DH10B and transformed together with F107S (27, 38) and pAM1900 (containing tetracycline at 5 μg/ml), and the cultures were grown at 37 °C at a shaking rate of 100 rpm. When the cultures reached an absorbance (600 nm) of ~0.6 unit, an aliquot (1 ml) was inoculated into fresh 200 ml of LB (with tetracycline as before). The cultures were grown until they had reached an A600 amount of 0.7 units. A final, fresh 200 ml of LB (with tetracycline as before) was added to ensure that the cultures were cured of the TS plasmid pJW289 by several passages on plates incubated overnight.

**Results**

Transformation of TRS-containing Plasmids into *E. coli* Cells—In order to study the transformation frequency, the pFW25 derivatives were then transformed into *E. coli* AB1157 cells containing pBR322 derivatives (see “Results”). The pFW25 derivatives cannot replicate by themselves; the only way to obtain *Cm*<sup>®</sup> colonies is when the two plasmids recombine, and the recombinant molecule uses the pBR322 origin of replication. However, the formation of *Cm*<sup>®</sup> colonies is contingent not only upon the formation of recombinant plasmids after transformation but also their establishment and maintenance, particularly in the face of competition from multiple copies of the resident plasmid which is in competition with the newly transformed plasmid. Thus, the following questions were raised: is the establishment of the recombinant molecule influenced by the presence of an incompatible plasmid in the cell? Does the efficiency of establishment of the recombinant vary with the sequence composition of the insert? In order to address these questions, we measured the ability of isolated recombinant molecules (examples shown in Fig. 2) to transform *E. coli* cells harboring the same sequence insert. The *Cm*<sup>®</sup> plasmid containing the undeleted CTG-CAG sequence was excised and electro-processed into *E. coli* DH10B, and the homogenous, undeleted plasmid was used for all the transformation experiments. pBR322 derivatives containing TRS or non-repeating tracks were transformed into the appropriate *E. coli* strain by electroporation (35). The pBR322 and pFW25 vectors were electrophoresed into *E. coli* DH10B along with F107S (27, 38) and pAM1900 (containing tetracycline at 5 μg/ml), and the cultures were grown at 37 °C at a shaking rate of 100 rpm. When the cultures reached an absorbance (600 nm) of ~0.6 unit, an aliquot (1 ml) was inoculated into fresh 200 ml of LB (with tetracycline as before). The cultures were grown until they had reached an A600 amount of 0.7 units. A final, fresh 200 ml of LB (with tetracycline as before) was added to ensure that the cultures were cured of the TS plasmid pJW289 by several passages on plates incubated overnight.
TABLE I

| Cells harboring pBR322 derivatives | Frequency of transformation of E. coli harboring pBR322 derivatives by recombinant molecules containing DMPK or (CTG-CAG)98 or (CTG-CAG)67 (× 10−4) |
|-----------------------------------|--------------------------------------------------------------------------------------------------|
|                                   | (CTG-CAG)67 sequences (× 10−4) | (CTG-CAG)98 sequences (× 10−4) |
| Plasmid-less AB1157               | 6.2                               | 0.8                             | 2.4                             |
| Plasmid-less ECF005               | 1.1                               | 0.17                            | 2.8                             |
| pRW4870 (DMPK) in AB1157         | 10                                | 2.4                             | 2.4                             |
| pRW4870 (DMPK) in ECF005         | 4.0                               | 4.0                             | 17                              |
| pRW4827 (CTG-CAG)67 in AB1157    | 1.2                               | 1.7                             | 1.4                             |
| pRW4827 (CTG-CAG)67 in ECF005    | 0.2                               | 0.52                            | 6.1                             |
| pRW4898 (CTG-CAG)98 in AB1157    | 230                               | 120                             | 250                             |
| pRW4898 (CTG-CAG)98 in ECF005    | 73                                | 22                              | 74                              |

Frequency of Recombination of Triplet Repeats—Two plasmid systems have been used previously to measure the frequency of intermolecular recombination between homologous sequences (43). Here, two plasmids, each containing a specific triplet repeat insert, belonging to different incompatibility groups, were introduced sequentially into an appropriate host strain. The plasmids and the host strains were chosen such that neither plasmid could exist independently; although the replication origin of one plasmid was non-functional in the chosen strain, the presence of the other plasmid by itself was selected against using an appropriate antibiotic. Thus, the selection ensured the survival of only those cells in which the two plasmids had recombined to form a co-integrate that would replicate using the origin of one plasmid and would survive on the appropriate antibiotic using the antibiotic resistance gene from the other plasmid.

| RESULTS |

Biological Assay for the Apparent Frequency of Intermolecular Recombination—Two plasmid systems have been used previously to measure the frequency of intermolecular recombination between homologous sequences (43). Here, two plasmids, each containing a specific triplet repeat insert, belonging to different incompatibility groups, were introduced sequentially into an appropriate host strain. The plasmids and the host strains were chosen such that neither plasmid could exist independently; although the replication origin of one plasmid was non-functional in the chosen strain, the presence of the other plasmid by itself was selected against using an appropriate antibiotic. Thus, the selection ensured the survival of only those cells in which the two plasmids had recombined to form a co-integrate that would replicate using the origin of one plasmid and would survive on the appropriate antibiotic using the antibiotic resistance gene from the other plasmid.

Also, experiments in a strain that could support the independent replication of both plasmids served to establish a base line. Determination of the transformation efficiencies of the two strains was accomplished by transforming each strain with a control plasmid and by using these numbers to normalize the efficiencies obtained with the experimental plasmids. The recombination frequency was then calculated by comparing the number of colonies obtained after the two-step transformation of the two different strains normalized for the transformation efficiency differences.

This strategy requires that the following events must occur for the antibiotic resistant colonies to appear. First, the incoming plasmid must integrate into the resident plasmid by homologous recombination to link the antibiotic resistance gene to a functional replicon. Second, the resulting recombinant molecule must get established and be maintained in the cell in the face of competition from the pre-existing incompatible multi-copy plasmid. Experiments done previously by Bierner and co-workers (44) had suggested that the measurement of recombination frequencies could be seriously jeopardized by differences in the fitness between the parent and recombinant plasmids. Their studies using a Tus/Ter system showed that facilitated establishment of some recombinant plasmids occurred in part...
due to the reduction in the copy number of the resident incompatible parental plasmid. To investigate the possibility that similar processes may play a role in our intermolecular recombination system, we analyzed the influence of sequence composition and length on the establishment and maintenance of recombinant molecules in the face of competition from the resident incompatible parental plasmid. This was accomplished by measuring the relative efficiencies with which recombinant plasmids transform cells that already harbor an incompatible parental plasmid (see “Experimental Procedures”). Our results (described under “Experimental Procedures”) enabled the conclusion that plasmid establishment and maintenance do not significantly influence the measurement of recombination frequencies for the sequences investigated herein. Nevertheless, we describe the recombination frequencies as “apparent frequencies.”

Accordingly, we used a pFW25 vector that contains the R6K \( \gamma \) origin (\( \gamma \) ori) of replication (34). The \( \gamma \) ori is a unidirectional origin, which can function only in the presence of the \( \pi \) protein encoded by the \( \text{pir} \) gene (45). In E. coli ECF005 and ECF006, the \( \pi \) protein is expressed from a chromosomal \( \text{pir} \) gene, which is under the control of an arabinose promoter (see “Experimental Procedures”). The pFW25 plasmid also contains a chloramphenicol resistance marker. For the second plasmid, we used pBR322 which contains the \( \pi \) protein independent ColE1 origin of replication as well as genes conferring resistance to ampicillin and tetracycline.

In order to study the apparent frequency of intermolecular recombination between plasmids harboring triplet repeat sequences, derivatives of pBR322 containing different lengths of TRS were transformed into E. coli AB1157 and ECF005 (AB1157\( \text{pir} \)) cells. This was followed by a second transformation with pFW25 derivatives containing various TRS inserts (see “Experimental Procedures”). In E. coli ECF005, both plasmids can co-exist without recombining and can give rise to Tet\(^R\) and Cm\(^R\) colonies (Fig. 1). These plasmids can also recombine to form co-integrants at a certain frequency. To establish the frequency of these recombination events, the (CTG-CAG)-containing pBR322 and pFW25 derivatives were successively transformed into E. coli AB1157. Because this was a two-step transformation, the pBR322 derivative was already established in the cell and could therefore exist independently in the presence of tetracycline. The pFW25 derivatives cannot replicate in this strain because of the absence of the \( \pi \) protein. Thus, after the transformation of the Tet\(^R\) cells with the pFW25 derivatives, the only way to obtain Tet\(^R\), Cm\(^R\) colonies is by recombination between the TRS-harboring plasmids. This could only be due to co-integrants that not only replicate using the ColE1 replicon but also carry the chloramphenicol resistance gene. The apparent frequency of recombination was the fraction of plasmids co-existing in E. coli ECF005 that underwent recombination. Thus, the number of colonies obtained from E. coli AB1157 (Fig. 1B) divided by the number of colonies obtained from E. coli ECF005 (Fig. 1A) gives the apparent frequency of recombination between plasmids containing CTG-CAG tracts.

Because two different strains were used in the experiment, it was possible that their different transformation efficiencies could affect the results. Therefore, the efficiencies of transformation of both E. coli strains were normalized with pACYC184 as a control plasmid. The use of pACYC184 was advantageous because it contains the \( \pi \)-independent p15A origin of replication (40) and therefore can be maintained in both AB1157 as well as ECF005 strains. Also, pACYC184 has a chloramphenol-resistant gene that enables the conclusion that plasmid establishment and maintenance do not significantly influence the measurement of recombination frequencies for the sequences investigated herein.
Frequency of Recombination of Triplet Repeats

**Table II**

**Plasmids used in this study**

The two families of plasmids used in this study were derivatives of pBR322 and pFW25. The different lengths of the CTG-CAG tracts as well as two non-repeating sequences were cloned into the PvuII site of pBR322 or into the HincII site of pFW25 (for details see “Experimental Procedures”). Orientations I and II were defined (5–7) by the presence of CTG or CAG repeats, respectively, on the leading strand template for DNA replication. The (CTG-CAG)n58 and (CTG-CAG)n80 sequences are pure CTG-CAG tracts; however, (CTG-CAG)n2 contains two G to A interruptions at repeats 28 and 69 and (CTG-CAG)n7 contains 2 G to A interruptions at positions 28 and 59 (5). The right column shows the number of G to A interruptions in each repeat sequence. Orientations A and B (for the 354-bp non-repeating sequence fragment) were defined by the presence of a KpnI recognition site, in the sequence, distal or proximal to the origin of replication, respectively.

| pBR322 derivatives | Sequence | Orientation of TRS | pFW25 derivatives | Sequence | Orientation of TRS | Number of G → A interruptions |
|--------------------|---------|-------------------|-------------------|---------|-------------------|-----------------------------|
| pRW4827           | (CTG-CAG)67 | I                  | pRW4316          | (CTG-CAG)67 | I                  | 0                           |
| pRW4828           | (CTG-CAG)73 | II                 | pRW4317          | (CTG-CAG)73 | II                 | 0                           |
| pRW4334           | (CTG-CAG)73 | I                  | pRW4332          | (CTG-CAG)73 | I                  | 2                           |
| pRW4333           | (CTG-CAG)73 | II                 | pRW4331          | (CTG-CAG)73 | II                 | 2                           |
| pRW4898           | (CTG-CAG)98 | I                  | pRW4324          | (CTG-CAG)98 | I                  | 0                           |
| pRW4899           | (CTG-CAG)98 | II                 | pRW4323          | (CTG-CAG)98 | II                 | 0                           |
| pRW4412           | (CTG-CAG)175 | I              | pRW4318          | (CTG-CAG)175 | I                  | 2                           |
| pRW4313           | (CTG-CAG)175 | II              | pRW4319          | (CTG-CAG)175 | II                 | 2                           |
| pRW4829           | 564-bp λ DNA | A                  | pRW4322          | 564-bp λ DNA | A                  | 0                           |
| pRW4870           | 354-bp DMPK DNA | A              | pRW4325          | 354-bp DMPK DNA | A                  | 0                           |
| pRW4869           | 354-bp DMPK DNA | B              | pRW4329          | 354-bp DMPK DNA | B                  | 0                           |

In summary, the apparent frequency of recombination between plasmids containing the TRS or non-repeating sequences was calculated as shown in Equation 1.

\[
\text{apparent frequency} = \frac{\text{No. colonies from AB1157}}{\text{No. colonies from ECF005 (AB1157pir)}} \times R
\]

where \( R \) is the ratio of the efficiency of transformation of \( E. coli \) ECF005 to that of \( E. coli \) AB1157 by the control plasmid pACYC184.

In order to determine whether the triplet repeats had any influence on the apparent recombination frequency, it was necessary to estimate the capacity of homologous but non-repetitive sequences to recombine in this system. Therefore, pRW4870 and pRW4328 containing a 564-bp segment of the human DMPK gene or pRW4829 and pRW4322 harboring a 564-bp fragment of phage \( \lambda \) DNA (Table II) were transformed into \( E. coli \) AB1157 or ECF005. The apparent frequency of recombination between plasmids containing non-repeating sequences was calculated as the number of TetR and CmR colonies obtained from both \( E. coli \) strains divided by the number of TetR and CmR colonies obtained from \( E. coli \) AB1157, because pFW25 cannot replicate in AB1157, the non-replicative circle and give rise to CmR colonies when subjected to Cm selection.

Considering instabilities from the human genetics standpoint, the severity and age of onset of the TRS diseases has been correlated with an increase in the length of the repeats in certain genes in patients (1). The stability of CTG-CAG in plasmids in \( E. coli \) depends on the length of the repeats (5, 33). Recombination of the tracts has also been shown to depend on their length (27, 28). Furthermore, the relationship between the length of homology and the apparent frequency of recombination has been well established in various systems (46, 47). Therefore, we investigated the effect of CTG-CAG tract lengths on their recombination frequency.

The apparent frequency of recombination between the (CTG-CAG)n70 tracts in pRW4828 and pRW4317 is \( 100 \times 10^{-4} \) (Table III). Thus, these tracts recombine at half the frequency at which the (CTG-CAG)n80 tracts recombine \( (p = 0.007) \). Also, the apparent recombination frequency of the (CTG-CAG)n67 tracts, which have 317 bp of homology, is ~30-fold higher than that of the 354-bp control DMPK sequence. Thus, we conclude that a lengthening of the CTG-CAG tracts substantially increases the intermolecular homologous apparent recombination frequency.

**Orientation of the CTG-CAG Tracts Influences Recombination Frequency**—Previous in vivo studies demonstrated that the genetic instabilities of (CTG-CAG)n, sequences are determined by their orientation relative to the origin of replication (5–7, 33, 42). The expansions and deletions of the TRS due to replication were shown to be dependent on the location of the CTG tracts on the leading (orientation I) or on the lagging (orientation II) strand template (5–7, 33, 42). Also, the orientation of the (CTG-CAG)n repeats strongly influenced the pausing of the replication fork in vivo (48).

To test the effect of triplet repeat orientation on the apparent frequency of recombination, we performed experiments with the two (CTG-CAG)n80 tracts in orientations I or in orientations II (Table III, 3rd and 4th lines). Interestingly, the (CTG-CAG)n80
tracts recombined 3 times more frequently in orientations II than in orientations I (Table III) \( (p = 0.002) \). Similar results were obtained with the (CTG-CAG)\textsubscript{67} tracts; the two repeats in orientation II were twice as recombinogenic as in orientation I. The higher apparent recombination frequencies of sequences in orientation II were not due to lower transformation efficiencies for the EcoF005 strain in which both plasmids could replicate. For example, the ratio of the transformation efficiency of pRW4316 ((CTG-CAG)\textsubscript{67} in orientation I) to that of the control plasmid pACYC184 was 0.48 ± 0.2 in \textit{E. coli} EcoF005. The equivalent ratio for pRW4317 ((CTG-CAG)\textsubscript{67} in orientation II) was 0.41 ± 0.4. In another experiment, the measured ratios for pRW4323 ((CTG-CAG)\textsubscript{98} in orientation II) and pRW4324 ((CTG-CAG)\textsubscript{98} in orientation I) were 3.0 ± 0.5 and 2.6 ± 1.0, respectively. Thus, there was no significant influence of triplet repeat orientation on the transformation efficiency.

Because orientation is defined relative to the origin of replication, the strong influence of orientation on the apparent recombination frequency suggested a role for DNA replication in the process. However, it was also possible that inverting the orientation of any sequence on a plasmid might influence the recombination frequency. To test this idea, we constructed pBR322 and pFW25 derivatives in which the 354-bp human DMPK gene sequences were oriented in the reverse orientations (orientations B) compared with pRW4870 and pRW4328 (orientations A) (defined in Table II). The recombination assay revealed no difference in the recombination frequencies for the two orientations of the DMPK fragment (Table III). Thus, these data strongly suggest that replication has a role in the increased apparent recombination frequency observed for the repeats in orientations II (see “Discussion”).

To elucidate further the role of replication on the homologous recombination frequency, we conducted experiments with the repeats in opposite orientations. We reasoned that because the pBR322 derivatives replicate in AB1157, the orientation of the (CTG-CAG)\textsubscript{67} tract in this vector would dictate the apparent recombination frequency. Hence, we performed a recombination assay in \textit{E. coli} AB1157 with a pBR322 derivative, pRW4899, containing the (CTG-CAG)\textsubscript{98} in orientation II and a pFW25 derivative, pRW4324, with the TRS in orientation I (Table II). Also, similar experiments were conducted with pRW4898 and pRW4323 wherein the (CTG-CAG)\textsubscript{98} tracts were in orientations I and II, respectively (Table II). We were surprised to find that, in general, the apparent recombination frequency between the plasmids containing repeating tracts in the opposite orientations was lower than between plasmids harboring TRS in the same orientation (Table III). To determine whether this behavior also extended to non-repeating sequences, we measured the apparent recombination frequency between the derivatives of pBR322 and pFW25 that contained the 354-bp DMPK fragment in orientation A and orientation B, respectively. The opposing orientations of the DMPK fragment had no influence on the homologous apparent recombination frequency. The frequency of recombination between the DMPK fragments in orientation A in pBR322 and in orientation B in pFW25 was 4.1 × 10^{-4}. For the reciprocal cross, the frequency of recombination between DMPK fragments in orientation B in pBR322 and in orientation A in pFW25 was 2.8 × 10^{-4}. By using the same two-plasmid system, other experiments were conducted to determine the frequency of intermolecular recombination between two (GAA-TTC)\textsubscript{176} or two (GAA-TTC)\textsubscript{60} tracts. The results revealed that the relative orientations of two GAA-TTC repeat tracts had no influence on the recombination frequency. Thus, the inhibition of recombination between oppositely oriented tracts appears to be a (CTG-CAG) triplet repeat-dependent phenomenon (see “Discussion”). Nevertheless, when the tracts were in opposite orientations, the apparent recombination frequency was the highest when the (CTG-CAG)\textsubscript{98} repeats in the replicating plasmid (the pBR322 derivative) were in orientation II (Table III).

These results clearly demonstrate that the intermolecular apparent recombination frequency is influenced by the orientation of the repeating tracts and is significantly higher when the CAG tract is on the leading strand template for pBR322 derivatives. These findings suggest that events that occur during replication, presumably replication fork arrest and pausing at unusual DNA structures (48–50), could stimulate intermolecular homologous recombination between the triplet repeat tracts.

Interruptions in the CTG-CAG Tracts Decrease the Recombination Frequency—Previous studies (51) suggested that interruptions stabilized the TRS sequences by interfering with the formation of slipped strand structures. It was also shown that interruptions in the repeating tracts inhibit the recombination between TRSs (27, 28). Hence, we postulated that interruptions in the (CTG-CAG)\textsubscript{67} tracts would reduce their recombination frequency. To test this hypothesis, we assayed the apparent frequency of recombination for CTG-CAG tracts containing G to A interruptions (Table IV). In the case of (CTG-CAG)\textsubscript{176}, the

### Table III

| pBR322 derivatives | Inserts | Orientation of (CTG-CAG)\textsubscript{67} | pFW25 derivatives | Inserts | Orientation of (CTG-CAG)\textsubscript{98} | Frequency of recombination x 10^{-4} (S.D.) |
|-------------------|--------|-----------------------------------------|----------------|--------|-----------------------------------------|---------------------------------------------|
| pRW4827           | (CTG-CAG)\textsubscript{67} | I | pRW4316 | (CTG-CAG)\textsubscript{67} | I | 317 | 50 (36) |
| pRW4828           | (CTG-CAG)\textsubscript{67} | II | pRW4317 | (CTG-CAG)\textsubscript{67} | II | 317 | 100 (26) |
| pRW4898           | (CTG-CAG)\textsubscript{98} | I | pRW4324 | (CTG-CAG)\textsubscript{98} | I | 410 | 60 (28) |
| pRW4899           | (CTG-CAG)\textsubscript{98} | II | pRW4323 | (CTG-CAG)\textsubscript{98} | II | 410 | 190 (15) |
| pRW4898           | None | NA \(^a\) | pFW25 | None | NA | NA | 0.02 |

\(^a\) Data are based on two experiments.  
\(^b\) NA, not applicable.

2 M. Napierala, R. Dere, and R. D. Wells, unpublished data.
apparent frequency of recombination was \( \sim 2 \times 10^{-4} \); there was no significant effect of orientation on the frequencies. Because these tracts had 654 bp of homology, we used a 564-bp non-repetitive sequence from the genome of bacteriophage \( \lambda \) as a control. The control sequence recombined at a frequency of \( 55 \times 10^{-4} \). Thus, the frequency of recombination of the triplet repeat tract was \( \sim 25 \)-fold less than observed for the phase \( \lambda \) fragments.

It was possible that the presence of the interruptions caused the \( 25 \)-fold lower frequency of recombination. However, an examination of the \( \lambda \) sequence revealed that it was \( \sim 60\% \) A + T-rich (data not shown). Because A + T-rich regions are known to be highly recombinogenic (52), the lower frequency of recombination might have been, in part, due simply to a higher recombinogenicity of the \( \lambda \) sequence. To clarify this, we determined the apparent recombination frequency between the 335-bp (CTG\(_{\lambda\text{77}}\/)H\_11011\) inserts that contain two G to A interruptions. The frequency was \( \sim 6 \times 10^{-5} \). This was \( 5 \)-fold less than compared with the 354-bp long DMPK sequence (Table III). Thus, we conclude that interruptions do, in fact, reduce the recombination frequencies between the triplet repeat tracts.

Interestingly, we also observed that the frequency of recombination between an interrupted (CTG\(_{\lambda\text{77}}\/)tract and an uninterrupted pure tract containing (CTG\(_{\lambda\text{77}}\/)was \( 5.8 \times 10^{-4} \) (Table IV). This was about 2-fold higher than the frequency measured between two interrupted tracts containing (CTG\(_{\lambda\text{77}}\/). Thus, a G to A interruption in one of the tracts reduces the recombination frequency but to a lesser extent than when both recombining tracts have the interruption.

We postulate that the G to A interruptions generate G-T and A-C mismatches during the formation of heteroduplex recombination intermediates. These intermediates may be destabilized by the mismatch repair system, thereby reducing the recombination frequency (see “Discussion”).

### Table IV

| pBR322 derivatives | Inserts | Orientation of (CTG\(_{\lambda\text{77}}\)//H\_11011\) | pFW25 derivatives | Inserts | Orientation of (CTG\(_{\lambda\text{77}}\)//H\_11011\) | bp of homology | Frequency of recombination \( \times 10^{-4} \) (S.D.) |
|-------------------|---------|-----------------------------------------------|-------------------|---------|-----------------------------------------------|-------------|-----------------------------------------------|
| pRW4334 I         | (CTG\(_{\lambda\text{73}}\/) | I                                             | pRW4332 I         | (CTG\(_{\lambda\text{73}}\/) | I                                             | 335         | 0.69 (0.25)                                   |
| pRW4333 I         | (CTG\(_{\lambda\text{73}}\/) | I                                             | pRW4331 I         | (CTG\(_{\lambda\text{73}}\/) | I                                             | 335         | 0.63 (0.19)                                   |
| pRW4312 I         | (CTG\(_{\lambda\text{77}}\/) | I                                             | pRW4316 I         | (CTG\(_{\lambda\text{77}}\/) | I                                             | 317         | 5.8 (2.1)                                    |
| pRW4312 I         | (CTG\(_{\lambda\text{77}}\/) | I                                             | pRW4318 I         | (CTG\(_{\lambda\text{77}}\/) | I                                             | 654         | 1.8 (1.5)                                    |
| pRW4313 I         | (CTG\(_{\lambda\text{77}}\/) | I                                             | pRW4319 I         | (CTG\(_{\lambda\text{77}}\/) | I                                             | 654         | 2.2 (1.6)                                    |

### Intermolecular Recombination Is a RecA-dependent Process

It was demonstrated previously (53) that intermolecular recombination between two compatible plasmids is diminished in RecA-deficient cells. This stands in contrast to intramolecular recombination that was demonstrated to occur by both RecA-dependent and -independent processes (26, 53, 54). In order to determine whether intermolecular homologous recombination between TRSs involved the RecA protein, we measured the apparent recombination frequency between CTG-CAG tracts in RecA+ cells (E. coli JC10287). The plasmids containing (CTG-CAG\(_{\lambda\text{77}}\/) or (CTG-CAG\(_{\lambda\text{77}}\/) were sequentially transformed into E. coli JC10287 as well as into ECF006 (Fig. 1, for details see “Experimental Procedures”). Similar experiments were performed using plasmids harboring non-repeating sequences (the 564 bp \( \lambda \) DNA fragment and the 350-bp DMPK fragment). For repeating as well as non-repeating sequences, we did not observe any recombination events (data not shown).

Our assay allows the selection for recombination events that take place with a frequency of \( 10^{-6} \) or higher. Therefore, we cannot rule out the possibility that recombination does take place in the recA cells but with a frequency of \( < 10^{-6} \).

In summary, our results agree with previous observations (27, 28, 53) demonstrating that intermolecular recombination between homologous sequences is greatly reduced in recA cells.

### A Single Crossover Event between Two Plasmids Occurs through CTG-CAG Tracts

In order to characterize the products of recombination, we analyzed the plasmids recovered from E. coli AB1157 and ECF005 cells that had been transformed with the pBR322 and pFW25 derivatives. Thus, pRW4312 containing (CTG-CAG\(_{\lambda\text{77}}\/) and pRW4316 harboring (CTG-CAG\(_{\lambda\text{77}}\/) were transformed sequentially into E. coli AB1157 and ECF005 (e.g. E. coli AB1157 and ECF005 were each transformed with pRW4312; subsequently, these cells harboring this plasmid were also transformed with pRW4316). The plasmids were isolated from the TetR and CmR colonies obtained from both E. coli strains.

Agarose gel electrophoresis of the supercoiled DNA revealed the presence of monomers of pRW4312 and pRW4316 as well as their multimeric forms when the plasmid DNAs were isolated from E. coli ECF005 (Fig. 2, lanes 7–12). This result was as expected because both pBR322-derived constructs as well as the pFW25 derivatives can co-exist and replicate independently in ECF005. Restriction analysis (see below) of these plasmids revealed that recombination (single crossover events) had occurred between the two plasmids (data not shown). These events were observed for plasmids of all lengths. Thus, plasmids that can co-exist independently in a cell also undergo homologous recombination.

When the DNAs were isolated from E. coli AB1157, the monomeric form of pRW4316 (pFW25 derivative) was not present (Fig. 2, lanes 1–6). This was in agreement with previous findings that the \( \pi \) protein is essential for the replication and maintenance of R6K plasmids. Interestingly, the monomer of pRW4312 was able to co-exist (Fig. 2, lanes 1–6) with the recombinant (co-integrant) species (composed of pRW4316 and pRW4312). The co-existence of pRW4312 and the recombinant plasmid, despite both being replicated by the ColE1 replicon, may be attributed to the ability of antibiotic selective pressure to overcome the statistical plasmid incompatibility observed in the absence of selection (55, 56).

It should be noted that plasmid multimers were observed in both AB1157 and ECF005. The pBR322 constructs, the pFW25 derivatives, as well as the recombinant co-integrants were all able to form multimers efficiently (Fig. 2 and data not shown). Therefore, we can rule out the possibility that some selective advantage accrued to some of the constructs that might have skewed the data.

To analyze the products of the recombination process, plasmids obtained from E. coli AB1157 were digested with SnaBI and NdeI that have unique recognition sites on pRW4316 and
pRW4312, respectively (Fig. 3). Digestion of the individual plasmids with these enzymes resulted in the expected linear products (Fig. 3, lanes 9 and 10). For the recombination products, those plasmids that underwent a single crossover would be expected to release a "short fragment" containing one copy of recombinant triplet repeat tract as well as a "long fragment" harboring the second copy of the TRS with the remaining vector sequence (Fig. 3). If the crossover took place within the triplet repeats, the short fragment would contain a CTG/H18528/CAG tract flanked on one side by 294 bp of non-repeating human sequence plus a segment of pBR322, and on the other side by 106 bp consisting of the non-repetitive human sequence and a fragment of pFW25. The size of this short fragment would be 601 bp if the fragment contained 67 repeats of CTG/H18528/CAG tract or 925 bp if the fragment harbored the (CTG/H18528/CAG)175 tract. In all cases, the sizes of the bands were in this range suggesting that the fragments did indeed contain the TRS (Fig. 3). For a detailed analysis of the triplet repeat lengths and instabilities, see Fig. 6 (discussed below). The size of the long fragment was 7500 bp. In all cases, the band corresponding to the linear pRW4312 was present, as expected. Because the transformations were done in a two-step manner, pRW4312 could continue to exist in the cell as a monomer and give rise to the linear product after digestion.

In order to conclusively establish that the "short bands" contain CTG-CAG tracts, the restriction fragments were iso-
lateral from the gel and sequenced. The DNA sequence analysis confirmed that all the fragments contained the CTG-CAG tracts, as expected (data not shown).

These results clearly demonstrate that pRW4312 and pRW4316 recombine through the CTG-CAG tracts by a single crossover event and give rise to the TetR and CmR colonies. The lack of additional unique recognition sites on both plasmids did not allow us to release the second TRS-containing recombinant fragment present in the ~7500-bp linear molecule (Fig. 3).

In this assay, ~5% of colonies of AB1157 after the two-step transformation contained only trace amounts of the co-integrant plasmids, which were isolated and characterized (data not shown). The restriction analysis for single crossover events performed on these DNAs revealed the existence of little or no TRS-containing recombinant fragment. It is possible that the CmR colonies arose due to the integration of the pFW25 derivatives into the chromosome in the presence of Cm selection.

Assay for a Second Crossover Event — The co-integrants obtained as a result of a single crossover between pRW4312 and pRW4316 can replicate in the cell using the ColE1 origin of replication. These recombinant molecules are able to recombine further via a second intermolecular crossover event (as shown in Fig. 4). If the second crossover takes place, the sequences flanking the TRS for the short bands should both be derived from either pBR322 or from pFW25. This would also be the case for 4, 6, and higher even-numbered crossover events. To identify even numbers of crossovers, HindIII or NdeI/XmnI digests were performed. The NdeI/XmnI restriction releases the TRS-containing insert from pRW4912, whereas the HindIII digestion excises the CTG-CAG-containing tracts from pRW4316. Because pRW4316 cannot exist in E. coli AB1157 by itself, the only way to possibly excise a TRS-containing insert with HindIII is from products of recombination between co-integrants as shown in Fig. 4. In seven of eight cases (Fig. 4), we observed that the second crossover did indeed take place for pRW4312 and pRW4316. These co-integrants can also undergo intramolecular recombination because each molecule contains two copies of the TRS (31). We were unable to assay for these events due to the lack of selection for the products formed.

The assay for an even number of crossover events was performed for all lengths of triplet repeat inserts. For (CTG-CAG)7, in 3 of 30 analyzed colonies, even-numbered crossover events were observed. Similarly, 4 of 35 colonies were scored for such events in the case of the (CTG-CAG)14 tract. Thus, these results demonstrate that the co-integrants can further recombine through subsequent intermolecular crossover events.

Instability of the CTG-CAG Tracts in the Recombination Products — In order to study the instability of (CTG-CAG)n tracts, we analyzed ~30 colonies obtained from E. coli AB1157 after successive transformation with pBR322 containing (CTG-CAG)n tracts and pFW25 harboring (CTG-CAG)n sequence. The plasmids were assayed for an odd number of crossover events with SnaBI/NdeI digestion. Fig. 5A shows the analyses of products (co-integrants) after single or odd numbers of crossover events, which took place between pRW4827 and pRW4316 (both contain (CTG-CAG)77 tracts). The length of the products after SnaBI/NdeI digestion (short fragments) should be 601 bp if the band contains 67 repeats of CTG-CAG tract or 802 for the band harboring 134 repeats (e.g. twice the length of (CTG-CAG)77). This would be possible if the crossover took place on the distal ends of the repeating tracts on both plasmids and the recombinant products contained both TRSs. The increase in the length of TRS could also be explained by the expansions of repeating tracts during the recombination process. In all cases, the sizes of the recombination products were in this length range. These products (after single or odd numbers of crossovers) differed in length because the crossover could take place in different parts (registers) of the repeating tracts. Thus, the products of recombination between two (CTG-CAG)77 tracts ranged from (CTG-CAG)14 (deletion of 40 repeats) to (CTG-CAG)104 (expansion by 37 repeats) (Fig. 6A).

Similar data were obtained from co-integrants between pRW4898 and pRW4324 (both harbor (CTG-CAG)14 tracts). The length of the TRS containing recombinant products after SnaBI/NdeI digestion should be 694 bp if the fragments contained 98 repeats of CTG-CAG or 888 bp if the fragments harbored 196 repeats (two copies of the CTG-CAG tracts) (Fig. 5B). The products obtained after a single crossover varied in length from (CTG-CAG)15 (deletion of 83 repeats) to (CTG-CAG)143 (ex-
Frequency of Recombination of Triplet Repeats

We have shown by using a biological assay that the apparent frequency of homologous intermolecular recombination between two CTG-CAG tracts is up to 60-fold higher than between two non-repeating sequences of similar length. The data also reveal the following. First, the apparent frequency of recombination is proportional to the length of the repeating tract; the longer the tracts, the higher the recombination frequency. Second, the frequency depends on the orientation of the CTG-CAG sequences; recombination is more frequent when the TRS in orientation II relative to the origin of replication (CTG tracts on the lagging strand template). Third, sequence polymorphisms that interrupt the homogeneity of the TRS are in orientation II relative to the origin of replication. Fourth, the recombination process can genetically destabilize the CTG-CAG tracts and result in both expansions and deletions. However, expansion products that are longer than the sum of the lengths of the two individual tracts were not observed (discussed below).

Repetitive sequences have been observed previously to stimulate homologous recombination (15–22, 57). The formation of unusual secondary structures such as left-handed Z-DNA and intramolecular triplexes was proposed to be responsible for the association of these sequences with recombination hot spots (16, 17, 58). In fact, triplexes can stabilize branch migration intermediates in vitro, suggesting a stimulatory role for these structures in recombination (59). Also, perfect inverted repeat sequences (which form cruciform structures (60)) stimulate recombination in bacteria (61, 62) and yeast (63). Triplet repeat sequences can form hairpin-loop (5–13, 64–66), tetraplex (67), and slipped (51) structures. The CTG-CAG repeats also exist in a highly flexible and writhed configuration (68–70), a property proposed to serve as a sink for localized negative supercoil density at these sequences and thereby promote duplex unpairing and strand slippage (32, 71). We hypothesize that the adoption of unusual secondary structures by the CTG-CAG tracts stimulates intermolecular recombination between homologous tracts. This is in contrast to the lower apparent recombination frequency observed for non-repeating sequences of similar length, which are unlikely to form unusual secondary structures. We favor the idea that the high negative supercoil density at the CTG-CAG repeats causes them to be transiently unpaired, whereas RecA mediated strand exchange may occur more frequently. However, we cannot rule out the alternate possibility that the higher recombinogenicity of the CTG-CAG repeats is because repetitive sequences can align with each other in multiple frames during the strand exchange reaction.

Surprisingly, the frequencies of recombination between non-repeating sequences with a pair of 354-bp DMPK fragments
and a pair of 564-bp bacteriophage λ DNA fragments were not the same. The frequency was 17-fold lower for the 354-bp DMPK fragments. The reason for this difference is uncertain, but an examination of the compositions of these sequences revealed that the phage λ fragment is 60% A + T-rich. In contrast, the DMPK sequence is 60% G + C-rich, and the (CTG-CAG)<sub>67</sub> repeats are 67% G + C-rich. Several prior studies (52, 72–74) have suggested that A + T-rich sequences are recombinogenic. Therefore, it is possible that the 60% A + T richness of the λ phage fragments (contrasted to the 40% A + T content of the DMPK sequence) is at least partly responsible for the higher apparent recombination frequency for the 564-bp λ fragments. Furthermore, the frequency of homologous recombination strongly corresponds to the length of the homology between the recombining sequences (46, 47, 75, 76). The phage λ fragment is 60% (210 bp) longer than the DMPK sequence. These two differences may be sufficient to increase the recombination frequency of the phage λ fragments. Neither the DMPK sequence nor the phage λ fragment contains CRS sites.

Our data show that the apparent recombination frequency of the CTG-CAG tracts depends on their orientation relative to the origin of replication. In orientation II (when the CTG tract is on the lagging strand template), the apparent recombination frequency was substantially higher. Because the two plasmids were introduced successively into the cell, we propose that the replication of the CTG-CAG repeats in the pBR322 derivatives (which were introduced in the first step) influences the recombination frequency. The CTG-CAG tracts arrest replication fork progression in vitro and in vivo, presumably due to the formation of unusual secondary structures (48–50, 67); this occurs predominantly when the CTG tract is located on the lagging strand template (orientation II) (48, 77). Hence, we propose a model wherein the stalling of the replication fork at the secondary structures leads to nicks and/or double-strand breaks in the repeating tract. Discontinuities in the duplex right-handed B-DNA structure inhibit replication fork progression (78, 79), and stalled replication forks induce DNA repair and recombination (78, 80, 81). The recombination proteins may then be recruited to the TRS loci due to the affinity of these proteins to unusual secondary structures or to the strand discontinuities. These events could result in the higher apparent recombination frequency for CTG-CAG

![Diagram of recombination frequency](http://www.jbc.org/)

**FIG. 6.** Distribution of the expansion and deletion products of different lengths of (CTG-CAG) after an odd number of crossovers. Several individual clones containing different lengths of the (CTG-CAG) tracts were isolated after an odd number of crossover events. The lengths of the (CTG-CAG)-containing fragments (as shown on Fig. 5) were measured, and the numbers of triplets were calculated as described (42). A, 26 individual clones after recombination involving (CTG-CAG)<sub>67</sub> versus (CTG-CAG)<sub>98</sub> (●) and 24 isolates of (CTG-CAG)<sub>67</sub> versus (CTG-CAG)<sub>175</sub> (▼) were analyzed. The measured lengths of the recombination products obtained after an odd number of crossover events are represented on the y axis as an increase or decrease in the number of repeats compared with the original starting length. Also, 23 clones of (CTG-CAG)<sub>67</sub> versus (CTG-CAG)<sub>175</sub> were characterized, and the lengths of the products are represented as changes in the length of (CTG-CAG)<sub>67</sub> (●) or in that of (CTG-CAG)<sub>175</sub> (■). The x axis depicts the numerical names that were arbitrarily assigned to the individual clones isolated from experiments involving each set of molecules. B, a quantitative description of the instability of the triplet repeat tracts after recombination was made by calculating the percentage of individual recombination products from A that harbored expanded, deleted, or unchanged TRS tracts for each set of recombining molecules.
tracts in orientation II. This model is supported by the observation of double-strand breaks in CTG-CAG repeats in yeast (82, 83).

A surprising observation was that when two inserts were oriented oppositely with respect to each other, the recombination frequency was substantially lower than when the inserts were in the same orientation. This effect was exclusively limited to inserts containing CTG-CAG repeats; oppositely oriented homologous control sequences from the DMPK locus as well as the GAA/TTG sequences recombined with frequencies similar to each other. The reason why the relative orientations of the CTG-CAG repeats have such a dramatic effect on their recombinogenicity is uncertain but may be due to a residual amount of replication from the R6K origin. However, literature exists (45, 84) that argues against this possibility. Another possibility, albeit remote, is that the secondary structure of the triplet repeat tract is somehow different in orientation I as compared with orientation II and that these differences may present a barrier to the recombination machinery. Because in vivo determinations of DNA conformations are exceedingly difficult (reviewed in Refs. 60 and 85), substantial new experimental strategies may need to be developed to address this question.

The apparent recombination frequency between the CTG-CAG repeats containing CTA/TAG interruptions was lower than the frequency with pure uninterrupted tracts. We postulate that the presence of interruptions results in the formation of imperfectly aligned heteroduplex recombination intermediates that contain G-T and A-C mismatches at the sites of the interruptions. These mismatches may attract the mismatch repair proteins MutS and MutL (42), which can inhibit RecA-mediated strand transfer (86). Thus, the recombination intermediates may be destabilized by the mismatch repair system, thereby diminishing the recombination frequency. This model is consistent with previous work (87, 88) that showed that recombination between homologous sequences was stimulated by the mismatch repair proteins MutS and MutL (42), which can inhibit intermediates that contain G-T and A-C mismatches at the sites.

TAG interruptions was...
Frequency of Recombination of Triplet Repeats

43. Michel, B., Niaudet, B., and Ehrlich, D. (1983) Plasmid 10, 1–10
44. Bierne, H., Ehrlich, D., and Michel, B. (1995) Plasmid 33, 101–112
45. Chen, D., Feng, J., Kruger, R., Urb, M., Inman, R. B., and Filutowicz, M. (1996) J. Mol. Biol. 262, 775–787
46. Singer, B. S., Gold, L., Gaus, P., and Doherty, D. H. (1982) Cell 31, 25–33
47. Shen, P., and Huang, H. V. (1986) Genetics 112, 441–457
48. Samadashwili, G. M., Raza, G., and Mirkin, S. M. (1997) Nat. Genet. 17, 298–304
49. Kang, S., Ohshima, K., Shimizu, M., Amirhaeri, S., and Wells, R. D. (1995) J. Biol. Chem. 270, 27014–27021
50. Ohshima, K., and Wells, R. D. (1997) J. Biol. Chem. 272, 16798–16806
51. Pearson, C. E., Eichler, E. E., Lorenzoetti, D., Kramer, S. F., Zoghbi, H. Y., Nelson, D. L., and Sinden, R. R. (1998) Biochemistry 37, 2701–2708
52. Svetlova, E. Y., Razin, S. V., and Debatisse, M. (2001) J. Cell. Biochem. 36, (suppl.) 170–178
53. Laban, A., and Cohen, A. (1981) Mol. Gen. Genet. 184, 200–207
54. Bi, X., and Liu, L. F. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 819–823
55. Novick, R. P. (1987) Microbiol. Rev. 51, 381–395
56. Nordstrom, K., and Austin, S. J. (1989) Annu. Rev. Genet. 23, 37–69
57. Benet, A., Molla, G., and Azorin, F. (2000) Nucleic Acids Res. 28, 4617–4622
58. Rooney, S. M., and Moore, P. D. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 2141–2144
59. Benet, A., and Azorin, F. (1999) J. Mol. Biol. 294, 851–857
60. Sinden, R. R. (1984) DNA Structure and Function, Academic Press, San Diego
61. Leach, D. R., Okely, E. A., and Pinder, D. J. (1997) Mol. Microbiol. 26, 597–606
62. Cromie, G. A., Miller, C. B., Schmidt, K. H., and Leach, D. R. (2000) Genetics 154, 513–522
63. Lobachev, K. S., Sher, B. M., Tran, H. T., Taylor, W., Keen, J. D., Resnick, M. A., and Gordenin, D. A. (1998) Genetics 148, 1507–1524
64. Chen, X., Huang, X., Smelt, R. K., and Zheng, M. (1998) in Genetic Instabilities and Hereditary Neurological Diseases (Wells, R. D., and Warren, S. T., eds) pp. 623–646, Academic Press, San Diego
65. Mariappan, S. V. S., Chen, X., Catasti, P., Bradbury, E. M., and Gupta, G. (1999) in Genetic Instabilities and Hereditary Neurological Diseases (Wells, R. D., and Warren, S. T., eds) pp. 647–676, Academic Press, San Diego
66. Mitas, M. (1997) Nucleic Acids Res. 25, 2245–2253
67. Umeda, K., and Watanabe, J. I. (1997) Nucleic Acids Res. 23, 4202–4209
68. Bacolla, A., Geliboblian, R., Shimizu, M., Amirhaeri, S., Kang, S., Ohshima, K., Larson, J. E., Harvey, S. C., Stollar, B. D., and Wells, R. D. (1997) J. Biol. Chem. 272, 16783–16792
69. Geliboblian, R., Bacolla, A., and Wells, R. D. (1997) J. Biol. Chem. 272, 16793–16797
70. Chastain, P. D., and Sinden, R. R. (1998) J. Mol. Biol. 275, 405–411
71. Bacolla, A., Bowater, R. P., and Wells, R. D. (1998) in Genetic Instabilities and Hereditary Neurological Diseases (Wells, R. D., and Warren, S. T., eds) pp. 467–484, Academic Press, San Diego
72. Hyrien, O., Dehaes, M., Buttin, G., and de Saint Vincent, B. R. (1987) EMBO J. 6, 2401–2408
73. Roch, F. A., Hobi, R., Berchtold, M. W., and Kuenzle, C. C. (1997) Nucleic Acids Res. 25, 2303–2310
74. Gerton, J. L., Delisi, J., Shroff, R., Lichten, M., Brown, P. O., and Petes, T. D. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 11383–11390
75. Watt, V. M., Ingles, C. J., Urdea, M. S., and Rutter, W. J. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 4768–4772
76. Fujitani, Y., Yamamoto, K., and Kobayashi, I. (1995) Genetics 140, 797–809
77. Iyer, R. R., and Wells, R. D. (1999) Chemtracts: Biochem. Mol. Biol. 12, 724–733
78. Cox, M. M. (1998) Genes Cells 3, 65–78
79. Kuzminov, A. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 8241–8246
80. Haber, J. E. (1999) Trends Biochem. Sci. 24, 271–275
81. Michel, B., Flores, M.-J., Viguera, E., Grompone, G., Seigneur, M., and Bidnenko, V. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 8181–8188
82. Freudenreich, C. H., Kantrow, S. M., and Zakian, V. A. (1998) Science 278, 853–856
83. Jankowski, C., Nasar, F., and Nag, D. K. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 2134–2139
84. Izuruka, M., and Helinski, D. R. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 5381–5385
85. Wells, R. D. (1988) J. Biol. Chem. 263, 1095–1098
86. Worth, L., Clark, S., Radman, M., and Modrich, P. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 3238–3241
87. Rayesniger, C., Thaler, D. S., and Radman, M. (1989) Nature 342, 396–401
88. Petit, M.-A., Dimpf, J., Radman, M., and Echols, H. (1991) Genetics 129, 327–332
89. Sopher, B. L., Myrick, S. B., Hong, J. Y., Smith, A. C., and La Spada, A. R. (2000) Gene (Amst.) 261, 383–390
Long CTG·CAG Repeats from Myotonic Dystrophy Are Preferred Sites for Intermolecular Recombination
Anna Pluciennik, Ravi R. Iyer, Marek Napierala, Jacquelynn E. Larson, Marcin Filutowicz and Robert D. Wells

J. Biol. Chem. 2002, 277:34074-34086.
doi: 10.1074/jbc.M202127200 originally published online June 26, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M202127200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 82 references, 46 of which can be accessed free at http://www.jbc.org/content/277/37/34074.full.html#ref-list-1