Stringent sequence requirements for the formation of human telomeres

(artificial chromosome/chromosome breakage/telomerase/telomeric protein/telomere healing)

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ABSTRACT In human cells, transfaction of telomeric T2A2G repeats induces the formation of functional telomeres at previously interstitial sites. We report that telomere formation has stringent sequence requirements. While (T2A2G)n telomere seeds formed telomeres in ~70% of the transfected cells, five T2A-G-related heterologous telomeric DNAs seeded new telomeres in <5% of the transfectants. Telomere formation did not correlate with the ability of human telomerase to elongate telomeric sequences in vitro. Homologous recombination is probably also not involved because a (T2A2G)n telomere seed with nontelomeric DNA at 160-bp intervals formed new telomeres frequently. Instead, the sequence dependence of telomere formation matched the in vitro binding requirements for the mammalian T2A2G3 repeat binding factor (TRF). Human TRF failed to bind ineffective heterologous telomere seeds and had a 4-fold lower affinity for (T2A2G)2T2A2G3 repeats, which seeded telomeres with reduced frequency. The results suggest that telomere seeds interact with TRF and predict that mammalian artificial chromosomes will require wild-type telomeric repeats at, or near, their termini.

The telomeres of most eukaryotic chromosomes carry a tandem array of short repeats, which are thought to form a protective nucleoprotein complex at chromosome ends (1, 2). The telomeric complex protects detection of chromosome ends by DNA damage checkpoints and protects the termini from exonucleases and ligases (1-3). In addition, telomeric DNA is important for the replication of linear chromosomes. Chromosome ends are predicted to shorten gradually with cell divisions due to the fixed direction of chromosomal DNA replication and the requirement for a primer. Telomeres can counter this effect by engaging telomerase, a ribonucleoprotein reverse transcriptase that adds telomeric repeat DNA to 3' telomere termini (reviewed in refs. 4 and 5).

Human telomeres contain several kilobase pairs of the highly conserved sequence (T2A2G)n with the G-rich strand oriented 5' → 3' toward the chromosome ends (6-9). These telomeric tracts progressively shorten from ~10 to ~1.5 kb during normal development, as well as in primary tumors, and in normal cells undergoing in vitro senescence (refs. 9-11; reviewed in refs. 5 and 12). As expected from the attrition of their telomeres, primary human cells lack telomerase activity (11). In contrast, immortal human cells express telomerase and the decline of their telomeres is halted (5, 11-14).

Human telomeres are expected to interact with specific telomeric proteins in addition to telomerase. Telomeric factors and specialized telomeric chromatin have been demonstrated in Saccharomyces cerevisiae and several ciliates (reviewed in refs. 1, 2, and 15). Human telomeres display an unusual chromatin structure and are attached to the nuclear matrix; a highly conserved candidate telomeric protein (TRF; T2A2G repeat binding factor) has been identified in mammalian cells (16-18).

The functional significance of telomeric repeats is demonstrated by the formation of new mammalian telomeres after transfaction of linear DNAs with a terminal (T2A2G)n stretch (19-21). These telomeric seeds become linked through internal sequences by illegitimate recombination between interstitial DNA and the nontelomeric end of the transfected molecules (refs. 19-21; this report). The resulting fragmented chromosomes apparently become stabilized when the (T2A2G)n DNA is recognized as a telomere seed and heals into a functional telomere. We have examined the cis-acting requirements for telomere formation in human HeLa cells. The results indicate that subtle alterations in the sequence of telomere seeds interfere with their conversion into functional telomeres. The sequences required for telomere formation match the binding site of TRF and implicate this factor in the formation of new telomeres in human cells.

MATERIALS AND METHODS

Construction of Telomere Seeds. Short telomeric arrays were derived from previously described plasmids (18) or formed by self-ligation of synthetic DNAs. Telomere seed plasmids were constructed by tandem ligation of these short (250-800 bp) telomeric repeat segments in pSXneo (see Fig. 1a and ref. 18). pSXneo-1.6-T2A2G (Fig. 1a) contains two tandem 0.8-kb T2A2G3 repeat arrays separated by a 23-bp nonrepeat polylinker segment. pSXneo-3.2-T2A2G3 contains four 0.8-kb T2A2G3 repeat arrays separated by 23-, 32-, and 23-bp segments. pSXneo-1.1-T2A2G3 contains two 540-bp T2A2G3 arrays interrupted by a 28-bp nonrepeat sequence. pSXneo-1.2-T2A2G3 contains three 385-bp T2A2G3 arrays interrupted by 19-bp nonrepeat segments. Additional heterologous telomere seeds were constructed from synthetic DNAs. For pSXneo-1.0-T2A2G3, DNA oligonucleotides with the sequence (T2A2G)n and (T2A2G)n were annealed and self-ligated to form a 250-bp T2A2G3 repeat array. A minipimer of this array was subcloned into pSXneo to form a 1.0-kb segment in which four 250-bp T2A2G3 segments are linked by 15-, 38-, and 15-bp nonrepeat segments. Similarly, oligonucleotides with the sequence (T2A2G)nC3 and (T2A2G)n were annealed and ligated to form a 400-bp array, which was multimerized to a stretch of five 400-bp T2A2G3 segments interrupted by 15-bp nonrepeat segments in pSXneo-2.0-T2A2G3. pSXneo-2.4-(T2A2G)n was generated by using the oligonucleotides (T2A2G)n and (T2A2G)nC3; resulting in a 2.4-kb repeat array with six 400-bp (T2A2G)nT2A2G3 units interrupted with non-

Abbreviations: MAC, mammalian artificial chromosome; TRF, TTAGGG repeat binding factor.

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pept segments of 15-bp. pSXneo-1.0-(T$_2$AG)$_{168}$N$_{14-22}$ contains 1.0 kb to T$_2$AG$_3$ repeats formed by the ligation of six 168-bp T$_2$AG$_3$ arrays from pTHS (11) joined by nontelomeric segments of 14 and 22 bp. All cloning steps were performed with *Escherichia coli* HB101 grown logarithmically at 30°C in brain/heart infusion broth. Precursor plasmids with short repeat arrays were sequenced and final telomere seed constructs were verified by partial sequence analysis.

**Transfection of HeLa Cells.** Plasmids were linearized at sites 4–20 bp 3' of the G-rich repeat strand and transfected into adherent HeLa cells (HeLa II in ref. 22) with Lipofectin (BRL). For each construct 100–500 G418$^8$ (400 μg/mL) colonies (from transfection of 1 pmol of DNA) were pooled for genomic analysis. Clonal cell lines were derived by limiting dilution or with cloning cylinders.

**Quantitation of Telomeric Neomycin Phosphotransferase (neo) Signals.** Genomic DNA from pooled colonies was treated with BAL-31 (IBI; 10 units of BAL-31-M/40 μg of DNA) for 6 hr at 30°C. Unlinearized samples were processed in parallel. HindIII-digested DNAs (14 μg per sample as measured by Hoechst fluorescence) was analyzed by blotting as described (9). neo signals on fragments larger than 1.2 kb (the size of the neo cassette) were quantitated with a PhosphoImager. The fraction of telomeric neo genes was calculated as

\[
\% \text{telomeric neo genes} = \frac{\text{neo signal before BAL-31} - \text{neo signal after BAL-31}}{\text{neo signal before BAL-31}} \times 100.
\]

Hybridization with a T$_2$AG$_3$ repeat probe (16) demonstrated that BAL-31 had removed 89–93% of the telomeric sequences in each case. The residual signal is due to interstitial T$_2$AG$_3$-related sequences. Hybridization with the human DEK gene (R. Bose and T.d.L., unpublished data) verified that BAL-31 had not degraded chromosome internal DNA.

**Pasmid Rescue.** *Xba I*-digested genomic DNA was circularized and plasmids were isolated that contained telomeric neo loci and several kilobases of flanking HeLa sequences. *Trf assays.* HeLa S100 extract was incubated with oligonucleotide primers (at 0.4 or 2.0 μM) and [γ-$^{32}$P]dGTP for 1 or 2 hr at 30°C and processed as described by Morin (14).

**TRF Assays.** HeLa TRF was assayed as described (18) with an end-labeled (T$_2$AG)$_{12}$ probe (12merA in ref. 18) in the presence of circular plasmids containing human and heterologous telomeric sequences. Pasmid concentrations were determined by Hoechst fluorescence and verified by ethidium bromide staining of agarose gels.

**RESULTS AND DISCUSSION**

**Frequent Telomere Formation with Telomere Seeds of 0.8–3.2 kb.** To determine which features of the (T$_2$AG)$_n$ telomere seeds are required for telomere formation in human cells, we measured the frequency with which different telomeric sequences are recovered at chromosome ends. In this assay, HeLa cells were transfected with linear DNAs containing the neo gene proximal to the telomeric sequence (Fig. 1a) and stable G418$^8$ cells were isolated. Clonal lines with telomeric neo genes were readily identified based on the typical heterogeneous size of telomeric restriction fragments (Fig. 1b). Telomere formation was also detected in pools of transfected colonies. In this case, the frequency of telomere formation was determined by quantitating the BAL-31 sensitivity of the neo loci (Fig. 1c and Table 1). These frequencies correlated well with those obtained from clonal cell lines (Table 1). We found that T$_2$AG$_3$ repeat arrays of 0.8–3.2 kb seeded telomeres in up to ~75% of the stably transformed cell lines (Table 1). The remaining 25% of the transfected cells had chromosome

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**Fig. 1.** Telomere formation in HeLa cells. (a) Telomere seed plasmid pSXneo-1.6-T$_2$AG$_3$. The wavy line represents 1.6 kb of T$_2$AG$_3$ repeats with a 23-bp interruption (solid box). 1.6N and 1.6P (Table 1) are generated by cleavage with *Not I* and *Pvu I*, respectively (9 and 900 bp 3' to the T$_2$AG$_3$ repeat strand, respectively). (b) Telomere formation with T$_2$AG$_3$, T$_2$AG$_3$C, and T$_2$AG$_3$ telomere seeds. Blots of neo fragments in HindIII-digested DNA of HeLa clones transfected with pSXneo-1.6-T$_2$AG$_3$ (lanes 1–8), pSXneo-2.0-T$_2$AG$_3$C (lanes 9–14), or pSXneo-1.0-T$_2$AG$_3$ (lanes 15–20). Arrowhead indicates expected position of pSXneo-1.6-T$_2$AG$_3$ telomere seeds without cleavage—i.e., the 2.8-kb HindIII/Not I fragment (see a). Molecular sizes (kb) of HindIII-digested λ DNA marker fragments are indicated. (c) BAL-31 sensitivity of neo loci in pools of colonies transfected with telomere seeds. DNA from pooled colonies was treated with BAL-31 for 0 hr (odd-numbered lanes) and 6 hr (even-numbered lanes), cleaved with HindIII, fractionated, blotted, and hybridized with neo. The following telomere seed plasmids were used: lanes 1 and 2, pSXneo vector; lanes 3 and 4, pSXneo-1.6-T$_2$AG$_3$; lanes 5 and 6, pSXneo-1.6-TAG$_3$; lanes 7 and 8, pSXneo-1.1-T$_2$AG$_3$G; lanes 9 and 10, pSXneo-1.2-T$_2$AG$_3$; lanes 11 and 12, pSXneo-1.0-T$_2$AG$_3$C; lanes 13 and 14, pSXneo-2.0-T$_2$AG$_3$C; lanes 15 and 16, pSXneo-2.4-(T$_2$AG)$_3$T$_2$AG$_3$; lanes 17 and 18, pSXneo-1.0-(T$_2$AG)$_3$G$^{22}$-N$_{14-22}$. (Table 1)
Table 1. Telomeres formed with human and heterologous telomeric DNAs

<table>
<thead>
<tr>
<th>pSXneo telomere seed derivative</th>
<th>Fraction of telomeric neo genes</th>
<th>In pooled colonies, %</th>
<th>In clonal lines</th>
<th>Median telomere length, kb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length, kb</td>
<td>Configuration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>TTAGGG Linear</td>
<td>67 ± 5</td>
<td>16/24</td>
<td>4.7 ± 1.5</td>
</tr>
<tr>
<td>2.</td>
<td>TTAGGG Linear Not 1*</td>
<td>69 ± 13</td>
<td>9/12</td>
<td>3.4 ± 2.0</td>
</tr>
<tr>
<td>3.</td>
<td>TTAGGG Linear Pru 1*</td>
<td>54, 61</td>
<td>7/11</td>
<td>3.0 ± 1.3</td>
</tr>
<tr>
<td>4.</td>
<td>TTAGGG Circular</td>
<td>45</td>
<td>13/33</td>
<td>2.7 ± 1.0</td>
</tr>
<tr>
<td>5.</td>
<td>TTAGGG Linear</td>
<td>69, 59</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>6.</td>
<td>TTAGGG Linear</td>
<td>≤ 10</td>
<td>0/22</td>
<td>NA</td>
</tr>
<tr>
<td>7.</td>
<td>TTAGGG Linear</td>
<td>≤ 10</td>
<td>0/20</td>
<td>NA</td>
</tr>
<tr>
<td>8.</td>
<td>TTAGGG Linear</td>
<td>≤ 10</td>
<td>0/19</td>
<td>NA</td>
</tr>
<tr>
<td>9.</td>
<td>TTAGGG Linear</td>
<td>≤ 10</td>
<td>1/31</td>
<td>7.5</td>
</tr>
<tr>
<td>10.</td>
<td>TTAGGG Linear</td>
<td>≤ 10</td>
<td>0/33</td>
<td>NA</td>
</tr>
<tr>
<td>11.</td>
<td>(T2AG3)2(T2AG3)2</td>
<td>55, 56</td>
<td>6/9</td>
<td>2.5 ± 1.2</td>
</tr>
<tr>
<td>12.</td>
<td>(T2AG3)2(T2AG3)2</td>
<td>37, ≤ 10</td>
<td>3/28</td>
<td>2.6 ± 0.8</td>
</tr>
</tbody>
</table>

See Materials and Methods and Fig. 1a for constructs. The fraction of telomeric neo genes (%) in pooled colonies was deduced from BAL-31 sensitivity. Values in lines 1 and 2 represent means ± SD (of three) and seven measurements, respectively. Lines 3, 5, 11, and 12 represent two measurements; line 4 represents a single experiment. Fraction of clonal cell lines with heterogeneous HindIII neo fragments (see Fig. 1b) is indicated. Most cell lines (72%) contained only one copy of neo; for multiple copies of neo, each was scored independently. Telomeres (median length) are assumed to be 1.2 kb shorter than the HindIII neo fragments (see Fig. 1). Shown are averages ± SD. ND, not determined; NA, not applicable.

*pSXneo-1.6-T2AG3 linearized with Not 1 or Pru 1 (see Fig. 1a).
†Telomere formation frequencies < 10% are not detected.
‡Median length of the single telomere formed with T2AG3 repeats.

internal integrations of the transfected DNAs. A similar high frequency of telomere formation was observed in two other human cell lines (HT1080 and a different HeLa subclone; data not shown). The results also demonstrated that T2AG3 repeat arrays of 0.8, 1.6, and 3.2 kb were equally effective (Table 1), indicating that the efficacy of telomere seeds in this size range is not strongly influenced by their length.

To verify that the telomere seeds used in these experiments induced the formation of new telomeres at interstitial sites (19–21), several kilobases of HeLa genomic DNA flanking two independently formed telomeres were isolated by plasmid rescue (see Materials and Methods). Both integration sites appeared interstitial in the parental HeLa cells based on the presence of restriction endonuclease sites around the integration site and the BAL-31 resistance of these loci (data not shown). In addition, a 404-bp region immediately adjacent to one of the newly formed telomeres lacked similarities to T2AG3 repeats or telomere-associated sequences (GenBank data base, accession no. U02502). These results are consistent with the occurrence of an illegitimate recombination between the nonterminal end of the telomere seed plasmids and an interstitial site in the HeLa genome.

Effects of Telomere Seed Configuration. We next examined the effect of different DNA configurations on telomere formation. The frequency of telomere formation was not significantly lowered when the segment 3' of a 1.6-kb telomere seed was increased from 9 to 900 bp (1.6N and 1.6P, respectively; see Table 1). Introduction of this plasmid in circular form (1.6C in Table 1) also resulted in new telomeres in a considerable percentage of the transfected cells (40–45%). In yeast, telomeres can be formed on nonterminal telomere seeds with retention of the distal segment (23, 24). Furthermore, occasional retention of distal nontelomeric DNA has been demonstrated in newly formed HeLa telomeres (21). However, the 20 telomeres formed with 1.6C and 1.6P all lacked a Bgl II site positioned 24 bp beyond the telomeric repeats in the transfected construct (data not shown). One possibility is that the telomeres seeds are modified during transfection, resulting in linear DNAs with T2AG3 at their termini. Regardless, our data indicate that the nontelomeric DNA distal to the telomere seeds does not inhibit telomere formation significantly.

Sequence Dependence of Telomere Formation. The sequence requirements for telomere formation were addressed using heterologous telomeric stretches of TAG3, T2AG3, T3AG3, T4AG4, and T2AG2C repeats (reviewed in refs. 1 and 2). For each sequence, a plasmid with a telomere seed of at least 1 kb was linearized close to the 3’ end of the G-rich strand and the ability of these constructs to form additional telomeres was measured in clonal cell lines and pooled colonies (Fig. 1c and Table 1). In most of the transfected cells, these heterologous telomeric repeats clearly failed to seed new telomeres, resulting in interstitial neo genes as evidenced by discrete neo fragments in clonal cell lines and BAL-31-resistant neo signals in pooled colonies. Telomere formation was not observed in the 20–30 clonal cell lines that were analyzed for each sequence. The only exception was a single clone (of 31 examined) in which the heterologous sequence T2AG3 had seeded a new telomere (Table 1). Restriction endonuclease analysis confirmed that this telomere was formed on a T2AG3 repeat array (data not shown). These results demonstrate remarkably stringent sequence requirements for human telomere seeds and suggest that the formation of telomeres requires a highly sequence-specific interaction.

Testing for Homologous Recombination. We considered three mechanisms that could explain the stringent sequence requirements for telomere formation. First, the requirement for T2AG3 repeats might be due to homologous recombination. The fact that telomere formation is accompanied by chromosome fragmentation indicates that the telomere seeds do not simply integrate into homologous DNA at endogenous telomeres. However, a one-sided homologous recombination with an endogenous telomere could be responsible for elongation of the transfected telomeres. Since the telomeres of the HeLa cell line used for these experiments contain 3–6.5 kb of T2AG3 repeats (22), homologous recombination could explain the observed addition of several kilobases to the transfected DNAs (Fig. 1b and Table 1). Furthermore, the presence of distal nontelomeric DNA in newly formed HeLa telomeres (21) does not rule out a role for homologous recombination in telomere healing, because the retention of
distal nonhomologous sequences is occasionally observed in homologous recombination (25).

To test for the involvement of homologous recombination in telomere healing, we created a 1.0-kb T2AG3 repeat array in which a 14- or 22-bp nonrepeat sequence occurs every 168 bp. Similar mismatches have been shown to inhibit homologous recombination 10- to 200-fold in mammalian cells (refs. 26 and 27; T. Jacks and R. Weinberg, personal communication). However, the infrequent interruptions of the T2AG3 repeat array did not inhibit telomere formation significantly and the new telomeres were elongated (Fig. 1c and Table 1). A more drastic departure from the T2AG3 repeat sequence was created by oligomerization of a synthetic (T2AG3)2T2AG3 DNA, which seeded new telomeres in 3 of the 28 cell lines we examined (Table 1). Telomere formation could also be detected in pooled colonies transfected with the (T2AG3)2-
T2AG3 telomere seed (Fig. 1c).

These results suggest that homologous recombination does not contribute to a rate-limiting step in the formation of telomeres. Furthermore, it is unlikely that the addition of 1.5 kb to the telomere seed that contained nonterminal interruptions (Table 1) involved homologous recombination. We do not believe that the abundance of telomeric loci in HeLa cells confounds the interpretation of these experiments because the search for homology is not rate-limiting for homologous recombination in mammalian cells (28). It cannot be excluded, however, that the telomere seeds are healed by some specialized recombination pathway active at telomeres. In yeast, telomere healing can occur by RAD52-independent recombination but, unlike telomere formation in human cells, this pathway can utilize heterologous telomeric DNA (see ref. 29 for review).

**Priming of Telomerase by Heterologous Telomeric DNAs.** As a second possibility, we considered that telomerase (refs. 11, 13, 14, and 30; reviewed in refs. 4 and 5) could be responsible for the sequence specificity of telomere formation. The involvement of telomerase is consistent with the occasional retention of distal segments in healed telomeres (21) because the enzyme is known to elongate G-rich primers with only 3 nt identity to T2AG3 repeats at their 3′ end (14).

To determine whether failure to engage telomerase limits the ability of the heterologous telomeric DNA to form new human telomeres, we performed in vitro telomerase assays with (T2AG3)4 and five similarly sized primers that represent the pertinent heterologous telomeric repeats (Fig. 2). As expected from previous work on the primer specificity of mammalian telomerasases (13, 14, 30), each of the heterologous telomeric oligonucleotides primed telomerase elongation in vitro. The length of the products and the overall incorporation of labeled guanine varied with the different primers. However, the size distribution and the total yield of telomerase products did not correlate with the capacity of the telomeric sequences to seed new telomeres in HeLa cells. Although it is possible that telomerase has a different sequence specificity in vivo, these in vitro assays suggest that the sequences of the heterologous telomere seeds per se do not prevent telomere elongation by telomerase.

**Matching Specificity of Telomere Formation and TRF.** A third interaction that could explain the stringent sequence requirements for telomere formation is the binding of a telomeric protein. HeLa cells express the candidate telomeric protein TRF, which binds (T2AG3)50 in linear and circular DNAs (18). To examine the interaction of TRF with the telomeric DNAs used in this study, the factor was allowed to bind to (T2AG3)50 probe in the presence of the relevant telomere seed plasmids and the residual TRF complex was quantitated by gel-shift analysis (Fig. 3). Plasmids with T2AG3 repeats were the most effective competitors for TRF. Each of the heterologous telomeric repeat arrays were at least 10-fold less active in this competition titration assay

![Fig. 2. Telomerase activity with human and heterologous telomeric repeat primers.](image-url)

(a) The sequence (5′ to 3′) of six single-stranded DNA oligonucleotides representing human (oligonucleotide 1) and heterologous (oligonucleotides 2–6) telomeric DNAs. (b) Telomerase products with the oligonucleotide primers depicted in a. See Materials and Methods for experimental details. Origin of the shortest strongly labeled product in lane 4 is unknown. Marker fragments are from Msp I-digested pHBr322 DNA. Numbers on left are bp.

(Fig. 3; ref. 18). An intermediate effect was observed with an artificial array of (T2AG3)2T2AG3 repeats. Although this mixed sequence competed for TRF, the competitor plasmid had to be present at an ≈4-fold higher concentration to achieve the same competition level as T2AG3 repeats (Fig. 3). These data indicate that the sequence specificity of TRF matches the requirements for telomere formation in HeLa cells. The involvement of TRF, which could bind along the length of the telomere repeat array rather than to its terminus, is in agreement with the observation that the terminal sequence of the transsected DNA is not important (Table 1) and with the occasional retention of distal nonterminal sequences in healed telomeres (21). Interestingly, in S. cerevisiae, efficient healing of short telomere seeds also requires a site for a telomeric DNA binding factor, RAP1 (31, 32). Whether TRF has any other similarities to RAP1 remains to be determined.

Mammalian cells contain nucleic acid binding activities that display some preference for single-stranded telomeric DNA in vitro. Several heterogeneous nuclear ribonucleoproteins bind single-stranded T2AG3 repeats with considerable specificity even though these proteins prefer the RNA version of this sequence (33–35). Since the exact sequence requirements of these factors is not known, we cannot rule out the involvement of heterogeneous nuclear ribonucleo-
proteins in the formation of human telomeres. Avian cells express a single-stranded DNA binding protein that forms a complex with single-stranded TTAGG repeats (36). However, it is not known whether HeLa cells contain a related activity and the sequence preference of the avian factor does not match the sequence requirements for telomere formation in human cells (36).

Implications for Mammalian Artificial Chromosomes (MACs). The stringent sequence requirements for human telomere sequences predict that heterologous telomeric sequences cannot stabilize the ends of MACs. Therefore, the development of MACs will require vectors that carry TTAGG repeat arrays. Specifically, the use of yeast artificial chromosomes for MACs is predicted to require TTAGG repeats at chromosome ends because the yeast telomeric (TGL3) tract is unlikely to seed mammalian telomeres. However, it may not be necessary to engineer the TTAGG repeats at a terminal position in MAC precursors, since 1.6 kb of TTAGG repeats seeded telomeres efficiently when positioned 900 bp from the end of the transfected DNA.

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