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Comparison of different kinds of probes used for analysis of variant telomeric sequences

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Abstract

In this work we aimed to compare and critically evaluate results obtained by different types of probes used for hybridisation to detect variant telomeric sequences with respect to their reliability and information value. Using slot-blot hybridisation we investigated three types of probes (oligonucleotides, cloned fragments and concatenated probes) under various conditions of hybridisation and washing. The concatenated probes exhibited the highest specificity although all three types are suitable for hybridisation of telomeric sequences under appropriate experimental conditions. We demonstrate how understanding generated from these data enables interpretation of hybridisation patterns of oligonucleotide probes to genomic DNAs.
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1. Introduction

In most eukaryotes studied, the DNA sequences that form the terminal parts of chromosomes (telomeres) are minisatellite repetitive sequences. The nature of the sequence can be conserved, e.g. $(TTAGGG)_n$ is found in vertebrates including human [1] while $(TTTAGGG)_n$ is found in many plant species [2]. However, in recent reports a number of organisms apparently have telomeres that are not "typical" of their phylogenetic position. The lack of typical plant telomeres $(TTTAGGG)_n$ has been shown in the genus *Allium* (family Alliaceae) [3] and the dicotyledonous genera *Cestrum, Vestia, Sessea* (Solanaceae) [4]. Further studies in a large set of species from Asparagales (including Alliaceae) have identified two switchpoints in the evolution of telomeres. At first, the original ("typical") plant telomeric repeats were replaced by the human-type telomeric sequence which is accompanied by other variants of telomeric repeats, namely *Tetrahymena*-type $(TTGGGG)_n$ and *Bombyx*-type $(TTAGG)_n$ [5]. The second evolutionary switchpoint within Alliaceae resulted in a loss of any detectable telomeric minisatellite motifs and telomerase activity. In these studies, the presence of variant minisatellite repeats was screened by slot-blot hybridisation and confirmed by other methods (fluorescence *in situ* hybridisation, primer extension reaction) in selected species. Since the reliability of all these techniques depends on the specificity of the hybridisation step of an assay, there is a need to understand better how a wider range (more complete representation) of known variants of telomeric minisatellite sequences interact under different hybridisation conditions. Such information, coupled with fast and reliable methods of sequence identification, is needed to identify minisatellite sequence abundance in genomic DNAs for taxonomic and diagnostic purposes.

Currently the probes used to detect these minisatellites are of three types: (i) oligonucleotide probes, (ii) cloned telomeric fragments of known DNA sequence, and (iii)

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"concatenated probes". While the classes (i) and (ii) need no further comment, the class (iii) is generated by repeated cycles of self-annealing of G- and C-rich telomeric primers in the absence of the DNA template. The annealed sequence is extended in each cycle by, e.g. *Taq* polymerase, as first described and used by Ijdo et al. [6]. However, there remains considerable concern that this approach might generate imprecise and variable sequence. There have also been doubts raised over the use of oligonucleotide and cloned probes due to our limited empirical understanding of the degree of cross-hybridisation between closely related minisatellite sequences. These doubts are especially justified for short oligonucleotide probes where high-stringency conditions cannot be applied.

The problem of multiple positive hybridisation signals provided by different sequence variants of telomeric repeat probes was first systematically addressed by Allshire et al. [7]. Here human telomeres were hybridised with $(TTGGGG)_n$, $(TTAGGG)_n$ and $(TTTAGGG)_n$ probes and the authors asked whether these sequences might just be cross-hybridising to a uniform tandem repeat or if there could be several types of repeat motifs which would contribute to a heterogeneous repetitive human telomeric region. Using Southern hybridisation with various stringency conditions and with, or without, the addition of competing cold oligonucleotides for subtraction of cross-hybridisation, they found that the signal of $(TTGGGG)_n$ on human DNA corresponds to the presence of this sequence rather than to cross-hybridisation, while significant amounts of $(TTTAGGG)_n$ repeats are probably absent [8].

The relative conservation of the telomeric sequences among species (see Table 1 for sequences used in this study), and among individual telomeres, arises from the mechanism of their synthesis. Typically this is by telomerase which functions as a reverse transcriptase with an internal RNA template [9]. However the homogeneity of the telomeric repeat arrays is naturally disrupted for the following reasons. First, it is only the most distal part of the telomere which is synthesised by telomerase. The proximal part of the array is synthesised by the conventional replication machinery and the two mechanisms differ in sequence fidelity. Secondly, telomerase is prone to errors (mainly in the number of T and G nucleotides in the synthesised sequence) as has been shown by analysis of products of *de novo* telomere synthesis [5,10]. This probably reflects the instability of the 3′ terminus of the telomeric substrate relative to the telomerase RNA template (T- or G-slippage). Thirdly, the proximal regions of telomeres may be subject to recombination events that can result in sequence degeneration and variation [11]. In addition, telomeric sequence heterogeneity is especially pronounced where alternative (recombination-mediated) lengthening of telomeres occurs, as is found in telomerase-negative tumours. Indeed in these cells the heterogeneity may serve as a marker of this process [12]. Therefore, telomeric DNA may be quite heterogeneous in detailed view even in the organisms with "known" telomeric sequence.

Table 1
Telomeric repeats used in this work

Telomeric repeat (5′to 3′)	Abbreviation	First described in organism
TTAGG	BO	*Bombyx*
TTAGGG	HU	*Homo*
TTTAGGG	AT	*Arabidopsis*
TTTTAGGG	CH	*Chlamydomonas*
TTGGGG	TT	*Tetrahymena*
TTTTGGGG	OX	*Oxytricha*
TTAGGC	AS	*Ascaris*

In this work we analyse the reliability of the three types of probes, concatenated, cloned and oligonucleotide, for the detection of typical and variant telomeric sequences. We compare the information the probes yield. The understanding generated enables a rational choice of probes in taxonomic and diagnostic studies of telomere arrays.

2. Materials and methods

2.1. Preparation of hybridisation probes

As probes for hybridisation either oligonucleotides BOSB, HUSB, ATSB, CHSB, TTSB, OXSB, ASSB (here termed XXSB according to abbreviations for different telomeric types given in Table 1), cloned DNA fragments pBO, pHU, pAT, pCH, pOX, pTT (here termed pXX), or concatemers BOTEL, HUTEL, ATTEL, CHTEL, TTTEL, OXTEL, ASTEL (here termed XXTEL) were used.

1) Oligonucleotides: G-strand specific oligonucleotides were end-labelled with α-[^{32}P]ATP (ICN Biomedicals) using T4 polynucleotide kinase (NEB).
2) Concatemers: Concatemers of individual telomeric motifs were prepared by template-free PCR [6] with some modifications such as the use of *Pfu* DNA polymerase (MBI Fermentas). The reaction mixture contained primers corresponding to both strands of a given sequence (see below), e.g. for *Arabidopsis*-type, ATSB and ATTC primers were used. PCR conditions were as follows: initial denaturation 94 °C/2 min, 10 cycles of 94 °C/30 s, 55 °C/30 s, 72 °C/20 s with 10-s extension per cycle, then 25 cycles of 94 °C/30 s, 55 °C/30 s, 72 °C/2 min and final extension at 72 °C for 10 min. For subsequent applications concatemers were purified with a PCR purification kit (Qiagen) and DNA concentration was determined by UV spectrophotometry. The concatemers were labelled by nick-translation with incorporation of α-[^{32}P]dATP (ICN Biomedicals) using a modified protocol for nick-translation based on similar protocols used for FISH probe labelling: the total 25 μl reaction mixture contained 1 μg concatemer DNA, dNTP mix (final concentrations 20 μM dATP, 46 μM dCTP, 46 μM dGTP, 46 μM

Table 2
Cloning of concatemers

Sequence	Positive clones	Total length (bp)	Errors	Error rate	pXX (errors/ length)
BO	4	936	6	6.4×10^{-3}	1/413
HU	3	644	0	0	0/324
AT	5	1223	1	8.2×10^{-4}	0/300
CH	1	599	1	1.7×10^{-3}	0/268
TT	3	803	2	2.5×10^{-3}	2/120
OX	3	874	0	0	0/324

dTTP), 50 μCi α-[^{32}P]dATP (6000 Ci/mmol), 2.5 μl 0.1M DTT and 10× nick-translation buffer (0.5M Tris–Cl, pH 7.8, 50 mM $MgCl_2$, 0.5 mg/ml BSA), then 10 units of DNA Polymerase I (NEB) and 1000× diluted RQ-DNaseI (1U/μl, Promega) were added. DNase I concentration and labelling time at 15 °C were optimised in a parallel nonradioactive reaction so that an optimal fragment length of 500–800 bp was reached. Reaction products were deproteinized with phenol/chloroform and precipitated by 0.3 M sodium acetate and ethanol, dissolved in water and used for hybridisation.

3) Cloned fragments of telomeric sequence: Concatemers were cloned into pZErO-1 vector (Invitrogen) and transformed into *E. coli* strain TOP10 (Invitrogen). Clones were sequenced on an automatic sequencer ABIPRISM (PE Biosystems). Selected clones were then used as double-stranded DNA samples of telomeric sequences (description pXX) with defined sequence and length. They were: *Bombyx*-type 82× (TTAGG), human-type 45× (TTAGGG), *Arabidopsis*-type 24× (TTTAGGG), *Chlamydomonas*-type 47× (TTTTAGGG), *Tetrahymena*-type 20× (TTGGGG), *Oxytricha*-type 40× (TTTTGGGG). We did not obtain any positive clone from *Ascaris*-type. DNA fragments for labelling were prepared from appropriate clones by PCR using vector universal primers M13F-40 and M13R, and then purified by agarose gel electrophoresis. The concentrations of products were determined spectrophotometrically. Cloned telomeric fragments were labelled with α-[^{32}P]dATP (ICN Biomedicals) with use of a DecaLabel DNA Labeling Kit (Fermentas).

Sequences of primers (all MWG Biotech, Germany) used for concatemer generation or slot-blot hybridisation were: BOSB 5′-TTAGGTTAGGTTAGGTTAGGTTAG-GTTAG-3′, HUSB 5′-TTAGGGTTAGGGTTAGGGTTAG-GGTTAG-3′, ATSB 5′-GGTTTAGGGTTTAGGGTTTAG-GGTTTAG-3′, CHSB 5′-GTTTTAGGGTTTTAGGGTTTT-AGGGTTTTAG-3′, TTSB 5′-TGGGGTTGGGGTTGGGG-TTGGGGTTG-3′, OXSB 5′-TTTTGGGGTTTTGGGGTT-TTGGGGTTTT-3′, ASSB 5′-TTAGGCTTAGGCTTAGGC-TTAGGCTTAG-3′, BOTC 5′-TAACCTAACCTAACCTA-ACCT-3′, HUTC 5′-AACCCTAACCCTAACCCTAAC-3′, ATTC 5′-CCTAAACCCTAAACCCTAAAC 3′, CHTC 5′-CTAAAACCCTAAAACCCTAAAA-3′, TTTC 5′-CCCAA-CCCCAACCCCAAC-3′, OXTC 5′-CAAAACCCCAAAA-CCCCAAAA-3′, ASTC 5′-AGCCTAAGCCTAAGCCTAA-GC-3′.

2.2. DNA samples and slot-blotting

Cloned fragments transferred onto a membrane were used in serial dilutions of 500 pg, 100 pg and 20 pg. Concatemers and genomic DNAs were loaded in amount of 200 pg and 1 μg, respectively. The transfer of DNA to nylon membrane Hybond-XL (Amersham) occurred under alkaline conditions (0.33 M NaOH) using a Bio-Dot SF

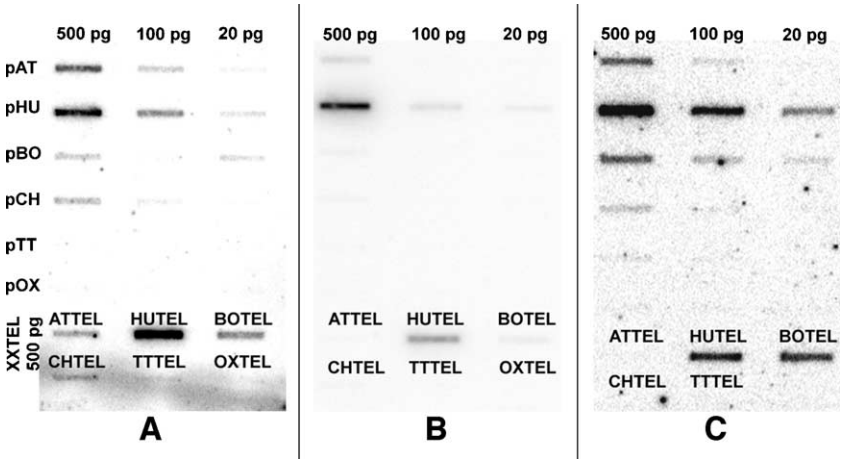

Fig. 1. Hybridisation of concatemers (XXTEL) and cloned DNA fragments (pXX) with three different human probes, HUSB* (A), HUTEL* (B) or pHU* (C).

Table 3
Hybridisation of individual oligonucleotide probes with 500 pg concatemers or cloned DNAs (signal in % of pXX value)

Probe	Sample													
	BO		HU		AT		CH		TT		OX		AS	
	pXX	XXTEL	pXX	XXTEL	pXX	XXTEL	pXX	XXTEL	pXX	XXTEL	pXX	XXTEL	pXX	XXTEL
BOSB*	100	23	6.5	2	2	1.5	2.5	1.5	2	1	1.5	1	N/A	<1
HUSB*	15	50	100	150	45	17	10	15	<1	5	<1	<1	N/A	<1
ATSB*	3.5	1	4	1.5	100	19	66.5	58	1	6	1	<1	N/A	<1
CHSB*	1.5	<1	<1	<1	11.5	<1	100	57	<1	<1	9	<1	N/A	<1
TTSB*	<1	<1	1	2.5	<1	<1	2	<1	100	55	4.5	7	N/A	<1
OXSB*	2.5	<1	2	<1	2	<1	22	5	100	50	7	<1	N/A	<1
ASSB*	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	N/A	100

apparatus (Bio-Rad) and membranes were fixed by heating at 80 °C for 1 h.

Cloned fragments were used without excision from the plasmids. The concentrations of products were determined spectrophotometrically. The relative proportion occupied by the insert determined the amount of plasmid used in the experiments. Where experiments were conducted that involved self-hybridisation, inserts were cut from the plasmid by *PstI/NotI* and gel-purified to avoid false positives. As positive controls we used total genomic DNAs from *Nicotiana sylvestris* (Queen Mary, University of London), *Bombyx mori* (a gift of Frantisek Marec, Institute of Entomology AS CR, Ceske Budejovice), *Chlamydomonas reinhardtii* (Queen Mary, University of London) and *Homo sapiens* (CAMBIO UK Ltd.). These control genomic DNAs also served as internal controls on individual membranes hybridised with the same type of telomeric probes.

2.3. Hybridisation procedure

All hybridisations were performed in 0.5 M phosphate buffer with 10 mM EDTA and 7% SDS. Hybridisation with end-labelled oligonucleotide probes was run at 55 °C for 16 h and the membranes washed with 2× SSC/0.1% SDS unless otherwise stated. Hybridisation with nick-translated labelled concatemers and cloned fragments was performed at 65 °C for 16 h and the membranes were washed with 0.2× SSC/0.1% SDS. Hybridisation signals were quantified using a STORM PhosphoImager with ImageQuant software (Amersham Biosciences Ltd); the rectangle or grid mode was used with comparable results. A signal intensity of 100% was designated as that derived from 500 pg pXX or 500 pg XXTEL sample where the probe is against the same species (see Results) and other signal intensities were compared to this value.

3. Results and discussion

3.1. Preparation and cloning of concatemers

Concatemer probes are widely used in the analysis of telomeres, especially in experiments involving fluorescent *in situ* hybridisation (FISH). These probes are generated by self-annealing of C- and G- telomeric oligonucleotides and amplification by *Taq* DNA polymerase in template-free PCR reactions [6]. We asked whether DNA fragments generated by this method consist of precise telomeric repeats. We used *Pfu* DNA polymerase instead of *Taq* DNA polymerase because it is thought to generate a lower error rate. Concatemers of different telomeric types resolved on an agarose gel were observed as a smear with mean lengths from 750 to 2000 bp (not shown). Reaction products were cloned and sequenced to determine error rates for different types of telomeric repeats and one clone from each group was chosen for hybridisation studies (as pXX, see below). Surveys of clone properties, including error rates for all telomeric types, are shown in Table 2. The calculated total error rate (for all clones) was 1.9×10^{-3} which appears

Table 4
Hybridisation of individual labelled concatenated probes with 500 pg cloned DNAs or 500 pg concatemers (signal in % of XXTEL value)

Probe	Sample													
	BO		HU		AT		CH		TT		OX		AS	
	pXX	XXTEL	pXX	XXTEL	pXX	XXTEL	pXX	XXTEL	pXX	XXTEL	pXX	XXTEL	pXX	XXTEL
BOTEL*	150	100	24	<1	2	<1	3	<1	<1	<1	<1	<1	N/A	<1
HUTEL*	<1	5	200	100	2	<1	<1	<1	<1	<1	<1	<1	N/A	<1
ATTEL*	<1	<1	<1	<1	300	100	<1	<1	<1	<1	<1	<1	N/A	<1
CHTEL*	<1	<1	<1	<1	<1	<1	300	100	<1	<1	<1	<1	N/A	<1
TTTEL*	<1	<1	<1	<1	<1	<1	<1	<1	50	100	<1	<1	N/A	<1
OXTEL*	<1	<1	<1	<1	6.5	<1	<1	7.5	<1	<1	90	100	N/A	<1
ASTEL*	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	N/A	100

surprising when compared to the declared accuracy of *Pfu* DNA polymerase (2.6×10^{-6}). However there was considerable variation in error rate, e.g. in the human-type and *Oxytricha*-type of cloned concatemers, we observed no misincorporation while the *Bombyx*-type of concatemers had an error rate of 6.4×10^{-3}, similar to [13] where a 550-bp-long sequenced fragment generated by *Taq* DNA polymerase contained three misincorporations (error rate of 5.4×10^{-3}). Perhaps some telomeric sequences are problematic templates to amplify. Further, sequencing of telomeric repeats is also difficult and, therefore, only short clones were chosen that could be read in one sequencing run. The oligonucleotide self-annealing phase of concatemer formation may also contribute to a relatively high, but still acceptable error rate.

3.2. Comparison of particular types of probes

Considering the mutual similarity of all telomeric motifs used, we might expect some level of cross-hybridisation [2,8]. The question is whether hybrid DNA molecules are formed that result in cross-hybridisation and if so, whether they can be removed with suitable washing conditions. The experimental design was influenced by the fact that short oligonucleotide probes cannot be reliably transferred and immobilised to membranes. Therefore cloned DNA (pXX) or concatemers (XXTEL) were loaded onto membranes and probed with radioactively labelled (indicated by *) oligonucleotides (XXSB*), cloned DNAs (pXX*) or concatemers (XXTEL*). Typical data are shown in Fig. 1 using all three types of probe against the human telomeric repeat. All data were evaluated for signal strengths as summarised in Tables 3–5. A value of 100% was assigned to the hybridisation signal from a particular kind of labelled probe to 500 pg of its corresponding DNA loaded to the membrane. Lower amounts of loaded DNA (100 pg and 20 pg) gave the expected reduction in signal intensity (data not shown).

In general, hybridisation of radioactively labelled oligonucleotides (Table 3) with cloned DNAs at low stringency gave some cross-hybridisation which is suppressed under more stringent conditions. The hybridisation of oligonucleotide probes to concatemers is much more specific, indeed cross-hybridisation occurs only between ATSB* and CHTEL. Although oligonucleotide hybridisation can be considered as specific, several cases of cross-hybridisation were observed, especially with ATSB*. A similar phenomenon was observed by Petracek et al. [14] using different hybridisation/washing conditions. ATSB*, under standard washing conditions, cross-hybridises with pCH and to a lesser extent pHU. These clones have repeat sequences that are very similar to those in pAT, i.e. one T base in *A. thaliana* versus the human repeat and one T base in the *A. thaliana* versus the *Chlamydomonas* repeat. When more stringent washing conditions were used, the cross-hybridisation was efficiently removed while the signal to pAT resisted this washing (for details, see Table 6 and Fig. 2).

Hybridisation of radioactively labelled concatemers with cloned DNA fragments and concatemers (Table 4) was generally more specific than with oligonucleotide probes. Concatemer-to-concatemer interactions were specific in our studies. In several cases of concatemer-to-cloned fragment hybridisation, there was very low cross-hybridisation. The reason for these differences could be that concatenated probes are of optimal length range ($\sim 10^2$ bp) for high-stringency hybridisation and washing conditions.

Table 6
Influence of washing stringency on non-specific hybridisation signals

AT		CH	
2× SSC/0.1% SDS			
500 pg pXX	100%	500 pg pXX	66.5%
100 pg pXX	23%	100 pg pXX	13%
20 pg pXX	4%	20 pg pXX	2.5%
500 pg XXTEL	19%	500 pg XXTEL	58%
1× SSC/0.1% SDS			
500 pg pXX	55%	500 pg pXX	8%
100 pg pXX	13%	100 pg pXX	2.5%
20 pg pXX	2.5%	20 pg pXX	1%
500 pg XXTEL	9.5%	500 pg XXTEL	8%
0.5× SSC/0.1% SDS			
500 pg pXX	31%	500 pg pXX	1%
100 pg pXX	9%	100 pg pXX	0.5%
20 pg pXX	4%	20 pg pXX	0.4%
500 pg XXTEL	6.5%	500 pg XXTEL	0.6%

The values of % signal are all relative to 500 pg pAT.

Table 5
Hybridisation of individual labelled cloned DNA probes with 500 pg cloned DNAs or 500 pg concatemers (signal in % of pXX value)

Probe	Sample											
	BO		HU		AT		CH		TT		OX	
	pXX	XXTEL	pXX	XXTEL	pXX	XXTEL	pXX	XXTEL	pXX	XXTEL	pXX	XXTEL
pBO*	100	118	10	4.5	30	3.5	16	2.5	6	2	7	2
pHU*	14.5	25.5	100	32.5	15	2	5	2	1.5	1.5	2.5	1.5
pAT*	<1	<1	1.5	<1	<1	<1	<1	<1	<1	<1	<1	<1
pCH*	2	3	3,5	5	4	1	100	6	2	1	2	2
pTT*	<1	<1	<1	<1	1	<1	1.5	<1	100	1	<1	<1
pOX*	2	2	3	1	9	1.5	17.5	1.5	1.5	3	100	125

pXX samples represent the DNA fragments cut from the plasmids (see Materials and methods).

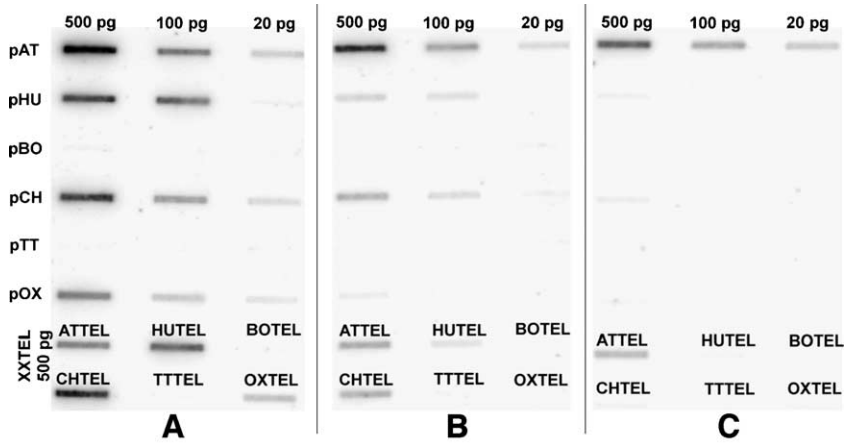

Fig. 2. Hybridisation with ATSB* as in Fig. 1 under different stringency conditions. The membranes were washed with 2× SSC/0.1% SDS (A), 1× SSC/0.1% SDS (B) or 0.5× SSC/0.1% SDS (C).

In some cases, the commercial nick-translation procedure (Roche Biochemicals Ltd.) was used for labelling of concatemers according to the manufacturer instructions, but no difference was found between results obtained by hand-made labelling protocols and commercial mixtures which are more user-friendly (not shown).

Hybridisation of radioactively labelled cloned telomeric sequences to cloned DNA and concatemers (Table 5) was less specific than hybridisation with concatenated probes under the same conditions. Cross-hybridisation occurred in several cases even though the washing stringency was high. On the other hand, the strength of the hybridisation signals using cloned telomeric sequences was considerably higher than those of concatenated probes. In concatemer preparations we aimed to obtain the most precise sequences and so used *Pfu* DNA polymerase but nevertheless there were misincorporations (see above). Cross-hybridisation occurred mostly in the cases of error-free clones (e.g. pAT) while the clones with sequence errors (e.g. pTT) exhibited good specificity. Therefore the choice of polymerase is not critical for hybridisation results since misincorporations were not related to levels of cross-hybridisation.

3.3. Hybridisation with genomic DNAs

Four genomic DNAs from (i) *N. sylvestris* (AT-type), (ii) *H. sapiens* (HU-type), (iii) *B. mori* (BO-type), and (iv) *C. reinhardtii* (CH-type) were probed with radioactively labelled oligonucleotides (XXSB*) in low stringency washing conditions. Concatemer samples served as positive controls for normalising signal strengths between individual membranes (Fig. 3). The most easily interpreted result was from genomic DNA of *B. mori* which hybridises strongly only with BOSB*. This probe was highly specific and did not cross-hybridise with other telomeric types as demonstrated by lack of labelling to all concatemers except BOTEL. The low hybridisation signal to *B. mori* genomic DNA of other oligonucleotide probes suggests either that some of these sequences are present in the genome, or that cross-hybridisation occurs to large quantities of *Bombyx*-type of telomeric sequence, which might be expected if there is sufficiency of these sequences (see Table 3).

Genomic DNA of *H. sapiens* showed positive hybridisation signals when hybridised with ATSB* and TTSB* and its own telomeric type HUSB* which labels the most strongly. The high signal intensities from ATSB* and TTSB* cannot be explained by cross-hybridisation (as in *B. mori* above) (see Table 3) but more likely reflects the presence of *Tetrahymena*- and *Arabidopsis*-types of sequences in the human genome, in agreement with the findings of Allshire et al. [7] and of the human genome sequencing project as reported in GenBank sequence records.

The green alga *C. reinhardtii* showed positive signals with the three probes: ATSB*, CHSB* and TTSB*, and the only signal representing cross-hybridisation for the other probes. The strong hybridisation of the TTSB* probe clearly demonstrates the presence of these sequences in the genome. However the interpretation of the ATSB* and CHSB* signals needs further consideration. The AT-type of probes cross-hybridised with the CH-type which resulted in signals of similar strength (compare the control concatemer samples probed by ATSB* in Fig. 3), but in contrast the CH-

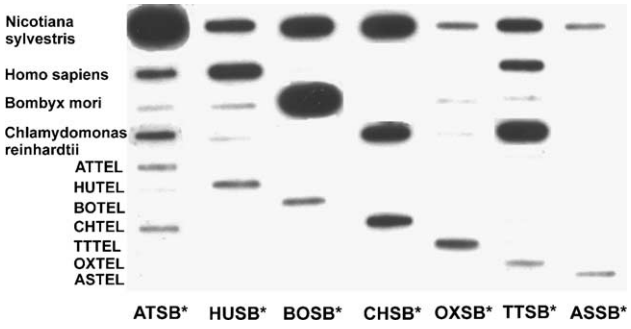

Fig. 3. Hybridisation of 1 μg genomic DNAs (rows 1–4) or 200 pg control concatemers (rows 5–11) with labelled oligonucleotide probes.

type probe did not show any cross-hybridisation with the AT-type (see control concatemers probed by CHSB* in Fig. 3). The ratio of signal strengths when *Chlamydomonas* genomic DNA and CHTEL were probed with ATSB* was similar to that when they were probed with CHSB*, and since ATSB* labels CHTEL and ATTEL equally these data suggest that there is no or very little ATSB sequence in the *Chlamydomonas* genome.

N. sylvestris is a plant with 20–200-kb-long telomeres [15] and its genomic DNA hybridised with nearly all kinds of telomeric sequence variant probes. The strongest signal was that for the *Arabidopsis*-type of telomeric sequence. Weak signals of ASSB* and OXSB* could be possibly caused by large amounts of AT-type telomeric sequence, as discussed above for *Bombyx* and the other signals could be attributed to the occurrence of these sequences in the genome of *N. sylvestris*. In the experiments with genomic DNAs, the genome size influences the strength of the signal, and therefore genome sizes must be taken into account when comparing the copy-number of telomeric repeats of a particular type among different organisms. Positive hybridisation signals provide information only about the absence/presence of the probed sequences in the genome, not about their chromosomal localisation, and therefore analysis by other methods should always accompany slot-blot studies.

4. Conclusions

All the types of probes studied here (oligonucleotides, concatemers and cloned DNA fragments) are suitable for probing telomeric sequences when applying appropriate hybridisation and washing conditions. Among them, concatemers show the highest specificity. The specificity of the concatenated and oligonucleotide probes may be further improved by increasing washing stringency. The cloned DNAs give more non-specific or cross-hybridisation signals even when high-stringency conditions are used but provide higher sensitivity.

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