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Immobilization of polynucleotides on magnetic particles

Factors influencing hybridization efficiency

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Immobilization of oligonucleotides containing 5′-terminal thiol groups on thiol-terminated paramagnetic Biomag beads via disulphide bond formation was investigated. Oligonucleotides are demonstrated to couple at high yields, the linkage is stable at 90 °C and is reversible, and the immobilized oligonucleotide is available for complementary, but not non-complementary, hybridization. Specific hybridization capacity per μg of immobilized oligonucleotide exceeds that achieved with other forms of immobilization chemistries employing random attachment and/or specific end attachment of the oligonucleotide to the solid support. Adsorption of DNA on the surface of the beads was decreased by incubation in 0.2% SDS; other non-specific blocking agents had no effect. Brief heating of the beads possessing immobilized oligonucleotides at 90 °C before hybridization increased the amount of specific hybridization dependent upon the inclusion of poly(dT) spacer sequences 5′ to the immobilized oligonucleotide and 3′ to the thiol group. Increasing lengths of spacers [up to a poly(dT_{16}) spacer] linearly increased hybridization of complementary sequences.

INTRODUCTION

Specific hybridization of nucleic acids to DNA immobilized on a solid support is commonly used in assays for detecting complementary target sequences in solution or immobilized on a variety of solid phases (Walker & Gaastra, 1985). The method used to link either target or capture DNA sequences (Polsky-Cynkin *et al.*, 1985) to the solid support is critical in determining the efficiency, and to a smaller degree the specificity, of Southern-blot and Northern-blot hybridization assays (Rosen *et al.*, 1990). Despite recent improvements (Durrant *et al.*, 1990), Southern-blotting techniques, employing heat or u.v. fixation of DNA to membranes, do not bind DNA in any specific orientation. Although random immobilization is sufficient for qualitative and rapid purification of nucleic acids (Bresser & Gillespie, 1983), it remains sub-optimal for quantification of target DNA by using e.l.i.s.a. or sandwich-type assays (Kemeney & Challacombe, 1980), which require multiple spatially discrete sequences available for hybridization.

Several methods commonly employed for immobilizing proteins without resulting in substantial loss of activity (Kennedy & Cabral, 1983) have succeeded in chemically attaching polynucleotides to solid supports, including glass (Bresser & Gillespie, 1983; Ghosh & Musso, 1987), latex (Wolf *et al.*, 1987), styrene (Lund *et al.*, 1988; Jakobsen *et al.*, 1990), nitrocellulose (Meinkoth & Wahl, 1984), plastic and agarose (Polsky-Cynkin *et al.*, 1985) and cellulose (Gilham, 1968; Bertazzoni *et al.*, 1971; Goldkorn & Prockop, 1986). Many of these methods, however, render the majority of immobilized sequences inaccessible to base-pairing with nucleic acid probes, since the linkage is formed randomly with reactive groups present on the target nucleic acid backbone (Bünemann *et al.*, 1982; Polsky-Cynkin *et al.*, 1985). Stable covalent linkages mediated by CNBr or CM-cellulose or intercalation of attached ligands with immobilized receptor (e.g. biotin–streptavidin) (Lund *et al.*, 1988; Syvänen *et al.*, 1988; Wilchek & Bayer, 1988; Wahlberg *et al.*, 1990) have demonstrated an increased availability of immobilized DNA for subsequent hybridization. Introduction of such reactive/ligand groups to the 3′-end or 5′-end of DNA (Bertazzoni *et al.*, 1971) has increased hybridization capacities; however, they often produce a high degree of non-specific binding (Lund *et al.*, 1988).

Paramagnetic beads (Lea *et al.*, 1985; Kemshead & Ugelstad, 1985) have been demonstrated to have considerable utility in fundamental and applied research, and in clinical diagnostics for the separation of cells (Kvalheim *et al.*, 1989), analyte protein (Gabriesen *et al.*, 1989) and nucleic acid sequences (Jungell-Nortamo *et al.*, 1988). We have therefore explored the ability to directionally link oligonucleotides to paramagnetic beads via disulphide bond formation between thiol groups on the beads (Hermentin *et al.*, 1990) and a 5′-terminal thiol group introduced into the oligonucleotide during synthesis. The efficiency of coupling, stability of bond and subsequent capacity to hybridize specifically to complementary target sequences under a variety of conditions has been investigated. The data suggest that the chemistries may have widespread applicability to the specific detection of nucleic acid sequences in simplified formats.

MATERIALS AND METHODS

Synthesis of oligonucleotides

An oligonucleotide (D99, 5′-TCAAAAGCCACTGTGTCC-TG-3′) that also acts as forward primer for the polymerase-chain-reaction (PCR) amplification of a 120 bp fragment of the E6 region of human papillomavirus type 16 (Young *et al.*, 1989) was synthesized with a 5′-terminal trityl-protected thiol group (Clonetech), termed S-D99, by using a Biotech Instruments BT8500 DNA synthesizer (Alta Biosciences, Birmingham, U.K.). Poly(dT)s having 4, 8 and 16 nucleotide residues were synthesized 5′ to the sequence of D99 and 3′ to the thiol group to study their effect on hybridization. cD99 (5′-CAGGACACAGTGGCT-TTTGA-3′) and D100 (5′-CGTGTTCTTTGATGATCTG-CA-3′) were synthesized respectively for use as complementary and non-complementary hybridization probe sequences to D99.

Abbreviations used: TTB, thiol-terminated Biomag beads.
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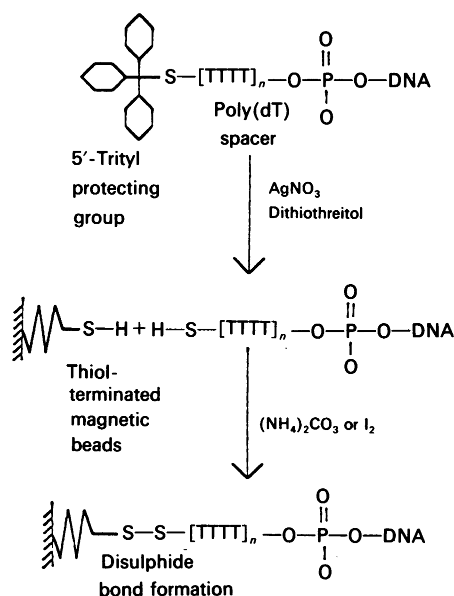

Scheme 1. Schematic representation of the detritylation of oligonucleotide D99 before co-oxidation with thiol-terminated magnetite beads with the use of I_2 or $(NH_4)_2CO_3$ as oxidant

Labelling of hybridization probes

A 1 μl portion of oligonucleotide solution (diluted to 200 μM in deionized water) was added to an Eppendorf tube containing 5 μl of buffer (Amersham kit N-4020), 5 μl of [α-^{32}P]dATP (Amersham PB-10235) and 4 units of terminal transferase, and the mixture was adjusted to 50 μl with deionized water before incubation at 37 °C for 1 h. The reaction was terminated by purification through a Sephadex G-25 or Sephadex G-50 column. Labelling efficiencies were almost exclusively $\geqslant$ 85%.

Coupling of oligonucleotides to thiol-terminated Biomag beads

A 20 nmol portion of oligonucleotide S-D99 in either distilled water or triethylammonium acetate buffer, pH 7.5 (TEAA buffer; made by diluting 1 ml of triethylamine in 72 ml of water and adjusting to pH 7.5 with acetic acid), was deprotected by cleaving the 5′-terminal trityl protecting groups with 10 μl of 1 M-$AgNO_3$ for 1 h at room temperature. Excess $AgNO_3$ was precipitated by incubation with 5 μl of 1 M-dithiothreitol for 1 h at room temperature (Scheme 1). The precipitate was removed and washed with 100 μl of TEAA buffer, and the two supernatants were pooled. Then 50 mg of thiol-terminated Biomag beads (TTB), which were thiol-modified silane-coated iron oxide particles (Hermentin *et al.*, 1990), were added to the supernatant, and the excess dithiothreitol was extracted with six 500 μl portions of diethyl ether. Traces of ether were removed with a stream of N_2 then I_2 (100 mg/ml in acetic acid) was added to colour the supernatant brown and the mixture was incubated at room temperature with occasional mixing for 1 h. The excess I_2 was removed by extraction with diethyl ether until the ether phase was clear. Traces of ether were removed with N_2, the supernatant was retained for gel-electrophoretic analysis, and the beads were washed in 200 μl of TEAA buffer followed by several washes in 200 μl portions of deionized water. After the coupling, the beads were stored at −20 °C, 4 °C or room temperature in deionized water.

$(NH_4)_2CO_3$ was used as an alternative oxidizing agent to I_2, although it has weaker oxidizing properties and requires a longer incubation. A 400 μl portion of 0.5 M-$(NH_4)_2CO_3$ was added to the TTB and S-D99 mixture, and incubated at room temperature for 2–6 days. The supernatant was removed, and the beads were washed several times with deionized water to remove excess $(NH_4)_2CO_3$. The coupled S-D99–TTB were stored at −20 °C, 4 °C or room temperature in deionized water.

Use of immobilized polynucleotides as hybridization capture probes

Portions (3 mg) of S-D99–TTB were placed into 0.6 ml tubes, all supernatants were removed (by using a Dynal Magnetic Particle Concentrator) and the beads were washed with sterile deionized water. Hybridization of ^{32}P-labelled cD99 and D100 oligonucleotides to immobilized S-D99 was performed during a 3 h incubation at 45 °C with 4×10^6 c.p.m. of the appropriate 3′-labelled oligonucleotide. The supernatants were decanted, and the beads were incubated four times in 200 μl of 2 × SSC (1 × SSC is 0.75 M-NaCl/0.075 M-sodium citrate buffer, pH 7) for 5 min each at 40 °C, then once at 50 °C, 60 °C, 70 °C, 80 °C and 90 °C for 5 min each. The radioactivities (c.p.m.) of each supernatant and the beads were counted on an LKB Rack Beta scintillation counter with Čerenkov counting. S-D99–TTB beads in 200 μl of 2 × SSC were used for hybridization either directly at room temperature or following pre-heating at 90 °C for 10 min. All assays were performed in duplicate and results represent means from several analyses.

RESULTS

In considering the data, it is noteworthy that the 3′-labelling technique used was extremely consistent, but was insufficiently accurate to compare radioactivity measurements directly between repeated analyses, the actual amounts of radioactivity retained by individual batches of beads differing by up to 20%. If, however, the cumulative radioactivities liberated from the various heat treatments (i.e. into the supernatants from 50–90 °C washes) are expressed as percentages of the amount remaining on the beads, there is consistency between the results for a given experiment (see Table 1).

Formation of the disulphide linkage

TTB were linked to S-D99 with the use of the following bead/oligonucleotide preparations: TTB with deprotected S-D99 to represent the specific coupling, TTB with protected S-D99–TTB and non-thiol-containing D99, and TTB alone to give indications of non-specific binding. Examples of radioactivity released from 'melting' of hybridized ^{32}P-labelled cD99 from the various bead/oligonucleotide combinations following incremental temperature incubations are displayed in Fig. 1 and Table 2. Most of the radioactivity was released at 60–80 °C, peaking at 70 °C. Experiments in which I_2 was used revealed that deprotected S-D99 coupled to TTB gave a higher hybridization signal compared with the protected S-D99, non-thiol D99 and finally plain beads samples, which showed 78%, 67% and 1.2% respectively of the radioactivity retained by deprotected S-D99–TTB samples. Coupling with the use of the milder oxidation method employing $(NH_4)_2CO_3$ increased the hybridization of cD99 by 90% compared with the hybridization achieved with I_2 as the oxidant. Experimental controls for the $(NH_4)_2CO_3$ oxidant generally exhibited lowered non-specific hybridization, showing 30%, 33% and 3% respectively of the radioactivity hybridized to S-D99–TTB (see Table 2).

Hybridization of ^{32}P-labelled non-complementary oligonucleotide D100 (of similar length to cD99) to TTB coupled with S-D99 generated 0.4% of the hybridization signal compared with cD99 (Table 2). Background binding to the control samples (plain TTB, non-thiol D99–TTB or protected S-D99–TTB) was negligible ($\leqslant$ 1.5%) with ^{32}P-labelled D100, and smaller

Table 1. Consistency of hybridization assay

The percentage release of radioactivity was calculated from the radioactivity released in the 50–90 °C washes and the radioactivity remaining on the beads' surface. Values are given as means ± S.D. (to indicate consistency) for the numbers of determinations given in parentheses. Abbreviation: RT, room temperature.

Oxidant used	Radioactivity released from beads (%)						
	Plain TTB (RT or 90 °C)		Deprotected S-D99–TTB		Protected S-D99–TTB		Non-thiol D99–TTB (SDS)
	SDS	No SDS	RT	90 °C	RT	90 °C	90 °C
I_2	30.1 ± 5.6 (3)	60.3 ± 9.5 (6)	86.0 ± 0.6 (2)	97.2 ± 0.9 (5)	94.9 ± 0.8 (3)	98.2 ± 1.0 (2)	96.3 ± 0.8 (2)
$(NH_4)_2CO_3$	72.6 ± 13.9 (3)	86.2 (1)	95.0 (1)	97.5 ± 1.6 (4)	95.3 (1)	97.6 ± 1.4 (3)	97.3 ± 0.8 (2)

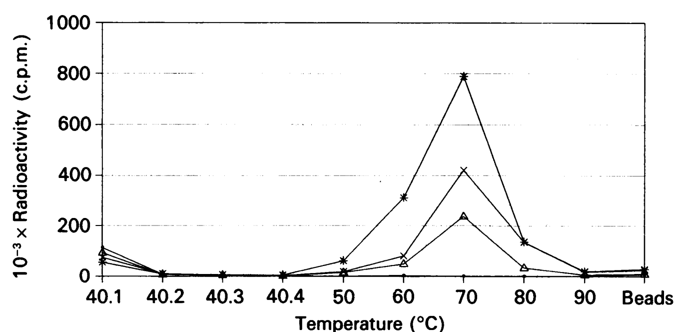

Fig. 1. Radioactivity released as a result of increasing temperature incubations from plain TTB (·), protected S-D99–TTB (×), deprotected S-D99–TTB (✱) and non-thiol D99–TTB (△) after hybridization to [^{32}P]dCTP-labelled cD99

than equivalent experiments in which ^{32}P-labelled cD99 was used as the hybridization probe.

Effect of blocking agents

A number of blocking agents commonly employed to decrease non-specific binding of proteins and nucleic acids (including BSA, milk powder, salmon sperm DNA and nucleotide triphosphates) did not decrease background binding of cD99 to control TTBs or S-D99–TTB coupled by either chemistry (results not shown). SDS, however, decreased binding of all probe sequences to S-D99–TTB and control TTBs (Table 2). The presence of 0.2% SDS during the formation of the disulphide linkage significantly decreased subsequent hybridization capacity. Inclusion of SDS in the hybridization reaction generated a significant decrease in non-specific hybridization to experimental controls (by 54–79%) and a 21% decrease in hybridization to the S-D99–TTB. The presence of SDS during both linkage and hybridization had a cumulative effect in decreasing background signals and/or specific hybridization signals. Although the overall hybridization signal with cD99 increased in the absence of SDS, this probably results from increased non-specific hybridization, since a similar increase was observed with D100 as the hybridization probe (results not shown).

Effect of temperature on coupling and hybridization

Pre-heating the TTB before coupling to S-D99 had the effect of decreasing the hybridization signal produced by cD99 to 25% of the value achieved by coupling at room temperature. Initial

Table 2. Effect of hybridization to complementary and non-complementary sequences, effect of oxidant used and effect of the use of SDS during hybridization and/or coupling

For the experiments with complementary and non-complementary sequences, the values are also expressed as percentages of the value for the deprotected S-D99–TTB sample probed with ^{32}P-labelled cD99. For the experiments with the two oxidants, the values are also expressed as percentages of the values for the deprotected samples. Abbreviation: N.D., not done.

Treatment	Probe used	Radioactivity released from beads (c.p.m.)			
		Deprotected S-D99–TTB	Plain TTB	Protected S-D99–TTB	Non-thiol D99–TTB
Complementary	cD99	1.3×10^6 (100%)	6.1×10^3 (0.5%)	1.6×10^5 (12.3%)	1.2×10^5 (9.2%)
Non-complementary	D100	4.8×10^3 (0.4%)	4.1×10^3 (0.3%)	2.0×10^4 (1.5%)	5.1×10^3 (0.4%)
Oxidant used					
I_2	cD99	9.4×10^5 (100%)	1.1×10^4 (1.2%)	7.3×10^5 (78%)	6.3×10^5 (67%)
$(NH_4)_2CO_3$	cD99	1.8×10^6 (100%)	5.4×10^4 (3.0%)	5.4×10^5 (30%)	5.9×10^5 (33%)
Use of SDS					
No SDS	cD99	1.4×10^6	4.2×10^4	9.7×10^5	4.6×10^5
SDS during hybridization	cD99	1.1×10^6	8.9×10^3	2.5×10^5	2.1×10^5
SDS during coupling	cD99	7.9×10^5	N.D.	N.D.	N.D.
SDS during coupling and hybridization	cD99	5.8×10^5	N.D.	N.D.	N.D.

Table 3. Effect of increasing temperature during pre-hybridization incubation of S-D99–TTB before hybridization to [^{32}P]dCTP-labelled cD99

Values are also expressed as percentages of the room-temperature value in 2 × SSC.

Temperature (°C)	Length of incubation (min)	Radioactivity released from beads in the 50–90 °C washes (c.p.m.)
Room temperature*	60	1.9×10^5(83 %)
Room temperature	60	2.3×10^5 (100 %)
45	10	3.1×10^5 (135 %)
45	60	3.5×10^5 (152 %)
90	10	4.3×10^5 (187 %)
90	60	3.1×10^5 (135 %)

* No 2 × SSC used during hybridization.

Table 4. Effects of increasing the length of 5′-poly(dT) spacer and pre-heating at 90 °C before hybridization

No. of residues in poly(dT) spacer arm	Radioactivity released from beads (c.p.m.)	
	After 90 °C pre-heating	No heat treatment
0	5.2×10^5	1.7×10^5
4	6.0×10^5	1.0×10^5
8	9.2×10^5	1.9×10^5
16	1.4×10^6	2.1×10^5

investigations demonstrated that hybridization of cD99 in 2 × SSC buffer to the S-D99–TTB (after 1 h at room temperature) produced a 21 % increase in signal compared with hybridization in water (Table 3). Heating of the S-D99–TTB in 2 × SSC at 45 °C for 10 min or 60 min before hybridization of cD99 steadily improved the hybridization signal by 35 % and 52 % respectively. An 87 % improvement in hybridization signal was evident after pre-heating of S-D99–TTB at 90 °C for 10 min; however, the signal was decreased to levels obtained with a pre-heating treatment of 45 °C for 10 min if the S-D99–TTB were heated at 90 °C for 60 min before hybridization.

Effect of the poly(T) spacer on hybridization

Addition of poly(dT) spacers of different lengths (4, 8 and 16 bases) 5′ to the immobilized S-D99 sequence and 3′ to the thiol group had an inconsistent but significant effect upon hybridization capacity to cD99 if the S-D99–TTB were kept at room temperature before hybridization (Table 4). However, if the S-D99–TTB were pre-heated at 90 °C for 10 min before hybridization a marked linear increase in hybridization signals was observed as the length of poly(dT) spacer increased.

DISCUSSION

Many strategies for immobilizing nucleic acids on solid supports severely limit their subsequent application in a number of assay formats, especially where availability of immobilized complementary sequence for hybridization or high temperatures are required, for example in the PCR (Saiki *et al.*, 1985; Erlich, 1989; Milbourne *et al.*, 1989). We have investigated the use of thiol group oxidation to link synthetic oligonucleotides specifically to the solid phase by formation of a disulphide bridge with thiol groups introduced on to the solid-phase surface. Here we report the results of studies with paramagnetic spheres. The data demonstrate several parameters that are generally pertinent to the development of strategies for optimal immobilization of synthetic oligonucleotides to solid supports.

Specific attachment of an oligonucleotide via a 5′-terminal thiol group is possible by means of several different chemistries, often dependent upon the nature of the solid support. Since the essential feature of this type of linkage reaction is purely oxidation, we have used either I_2 or $(NH_4)_2CO_3$. Using $(NH_4)_2CO_3$ as oxidant, we have achieved linkage of 1.8 μg of oligonucleotide accessible for hybridization as determined by the amount of 3′-labelled cD99 and hexanucleotide-labelled D99 required to saturate 3 mg of bead. Other immobilization techniques evaluate attached DNA by criteria not pertinent to their eventual use, such as S1-nuclease digestion (Bünemann *et al.*, 1982; Langdale & Malcolm, 1985). The apparently lower coupling achieved by our protocol may reflect the fact that non-specifically bound (not end-attached) DNA is not necessarily discernible during hybridization. With an excess of complementary sequence, a maximum of 32 % was bound by the immobilized S-D99–TTB (see Table 5). Binding is a function of target concentration and not incubation time, since experiments in which the incubation times were increased did not alter overall hybridization signals. Maximum hybridization was achieved after 2.5 h. Hexanucleotide-labelled D99 and probe sequences having 5′-terminal sequences non-complementary to D99 (PCR products) have produced similar results (results not shown). It is therefore likely that the immobilized oligonucleotide is capable of capturing DNA or RNA sequences in which the complementary sequence is located internally.

Use of I_2, a more potent oxidizing agent, leads to a decrease in coupling and/or oligonucleotide availability for specific hybridization (see Table 2) and increases background non-specific binding, which may be connected with the formation of sulphonic acid (Lui, 1977). Despite the oxidant, 5′-disulphide-bridge-immobilized oligonucleotides maintain hybridization specificity, as demonstrated by the negligible hybridization signal observed with the D100 non-complementary oligonucleotide sequence (Table 2). Furthermore, use of a series of control situations whereby plain TTB (i.e. no oligonucleotide coupled) or TTB that had reacted with oligonucleotides having no available or active thiol groups (either absence of thiol group or with the protecting trityl group present) demonstrates that with all chemistries the extent of hybridization is related to the amount of specific 5′-linkage of the oligonucleotide. Omitting the oxidants produces a similar background to that produced by the protected controls (results not shown). Thus the protected S-D99–TTB produced about 30 % and 78 % of the radioactivity released by the equivalent deprotected S-D99–TTB with the $(NH_4)_2CO_3$ and I_2

Table 5. Radioactivity bound by 3 mg of S-D99–TTB after initial addition of various amounts of labelled cD99

The values in the last column indicate the radioactivities bound between 50 and 90 °C expressed as percentages of total radioactivity added.

Bead type	Radioactivity added (c.p.m.)	Radioactivity released from beads in the 50–90 °C washes (c.p.m.)	Radioactivity bound to beads (%)
S-D99–TTB	5×10^6	1.5×10^6	30
S-D99–TTB	10×10^6	3.2×10^6	32
S-D99–TTB	20×10^6	4.6×10^6	23
S-D99–TTB	40×10^6	4.5×10^6	11

oxidants respectively. The hybridization achieved by negative controls suggests non-specific adsorption of D99 to the TTB surface at an extent and orientation that allows it to remain available for hybridization to complementary sequences but not non-complementary sequences, i.e. passive adsorption of D99 or probe akin to the passive adsorption on nylon or nitrocellulose membranes in Southern blotting (Williams, 1990). However, this was unaltered by extensively washing the beads in either 0.2 × SSC or distilled water at room temperature or 40 °C.

In order to attempt to demonstrate the relationship between the amount of specifically 5′-attached oligonucleotide to the degree of hybridization achieved, we employed S-D99–TTB in hybridization experiments after treatment of the beads with conditions designed specifically to remove any disulphide-bridged oligonucleotide. Unexpectedly, S-D99–TTB subjected to 90 °C in either 0.2 M-dithiothreitol or 2-mercaptoethanol showed only a partial decrease in hybridization signal with cD99 (65–50 % of the hybridization signal generated by untreated S-D99–TTB). This suggests that the disulphide bridges are more stable in this situation or that specific hybridization results from oligonucleotide bound by an unknown linkage that cannot be removed from the bead surface by reducing agents.

Hybridization specificity and capacity were unaffected by treatment with many of the usual non-specific binding-blocking agents. However, SDS decreased background binding during hybridization if included in the hybridization buffer, and also decreased the immobilization of S-D99 on TTB if present during oxidization of the thiol groups. The SDS is presumed to be preventing weak ionic associations forming between the silane-coated beads and the polynucleotides, covering the beads with a slight negative charge, thus repelling polynucleotides.

Specific hybridization capacity of oligonucleotides immobilized by the methods reported here was consistently achieved when the same S-D99–TTB were employed in consecutive hybridizations. The specific hybridization could also be enhanced by pre-heating the immobilized oligonucleotide above 80 °C before hybridization (see Tables 3 and 4), suggesting that some of the immobilized oligonucleotides were unavailable to the aqueous phase by weak energy interactions either with other oligonucleotide sequences or with groups on the solid phase. A 10 min incubation at 90 °C was chosen as the standard pre-hybridization incubation temperature, and this consistently improved the specific hybridization of cD99 (by 87 %), whereas non-specific hybridization and general background binding were essentially unchanged. There was no evidence to suggest the immobilized oligonucleotides were being removed during this treatment or heating at 92 °C for 30 min, but prolonged heating at 100 °C for a minimum of 5 min decreased cD99's hybridization signal (results not shown). A further temperature stability exercise consisting of 40 tri-temperature cycles comprising 30 s at 94 °C, 30 s at 40 °C and 90 s at 72 °C was used to test the stability of the linkage for use in the PCR. After this treatment the beads displayed a marked decrease in hybridization capacity, but if the cycling denaturation temperature was lowered to 90 °C the hybridization capacity remained generally unaffected (results not shown). Therefore, provided that the requirement for denaturation in PCR is not too rigorous, the disulphide linkage is suited to application in the PCR.

Different lengths of poly(dT) sequences incorporated at the 5′-end of the specific oligonucleotide sequence functioned effectively as spacer arms, exhibiting positive correlation with hybridization signal (Table 4). A poly(dT_4) spacer was employed throughout all the remaining experiments, but longer spacer sequences would improve specific binding. Poly(dT) sequences appear to have limited interaction with TTB and should be suitable for the capture of polyadenylated RNA (Jakobsen *et al.*, 1990).

Use of a disulphide bridge to link oligonucleotides via their 5′-phosphate groups to magnetic particles compares similarly with previously reported polynucleotide immobilization techniques (Lund *et al.*, 1988), exceeding those utilizing non-specific adsorptive methods (Bünemann *et al.*, 1982). A potentially useful facet of the reported strategy with TTB is that the linkage remains stable over a wide range of temperature and reagent conditions, and has a long shelf life. To date the S-D99–TTB complex has demonstrated 9 months' stability at 4 °C with no appreciable loss of activity, unlike several other linkages (Bünemann *et al.*, 1982). However, storage at −20 °C destroys TTB integrity. This will facilitate their application in a range of nucleic acid-based assays for fundamental and applied research and should also contribute to the development of simplified diagnostic assays. Polynucleotides immobilized by this technique are ideally suited to function in capture probe assays (Jungell-Nortamo *et al.*, 1988) and any other technique requiring specific and potentially reversible immobilization of a polynucleotide.

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REFERENCES

Bertazzoni, U., Campagnari, F. & De Luca, U. (1971) Biochim. Biophys. Acta **240**, 515–521

Bresser, J. & Gillespie, D. (1983) Anal. Biochem. **129**, 357–364

Bünemann, H., Westhoff, P. & Herrmann, R. G. (1982) Nucleic Acids Res. **10**, 7163–7180

Durrant, I., Benge, L. C. A., Sturrock, C., Devenish, A. T., Howe, R., Roe, S., Moore, M., Scozzafava, G., Proudfoot, L. M. F., Richardson, T. C. & McFarthing, K. G. (1990) Biotechniques **8**, 564–570

Erlich, H. A. (1989) PCR Technology: Principles and Applications for DNA Amplification, pp. 7–22, Stockton Press, New York

Gabriesen, O. S., Hornes, E., Korsnes, L., Ruet, A. & Oyen, T. B. (1989) Nucleic Acids Res. **17**, 6253–6267

Ghosh, S. S. & Musso, G. F. (1987) Nucleic Acids Res. **15**, 5353–5390

Gilham, P. T. (1968) Biochemistry **7**, 2809–2813

Goldkorn, T. & Prockop, D. J. (1986) Nucleic Acids Res. **14**, 9171–9191

Hermentin, P., Doenges, R., Franssen, U., Bieva, C., Vander Brugghen, F. J., Stryckmans, P., Friesen, H.-J., Optaczy, B., Schneider, S., Ax, W., Enssle, K. H., Kurrle, R. & Seiler, F. R. (1990) Bioconjugate Chem. **1**, 411–418

Jakobsen, K. S., Brievold, E. & Hornes, E. (1990) Nucleic Acids Res. **18**, 3669

Jungell-Nortamo, A., Syvänen, A.-C., Luoma, P. & Söderlund, H. (1988) Mol. Cell. Probes **2**, 281–288

Kemeney, D. M. & Challacombe, S. J. (1988) ELISA and other Solid Phase Immunoassays: Theoretical and Practical Aspects, pp. 155–180, John Wiley and Sons, New York

Kemshead, J. T. & Uglestad, J. (1985) Mol. Cell. Biochem. **67**, 11–18

Kennedy, J. F. & Cabral, J. M. S. (1983) in Solid Phase Biochemistry (Scouten, W. H., ed.), pp. 253–391, Wiley–Interscience, New York

Kvalheim, G., Fjeld, J. G., Pihl, A., Funderud, S., Ugelstad, J., Fodstad, O. & Nustad, K. (1989) Bone Marrow Transplant. **4**, 567–574

Langdale, J. A. & Malcolm, A. D. B. (1985) Gene **36**, 201–210

Lea, F. V., Davies, C. & Ugelstad, J. (1985) Scand. J. Immunol. **22**, 207–216

Lui, T.-Y. (1977) in The Proteins (Neurath, H. & Hill, R. L., eds.), pp. 239–353, Academic Press, New York

Lund, V., Schmid, R., Rickwood, D. & Hornes, E. (1988) Nucleic Acids Res. **16**, 10861–10880

Meinkoth, J. & Wahl, G. (1984) Anal. Biochem. **138**, 267–284

Milbourne, A., Nakamura, S. & Nakamura, J. M. (1989) Hawaii Med J. **48**, 125–126

Polsky-Cynkin, R., Parsons, G. H., Allerdt, L., Landes, G., Davis, G. & Rashtchian, A. (1985) Clin. Chem. **31**, 1438–1443

Rosen, K. M., Lamperti, E. D. & Villa-Komeroff, L. (1990) Biotechniques **8**, 398–403

Saiki, R. A., Scharf, S., Faloona, F., Mullis, K. B., Horn, G. T., Erlich, H. A. & Arnheim, N. (1985) Science **230**, 1350–1354

Syvänen, A.-C., Bengtström, M., Tenhunen, J. & Söderlund, H. (1988) Nucleic Acids Res. **16**, 11327–11338

Wahlberg, J., Lunderberg, J., Hultman, T. & Uhlen, M. (1990) Proc. Natl. Acad. Sci. U.S.A. **87**, 6569–6573
Walker, J. M. & Gaastra, W. (1985) Techniques in Molecular Biology, Croom Helm, London
Wilchek, M. & Bayer, E. A. (1988) Anal. Biochem. **171**, 1–32
Williams, D. L. (1990) Biotechniques **8**, 14–15
Wolf, S. F., Haines, L., Fisch, J., Kremsky, J. N., Dougherty, J. P. & Jacobs, K. (1987) Nucleic Acids Res. **15**, 2911–2926
Young, L. S., Bevan, I. S., Johnson, M. A., Blomfield, P. I., Bromidge, T., Maitland, N. J. & Woodman, C. B. J. (1989) Br. Med. J. **298**, 14–18

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