and 100-fold dilutions onto a ProtranTM Nitrocellulose Membrane (Schleicher & Schuell GmbH, Dassel, Germany). The filter was blocked with 5% low-fat milk in 1× PBS for 1 h at room temperature. After blocking, the filter was hybridized overnight at 4°C in O2-tag protein extract. Subsequently, the filter was washed for 15 min with 1× PBS buffer and hybridized with RGS•HisTM Antibody (Qiagen, Chatsworth, CA, USA) for 1 h at 4°C.

Hybridization with RGS•His was followed by three washing steps of 15 min each in 1× PBS at 4°C. The filter was hybridized with alkaline phosphatase (AP)-conjugate secondary antibodies for 1 h at 4°C. After hybridization, the filter was washed twice for 15 min with 1× PBS and once more for 15 min with 1× PBS plus 0.1% Tween[®] 20 at 4°C. Excess liquid was removed, and the moist filter was treated with CDP-StarTM using Nitro-Block-IITM Luminescence Enhancer (Tropix, Bedford, MA, USA). Signal was revealed after 30 min of exposure (Figure 2).

Figure 2 shows the observed signals corresponding to the formation of O2 homodimers, O2-OHPI heterodimers and O2-PBF interacting proteins. No signals were observed for the negative control, consisting of protein extract from *E. coli* cells transformed with the expression vector pET3c. An additional control was performed by probing the filter with protein extract from *E. coli* cells that did not express O2-tag. Again, no positive signals could be observed (data not shown).

In conclusion, the method presented here allows the detection of protein-protein interactions between a known protein fused with an epitope and a list of candidate interacting proteins. The fact that this approach does not require protein purification or the use of radio-labeled material, makes this method an attractive alternative in comparison to previously cited methods. Although no attempt was made here to assess the relative strength of protein-protein interactions quantitatively, it is suggested that by putting a different epitope tag on the candidate protein than that on the known protein, it should be possible to quantitate the amount of candidate protein in each bacterial extract and load equivalent molar amounts of each

onto the blots. This would permit a direct comparison of the strength with which the tester protein interacts with each of the candidate proteins.

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Disruption of the Streptavidin Interaction with Biotinylated Nucleic Acid Probes by 2-Mercapto-ethanol

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Biotinylated oligonucleotides are now a well-established and very useful tool in many fields of biological research, particularly as probes for hybridization studies (3). Methods of biotin detection usually take advantage of the extremely tenacious interaction between streptavidin and biotin [$K_d = 1.3 \times 10^{-15}$] at pH 5.0 (4). It is crucial to ensure that this interaction remains stable under as many different buffer conditions as possible, which in practice normally poses few problems. The major problem encountered in the application of this system lies in the difficult reversal of the interaction between immobilized streptavidin and the adsorbed biotinylated material. Subsequently, rather drastic elution conditions usually have to be used.

Disrupting the biotin/streptavidin interaction is particularly desirable when, for example, biotin is used as an affinity tag for the purification of biological molecules. This includes the efficient, quantitative chromatographic purification of valuable biotinylated oligonucleotides (2). Another application in which the desired elution of biotinylated oligonucleotides from the streptavidin matrix plays a major role is in the screening of randomized RNA libraries for novel catalytic RNAs. This strategy involves the covalent modification of the active ribozymes with a biotin-tag

Benchmarks

and the subsequent purification by streptavidin chromatography (1,6). Current elution procedures either involve highly denaturing conditions using chaotropic reagents such as 50% guanidine isothiocyanate (GITC)/formamide (2) (the disadvantage being that these agents also release significant amounts of streptavidin) or competition of streptavidin-bound biotinylated oligonucleotides with high concentrations of free biotin. This second method involves incubation with biotin in solution at 95°C for 10 min and subsequent washes with large volumes of biotin solutions; conditions that can result in significant RNA molecule degradation (1,6). Another serious disadvantage of this elution method is that the excess biotin used as a competitor for streptavidin binding has to be removed in subsequent purification steps. In cases in which quantitative removal of free biotin is essential, this might prove to be

difficult and time-consuming.

We have observed an unexpected lability of the biotin/streptavidin interaction of streptavidin-immobilized biotinylated oligonucleotides (RNA and DNA) in the presence of 2-mercaptoethanol (2-ME) or dithiothreitol (DTT). This observation might lead to the establishment of a rapid, simple and inexpensive procedure for the release of streptavidin-bound biotinylated oligonucleotides under relatively mild and controllable conditions that avoids the presence of free biotin or chaotropic agents in the eluted sample. The method involves incubation of streptavidin-bound biotinylated oligonucleotides with a buffer adjusted to 2.0 M 2-ME and either incubation at room temperature or heating to 95°C for 2–3 min. With DTT instead of 2-ME, elution of oligonucleotides was also observed under the same conditions with similar results both at room temperature and at

95°C (data not shown). Our study provides, to our knowledge, the first example in which thiols such as 2-ME or DTT have been used to disrupt the biotin/streptavidin interaction. These results can be relevant for hybridization studies, allowing better evaluation of whether certain reducing buffer conditions are suitable or whether it is best to avoid them.

For our studies, we used various random or specific RNA sequences of different lengths (Table 1) that had a biotin moiety attached to an internal 2'-hydroxyl group (2'-OH) by a 3-atom spacer arm (5). ³²P-labeled biotinylated RNAs were generated as described previously (5). DNA oligonucleotides were biotinylated at the 5' end and were labeled with ³²P at the 3' end. The radiolabeled oligonucleotides [25 pmol, in a final vol of 200 µL of streptavidin coupling buffer (150 mM NaCl, 25 mM sodium phosphate, pH 6.9)] and ¹⁴C-

Figure 1. Qualitative analysis of biotinylated oligonucleotides 28-mer and 33-mer DNA on a 20% denaturing polyacrylamide gel after elution with 2-ME. E: Samples immobilized on streptavidin and eluted from the matrix by heating to 95°C for 2 min in the presence of 2.0 M 2-ME. For comparison, untreated oligonucleotides were included in various concentrations given as the percentage of total input DNA (100, 75, 50 and 25). Similar data were obtained for all other oligonucleotides shown in Tables 1 and 2. RNA oligonucleotides clone 11 RNA, clone 26 RNA, clone 29 RNA and 28-mer RNA were still fully active as ribozymes or ribozyme substrates, respectively, after elution with 2-ME.

biotin (Amersham Pharmacia Biotech, Braunschweig, Germany) as a control were coupled to 50 µL streptavidin agarose (SA; Pierce Chemical, Rockford, IL, USA) by incubating for 30 min. After immobilization, the agarose was transferred into a spin-filter tube and washed with 8 resin vol (400 µL) each of 3 different buffers (**W1**: 1 M NaCl, 5 mM EDTA, 25 mM K-3-[N-morpholino] propanesulfonic acid (MOPS), pH 7.4; **W2**: 4 M urea, 5 mM EDTA adjusted with K-MOPS to pH 7.4; **W3**: 3 M guanidinium-HCl, 5 mM EDTA; final wash: 400 µL water) until no further radioactivity could be detected in the wash fractions. Substrate bound oligonucleotides were eluted by incubating the agarose samples with 400 µL elution buffer (1.0 M NaCl, 50 mM MOPS, pH 7.4, 5.0 mM EDTA) in the presence of 0.2 or 2.0 M 2-ME for 3 min either at room temperature or including heating to 95°C. After centrifugation, virtually all free oligonucleotides were recovered in this single fraction of 400 µL. Eluted RNA or

Table 1. Oligonucleotides Used

Oligonucleotide	Sequence
Pool 7 RNA ^a	Mixture of various sequences described in Reference 5.
Pool 8 RNA ^a	Mixture of various sequences described in Reference 5.
Pool 10 RNA ^a	Mixture of various sequences described in Reference 5.
Clone 11 RNA ^a	164-mer ribozyme isolated as described in Reference 5.
Clone 26 RNA ^a	161-mer ribozyme isolated as described in Reference 5.
Clone 29 RNA ^a	160-mer ribozyme isolated as described in Reference 5.
28-mer RNA ^a	5'-GAGUCGGGGGCACCUGCCACGAGGGAUC-3'
20-mer DNA	5'-Biotin-ATGGATCCGACCGTGGTGCC-3'
28-mer DNA	5'-Biotin-GGGACGAATTCTAATACGACTCACTATA-3'
33-mer DNA	5'-Biotin-GGGACGAATTCTAATACGACTCACTATAGGAGC-3'

^aThe biotin tag is attached to an internal cytosine (5).

Table 2. Elution Behavior of Various Biotinylated Radiolabeled RNA and DNA Oligonucleotides and ¹⁴C-Biotin from Streptavidin Agarose in the Presence of 2-ME

Oligonucleotide	% of Bound Biotinylated Oligonucleotide Eluted			% of Eluted RNA Rebound to Streptavidin
	0.2 M 2-ME 23°C	2.0 M 2-ME 23°C	2.0 M 2-ME 95°C, 2 min ^c	
Pool 7 RNA ^a	6.3	54.0	nd	90
Pool 8 RNA ^a	5.5	53.0	nd	90
Pool 10 RNA ^a	3.8	61.2	96	82
Clone 11 RNA ^a	3.0 ± 0.7	55.6 ± 1.8	93 ± 4.2	91
Clone 26 RNA ^a	3.8	58.1	nd	90
Clone 29 RNA ^a	3.0	51.3	nd	85
28-mer RNA ^a	1.0 ± 0.2	58.4 ± 2.3	95 ± 3.7	86
20-mer DNA ^b	0.2 ± 0.1	2.8 ± 0.1	44.0 ± 6.7	94
28-mer DNA ^b	0.7	2.6	55.7	91
33-mer DNA ^b	0.5 ± 0.2	3.3 ± 0.3	64.5 ± 8.0	88
¹⁴ C-biotin	0	0	2.8 ± 0.3	

^aBiotin introduced by ribozyme catalysis (5) to internal 2'-OH by phenylalanyl ester linkage (3-atom spacer).

^bBiotin introduced by chemical synthesis to 5' end using a biotin-phosphoramidite with a 7-atom spacer between biotin and the 5'-phosphate (Glen Research, Sterling, VA, USA), DNA was labeled at the 3' end with [α-³²P]cordycepin triphosphate and terminal deoxynucleotidyl transferase (Stratagene, La Jolla, CA, USA).

^cHeating to 95°C for 2 min in the absence of 2-ME or DTT lead only to elution of traces of bound oligonucleotides (between 0.2% and 1.8%).

Values for which an error range is shown were determined in triplicate.

nd = not determined.

DNA was quantified by scintillation counting, precipitated with ethanol and resuspended in a streptavidin coupling buffer. To show that the biotin tag was still covalently bound to the eluted oligonucleotides, they were again coupled to fresh streptavidin agarose as described above. It was established that

each oligonucleotide eluted with 2-ME could be almost quantitatively re-immobilized after removal of 2-ME (Table 2). Pretreatment of streptavidin agarose with 2 M 2-ME for 30 min at 23°C, heating to 95°C for 2 min and washing with buffers W1–W3 did not affect the ability of streptavidin to bind biotinylated oligonucleotides. Thus, it should be possible to recycle the streptavidin matrix after bound oligonucleotides have been removed. Control, nonbiotinylated versions of the same oligonucleotides were not detectably immobilized on streptavidin. The fact that the eluted oligonucleotides were not degraded was confirmed by polyacrylamide gel electrophoresis (PAGE) analysis (Figure 1).

Table 2 shows that between 1% and 6% of the various biotinylated RNA molecules immobilized on streptavidin agarose can be eluted after 3 min incubation at room temperature with an elution buffer containing 0.2 M 2-ME. Up to 60% of immobilized biotinylated RNA is eluted when the concentration of 2-ME is raised to 2.0 M. Without 2-ME, the RNA could not be eluted. The similar data obtained with the different RNA oligonucleotides indicate that the elution behavior does not depend on the length or sequence of the RNA.

With 5'-biotinylated short synthetic DNA oligonucleotides (Table 1), which have a spacer arm of 7 atoms between the biotin moiety and the 5'-terminal phosphate, the disruption of the biotin/streptavidin interaction with 0.2 M 2-ME was below 0.5%. However, about 3% elution was detected with 2.0 M 2-ME after incubation for 3 min at ambient temperature and up to 72.5% elution after incubation with a buffer containing 2.0 M 2-ME heated to 95°C for 2 min. The labeled biotin control was not detectably eluted under any conditions at room temperature, and only 3% was eluted with 2.0 M 2-ME after heating to 95°C for 2 min. This suggests that an oligonucleotide attached to biotin might favor the disruption of the streptavidin/biotin interaction and/or prevents rebinding in the presence of 2-ME. It might also be possible that the 2'-OH groups on the RNA contribute to effects that facilitate the elution by 2-ME or DTT when compared to the less efficient elution prop-

erties of DNA oligonucleotides. The differences in spacer arm length might also contribute to the elution efficiency to some extent.

In summary, Table 2 shows that 2'-biotinylated RNAs could be eluted, although inefficiently, from streptavidin agarose with 0.2 M 2-ME at room temperature. With 2.0 M 2-ME again at room temperature, elution of between 50% and 60% of the RNA oligonucleotides, regardless of sequence or length, was possible. However, 5'-biotinylated DNA primers were only inefficiently eluted with 2.0 M 2-ME at room temperature, but after heating to 95°C for 2 min, more than 50% of the labeled DNA was released from the matrix. Low levels of labeled biotin alone were only eluted with 2.0 M 2-ME after heating. The oligonucleotides eluted with 2-ME were still covalently bound to the biotin tag, as demonstrated by their efficient re-immobilization on streptavidin agarose, while checking the integrity of the eluted oligonucleotides confirmed that they were not degraded.

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Inexpensive Alternative to M&S Medium for Selection of *Arabidopsis* Plants in Culture

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The vacuum infiltration method is widely used to introduce foreign genes into *Arabidopsis thaliana* plants (1). To identify transformants, large numbers of seeds from the infiltrated plants must be screened for the presence of the dominant selectable marker. Many commonly used transformation vectors use the neomycin phosphotransferase II gene as the selectable marker. This confers kanamycin resistance to transformed plants. Kanamycin selection of transformants is commonly carried out on agar-solidified Murashige and Skoog (M&S) medium (2) supplemented with kanamycin. Although the vacuum infiltration method is easy, the transformation frequency is low; usually only a few percent of the seed from the infiltrated plants are transformed. Because of this low frequency, the screening and generation of large populations of transformed plants can be expensive.

We have found an alternative to M&S salts for the growth of *Arabidopsis* on agar-solidified medium. This alternative fulfills four time- and money-saving criteria: (i) it supports the growth of *Arabidopsis* plants on agar-solidified medium comparable to M&S medium, (ii) it is inexpensive, (iii) it is easy to prepare and (iv) it can be used as a complete nutrient solution for plants grown in soil or a soilless potting medium.

To find an alternative, we plated