drug efficacy. The protecting groups, and not the nucleic acid itself, can elicit an antibody response[6].

Unfortunately, no simple, reproducible, sensitive, and inexpensive analytical method exists to identify and quantify every protecting group that may remain in an antisense sample. High-performance liquid chromatography (HPLC) nucleoside composition analysis identifies and quantifies protecting groups remaining on oligonucleotides. However, the analysis is insensitive because it depends on enzymatic cleavage of the oligonucleotide and on UV diode-array detection for identification and quantification. Capillary electrophoresis and mass spectrometry detect the aborted sequences, but are not easily adapted to identifying and quantifying the protecting groups that remain on the oligonucleotide[6].

To address this problem, we previously developed monoclonal antibodies (mAbs) for the specific identification and quantification of the nucleobase and sugar protecting groups commonly used in DNA and RNA chemical syntheses[6]. Using these mAbs, we now present a dot-blot assay and a microplate enzyme-linked immunosorbent assay (ELISA) for identification and quantification of protecting groups that remain in standard, intact DNA and RNA oligonucleotide samples (Fig. 1).

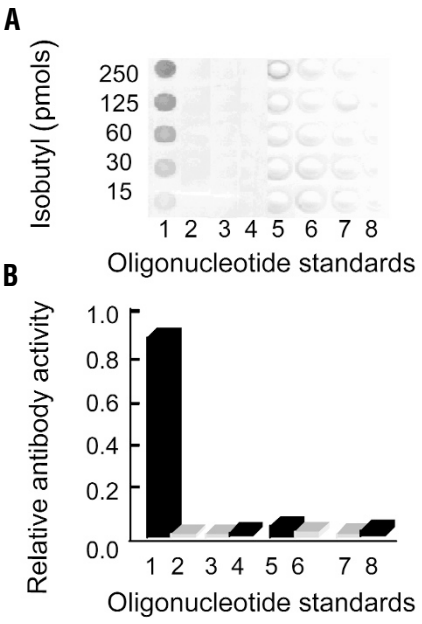

Figure 1. MAb detection of isobutyl protection on oligonucleotides. (A) Nitrocellulose dot-blot assay. (B) Microplate-based ELISA. MAb developed against the isobutyl-protecting group selectively recognizes a 20-mer oligonucleotide comprising isobutyl-protected dG. Protected homopolymer 20-mer standards validated by nucleoside composition and spectral analyses as having 80–95% protection included: isobutyl-protected dG (lane 1); isopropyl-phenoxyacetyl-protected dG (lane 4); benzoyl-protected dC (lane 5); and 8) 4,4′-dimethoxytrityl-protected dT (lane 8). The corresponding 20-mer homopolymer standards validated by HPLC as deprotected from the same protection groups were the following: dG from isobutyl group (lane 2); dG from isopropyl-phenoxyacetyl group (lane 3); dC from benzoyl group (lane 6); and dT from dimethoxytrityl group (lane 7).

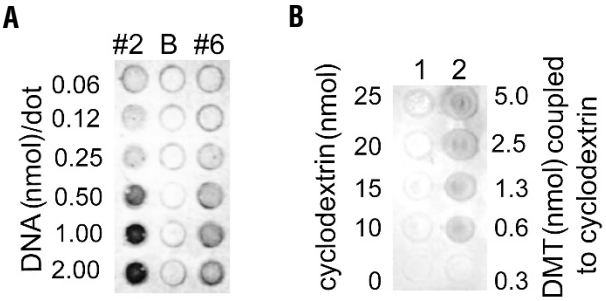

Figure 2. MAb detection of the benzoyl-protecting group on commercial samples, and the common hydroxyl-protecting group, dimethoxytrityl, on cyclodextrin. (A) In a dot-blot assay, the mAb against benzoyl group detects the contaminating protecting group on samples of 20-mer primer DNA purchased from two different companies (#2 and #6; B, blank lane). (B) The mAb against dimethoxytrityl group detects the protecting group on cyclodextrin using a dot-blot assay even though the immunological response to generate the antibody was initiated with an oligonucleotide derivative. Lane 1, cyclodextrin; lane 2, dimethoxytrityl-derivatized cyclodextrin. The amount of dimethoxytrityl coupled with cyclodextrin was determined by comparison of absorbance at 500 nm with that of a standard curve for dimethoxytrityl chloride.

Monoclonal antibodies have the advantage of identifying specific protecting groups that remain on intact oligonucleotides independent of the base or sugar. The assays are amenable to robotic analysis of hundreds of samples and should be applicable to oligonucleotides on solid supports. The antibodies could be used for the synthesis of affinity columns to separate incompletely deprotected nucleic acid from completely deprotected molecules.

Because the mAb reagents are group specific and not influenced by the polymer support, as demonstrated by the identification of 4,4′-dimethoxytrityl groups on cyclodextrin (Fig. 2B), the technology can be applied to the detection of protecting groups remaining from the synthesis of other biopolymers, dendrimers, and biopolymers on solid supports, such as oligonucleotide and peptide arrays.

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Erratum

The need for national centers for proteomics
Ruedi Aebersold and Julian D. Watts
Nat. Biotechnol. ***20,*** *651 (2002).*

Because of a proofreading error, Julian D. Watts' name was incorrect in the author affiliations.

The correct affiliations are as follows: Ruedi Aebersold is cofounder and Julian D. Watts is a senior research scientist, Institute for Systems Biology, 1441 North 34th Street, Seattle, WA 98103 (raebersold@systemsbiology.org). We regret the error.