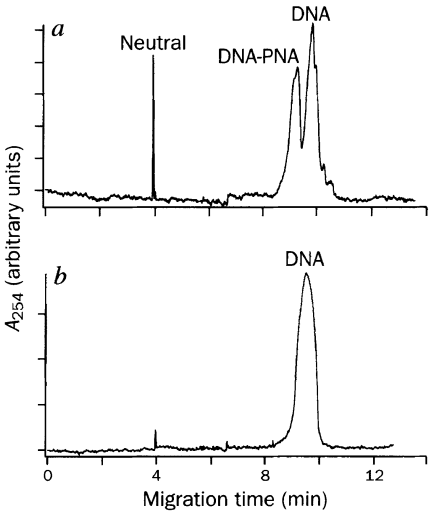
Screening for genetic mutations

SIR — A practical method for screening human genes for mutations must be rapid, inexpensive and have the capability of detecting single base substitutions. We report here the use of the DNA mimic, peptide nucleic acid (PNA)[1], as a probe for genetic mutations.

PNA differs from DNA in that the DNA ribose–phosphate backbone is replaced by its pseudo-peptide counterpart, in this case *N*-(2-aminoethyl)glycine. By choosing the sequence of the four bases in PNA to be complementary to one of the strands of DNA, the PNA probe hybridizes by Watson–Crick pairing. Such hybridization reactions can be rapid, and the hybridization products can be readily separated by free-solution capillary electrophoresis because the hybrid complex has a different ratio of electrical and frictional forces from the native DNA, because of the non-ionic nature of PNA[2]. We illustrate this procedure by using a PNA to distinguish normal (wild-type) and mutant sequences in the human cystic fibrosis gene.

The table gives the sequence of the 15-mer PNA probe we synthesized, as well as four test sequences, each comprising a 50-mer single-stranded DNA fragment, representing the wild-type and three mutant sequences of the cystic fibrosis transmembrane conduction regulator (CFTR) gene[3]. In particular, the three-base deletion ΔF508 accounts for two-thirds of the CF mutations, the percentage varying with different populations[4].



Comparison of electropherograms at 70 °C of wild-type gene hybridized with PNA (*a*) and DNA containing a single-base substitution (MU1) in the target region hybridized with PNA (*b*). Free PNA is not detected because it binds to the capillary wall.

The hybridizations were carried out for 5–10 min at room temperature, and the mixtures were then separated on a Beckman P/ACE Model 2000 capillary electrophoresis instrument (kindly made available by S. Pentoney Jr) modified to operate at high temperatures. The figure shows electropherograms for wild-type (*a*) and mutant MU1 (*b*) DNA where the latter is a single base substitution in the target region hybridized with PNA (see table), both recorded in absorption at 70 °C. At this temperature and ionic strength (50 mM) DNA–DNA hybridization is unlikely, and DNA–PNA hybrid complexes melt unless the sequence is a perfect match. The peaks in the figure were identified by varying the PNA concentration in the hybridization reaction (not shown). This ability to distinguish mismatches has been demonstrated for other sequences (not shown).

In other experiments, we added the PNA probe to a polymerase chain reaction preparation of human DNA of the 142-mer wild-type CF fragments and the 139-mer three-base deletion fragments. At high temperature (50 °C), a PNA complex of unknown structure is indicated with the wild-type but not with the mutant DNA. Before the method can be developed to its full potential, there must be further testing of genomic double-stranded DNA and exploitation of the concentration dependence of the PNA–DNA signal to allow distinction between homo- and heterozygosity.

Our results to date encourage us to believe that a PNA probe library, possibly with multiple fluorescence tags for multiplex testing of one or more exons, might form the basis for a universal screening strategy for any genetic disease with a known spectrum of mutations[5].

SYNTHETIC DNA FRAGMENTS OF THE CYSTIC FIBROSIS GENE

DNA fragment	Sequence*	Mutation
WT	5′-{a}-A TAT CAT CTT TGG TG-{b}-3′	None
ΔF508	5′-{a}-A TAT CAT ··· TGG TG-{b}-3′	Deletion of three bases
MU1	5′-{a}-A TAT CGT CTT TGG TG-{b}-3′	One-base substitution (underlined)
MU2	5′-{a}-A TAT ACT CTT TGG TG-{b}-3′	Two-base substitution (underlined)

The PNA used has the sequence H-CA CCA AAG ATG ATA T-Lys=NH_2.
*{a}, TGG CAC CAT TAA AGA AA; {b}, T TTC CTA TGA TGA ATA TA.

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Bomb signals in old Antarctic brachiopods

SIR — Skeletal check-marks are commonly used to assess the age and growth of organisms. They are usually assumed to be formed annually. By using radiocarbon bomb signals to calibrate growth checks in shells of Antarctic brachiopods, we show that they were laid down with a sub-biennial periodicity. The data also indicate that low Southern Ocean ^{14}C signals are probably not caused by upwelling deep water, but are more probably due to reduced atmospheric supply and long-term radiocarbon deposition in ice.

Articulate brachiopods dominate the macrofossil record from Cambrian times. They are sessile filter-feeders, present in all oceans of the world, and are classified as 'low-energy lifestyle' organisms[1]. Growth-rate investigations, which are performed rarely, are typically based on analyses of bands in their calcium carbonate shell valves[2]. All species with carbonate skeletons incorporate ^{14}C from the environment. Radiocarbon concentrations in oceans are low compared with atmospheric and terrestrial levels, because their dissolved carbon pool is around 60 times the atmospheric pool. Variability in oceanic signals has been used to indicate areas of upwelling water[3], and coral and bivalve mollusc skeletal signals to assess ^{14}C records in latitudes to 42 °N (ref. 4).