

# A new class of genome rare cutters

Alexei G. Veselkov, Vadim V. Demidov, Peter E. Nielsen<sup>1,\*</sup> and Maxim D. Frank-Kamenetskii

Center for Advanced Biotechnology and Department of Biomedical Engineering, Boston University, 36 Cummington Street, Boston, MA 02215, USA and <sup>1</sup>Center for Biomolecular Recognition, Panum Institute, University of Copenhagen, Blegdamsvej 3, DK-2200 Copenhagen N, Denmark

Received April 19, 1996; Accepted May 8, 1996

## ABSTRACT

Although significant efforts have been directed at developing efficient techniques for rare and super rare genome cutting, only limited success has been achieved. Here we propose a new approach to solve this problem. We demonstrate that peptide nucleic acid 'clamps' (bis-PNAs) bind strongly and sequence specifically to short homopyrimidine sites on  $\lambda$  and yeast genomic DNAs. Such binding efficiently shields methylation/restriction sites which overlap with the bis-PNA binding sites from enzymatic methylation. After removing the bis-PNA, the genomic DNAs are quantitatively cleaved by restriction enzymes into a limited number of pieces of lengths from several hundred kbp to several Mbp. By combining various bis-PNAs with different methylation/restriction enzyme pairs, a huge new class of genome rare cutters can be created. These cutters cover the range of recognition specificities where very few, if any, cutters are now available.

## INTRODUCTION

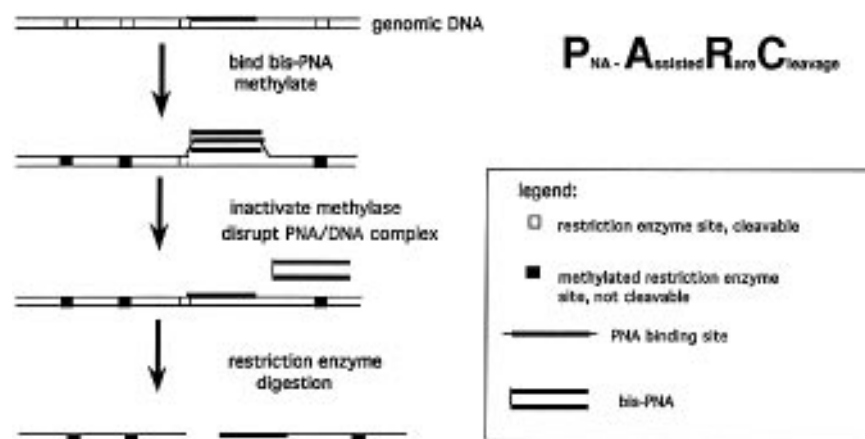
While pulsed-field gel electrophoresis (PFGE) solves the problem of separation of large DNA molecules (1,2), a recent optical mapping technology allows a rapid construction of ordered maps for rare cut individual chromosomes (3,4) and the problem of cloning of large DNA fragments has also been solved (5,6), cleavage of genomic DNA into a limited number of large pieces still remains a problem. Most restriction enzymes recognize only 4 or 6 nt (7), so the target sites are met too often in a particular genome. Although significant efforts have been directed towards developing efficient methods for rare and super rare genome cutting, only partial success has been achieved. Very few naturally occurring rare cutting restriction and intron-encoded endonucleases and other enzymes with recognition sites of 8 bp or longer have been identified (7–9). Therefore, further widening of the arsenal of rare DNA cutters is very desirable. Among the different approaches, the Achilles' cleavage general strategy has proven to be most efficient and versatile to increase the selectivity of restriction enzyme cutting (10,11). In principle, this strategy

permits the conversion of any restriction enzyme into a rare cutter. To this end, a sequence-specific tool is required, which shields one or a few of the methylation/restriction sites for the chosen pair of methylation/restriction enzymes on the genomic DNA. However, up to now this approach was limited to cleavage of genomic DNA at predetermined sites (12–18). We have recently proposed a new approach, which offers a general solution to the problem (19).

Our approach assumes a ligand which sequence specifically targets short DNA sites overlapping with restriction/methylation sites. After methylation of accessible sites and removal of the ligand, DNA is cut with the restriction enzyme in a very limited number of sites. To meet the goal of rare cleavage of an arbitrary genome it is necessary to be able to cut DNA of unknown sequence. Therefore, the ligand must target a short DNA region. If the target region is 15 bp or longer, at the most one cutting site in the entire genome is expected statistically, given its size equivalent to the human one. To cut an unknown genome into pieces in the range from several hundred kbp to several Mbp one needs the total length corresponding to the ligand target site plus the non-overlapping part of the restriction site to be 8–12 bp long. This requirement for targeting short sequences makes our approach radically different from the existing approaches, which permit cutting of DNA only at predetermined sites consisting of 20 bp or more (12–18). Within the framework of these methods shorter ligands either do not bind to DNA or are displaced at the methylation stage.

We achieve the goal of targeting short DNA sequences by using peptide–nucleic acid (PNA), which sequence specifically forms exceptionally stable complexes with duplex DNA. PNA is an oligonucleotide mimic in which the common DNA nucleobases are attached via a linker to an *N*-(2-aminoethyl)glycine backbone (20). As a result of sequence-specific binding to duplex DNA of homopyrimidine PNAs, a stable P-loop structure emerges consisting of a PNA<sub>2</sub>–DNA triplex and the displaced DNA strand (21,22). Furthermore, it has been shown that a restriction site adjacent to the P-loop is completely protected from cleavage by restriction endonuclease (23). The X-ray crystallographic structure of the triplex of the oligonucleotide with two PNA strands (24) has indicated why the DNA–PNA<sub>2</sub> triplex is so exceptionally stable. It appeared that, in addition to classical Hoogsteen pairing, this triplex is stabilized by hydrogen bonds

\* To whom correspondence should be addressed



**Figure 1.** Schematics of the PARC technique for rare enzymatic cleavage of genomic DNA mediated by a peptide-nucleic acid 'clamp' (bis-PNA).

formed by the peptide NH group of the PNA residues, which forms a Hoogsteen pair with an oxygen of the phosphate groups of the oligonucleotide.

Since two PNA molecules bind to the DNA single strand forming the P-loop, PNA 'clamps' (bis-PNAs) consisting of two PNA molecules connected with a flexible linker proved to be much more efficient in targeting short regions on duplex DNA than monomeric PNAs (25,26). Further stabilization of the P-loop at neutral pH can be achieved by replacing cytosines with pseudo-isocytosines in that half of the bis-PNA, designed for Hoogsteen recognition (25). We have demonstrated that such bis-PNAs, carrying extra positive charges to promote additional electrostatic stabilization of the PNA-DNA complexes bound strongly and sequence specifically to quite short target sites on genomic DNA and efficiently protected a limited number of methylation sites overlapping with them (19). After removing the bis-PNA, the restriction enzyme made very few cuts in the yeast genome (19). Here we present the detailed data, which strongly suggest that short, positively charged bis-PNAs combined with various methylation/restriction enzyme pairs provide a huge new class of genome rare cutters. This opens new opportunities for genome mapping, cloning and sequencing.

## MATERIALS AND METHODS

PNA oligomerization was performed as previously described (27) and the PNA monomers and egl linker were obtained from PerSeptive Biosystems. The PNA oligomers were purified by reversed phase HPLC and characterized by MALDI-TOF mass spectroscopy. The following bis-PNAs (J is pseudo-isocytosine and egl is 8-amino-3,6-dioxaoctanoic acid) were used:

PNA 1, H-T<sub>2</sub>JTJ<sub>2</sub>T<sub>2</sub>-egl<sub>3</sub>-T<sub>2</sub>C<sub>2</sub>TCT<sub>2</sub>-LysNH<sub>2</sub>;

PNA 2, H-Lys<sub>3</sub>-T<sub>2</sub>J<sub>2</sub>T<sub>3</sub>-egl<sub>3</sub>-T<sub>3</sub>C<sub>2</sub>T<sub>2</sub>-LysNH<sub>2</sub>;

PNA 3, H-Lys<sub>2</sub>-TJ<sub>4</sub>T<sub>2</sub>J-egl<sub>3</sub>-CT<sub>2</sub>C<sub>4</sub>T- LysNH<sub>2</sub>.

All enzymes were from New England Biolabs except methylase *Cvi*BIII and endonuclease *Sfi*I, which were purchased from Boehringer Mannheim.  $\lambda$  phage DNA was obtained from New England Biolabs. *Saccharomyces cerevisiae* yeast strains PSY316 and BP1 (28) were kindly provided by Dr N.Silverman (MIT, Cambridge, MA). Yeast DNA was isolated as described (29).

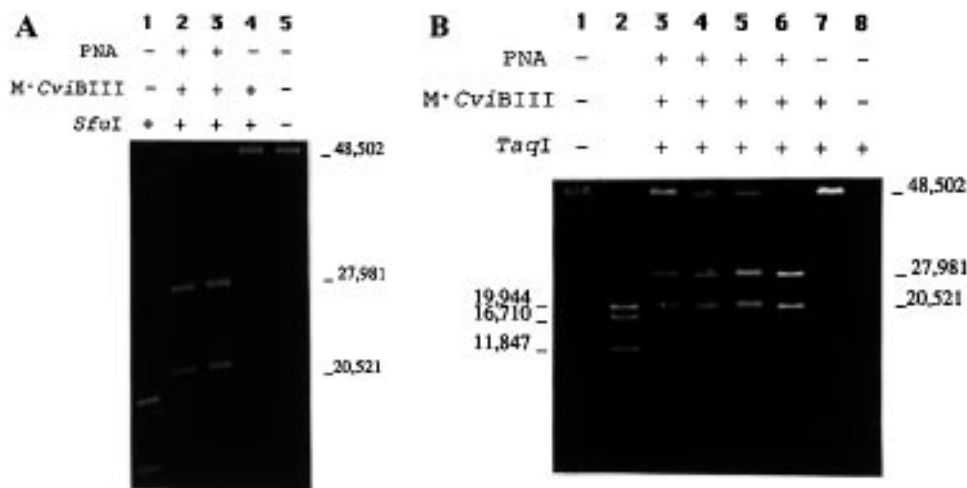
For PARC analysis,  $\lambda$  or yeast DNA embedded in a 0.65% low melting agarose plug was incubated with shaking at 37°C in

buffer solution (20 mM MES, 10 mM NaCl, 2 mM EDTA, pH 6.3) containing the desired PNA for 5–10 h. Then the samples were equilibrated in buffer containing either 25 mM sodium citrate, 10 mM NaCl and 5 mM  $\beta$ -mercaptoethanol, pH 7.2, or 25 mM sodium citrate and 5 mM  $\beta$ -mercaptoethanol, pH 7.0, and were incubated overnight with 1 U methylase *Cvi*BIII, with 15 U methylase *Hha*I or with 8–12 U methylase *Hpa*II at 4°C in the same buffers containing 100  $\mu$ g/ml BSA. After that, S-adenosyl-methionine was added up to 300  $\mu$ M and methylation was performed for 1.5–4 h at 37°C. Methylation was stopped and the PNA-DNA complex was dissociated by incubation for 50 min at 58°C in 1% SDS, 500 mM NaCl, 10 mM EDTA, 50 mM Tris-HCl, pH 8.9. Then the samples were equilibrated in an appropriate buffer and digestion with a desired restriction endonuclease was performed under optimal conditions in the presence of 100  $\mu$ g/ml BSA. The digestion was stopped by incubating for 10–20 min in 50 mM EDTA, pH 8. Finally, the samples were equilibrated with TE buffer, pH 7.5, and loaded onto a 1% agarose gel. The PFGE was run on a CHEF Mapper system (BioRad).

## RESULTS AND DISCUSSION

### Cleavage of $\lambda$ DNA using various bis-PNAs

The PNA-assisted rare cleavage (PARC) approach we describe follows the general scheme of the Achilles' cleavage strategy and uses PNA to protect a limited number of genomic sites from enzymatic methylation (Fig. 1). To check whether short bis-PNAs can be used within our approach, we first tested cleavage of  $\lambda$  DNA and used bis-PNA H-T<sub>2</sub>JTJ<sub>2</sub>T<sub>2</sub>-egl<sub>3</sub>-T<sub>2</sub>C<sub>2</sub>TCT<sub>2</sub>-LysNH<sub>2</sub> (PNA 1). This PNA contains pseudo-isocytosines in one half of the molecule for stabilization of the P-loop at neutral pH.  $\lambda$  DNA contains a unique site for binding PNA 1, **AAGAGGAATCGAT** at position 35 050, consisting of a homopurine tract, which has a 1 bp overlap with the *Cla*I methylation/restriction site (here and below, the PNA binding sites are in bold and the restriction sites are underlined). We applied the PARC strategy using the PNA 1/*M-Cla*I/*Cla*I combination to  $\lambda$  DNA predigested by *Hind*III restriction enzyme (the target site was located on the 9.4 kbp *Hind*III fragment of  $\lambda$  DNA).



**Figure 2.** Cleavage of  $\lambda$  DNA with restriction endonucleases after treatment of the PNA–DNA complexes with the methylase *CviBIII* (a low temperature isoschisomer of *M·TaqI*). (A) The PNA 2/*M·CviBIII*/*SfuI* combination was used. Lanes 2 and 3 present the results obtained for PNA concentrations of 0.43 and 0.51  $\mu$ M, respectively. Lanes 1 and 4 with no PNA added demonstrate complete digestion with the *SfuI* enzyme (the length of the larger *SfuI* fragment is 18 050 bp) and no digestion when *M·CviBIII* was used before the restriction enzyme, respectively. Lane 5 shows the untreated  $\lambda$  DNA. (B) The PNA 3/*M·CviBIII*/*TaqI* combination was used. Lanes 3–6 present the results obtained for PNA concentrations of 0.11, 0.13, 0.15 and 0.19  $\mu$ M, respectively. Lanes 8 and 7 demonstrate complete digestion with the *TaqI* enzyme and no digestion when *M·CviBIII* was used before the restriction enzyme, respectively. Lane 1 shows the untreated  $\lambda$  DNA. Lane 2 gives the DNA markers obtained by digestion of  $\lambda$  DNA with *EagI* restriction enzyme (the lengths of the fragments obtained are shown in bp at the sides of both patterns).

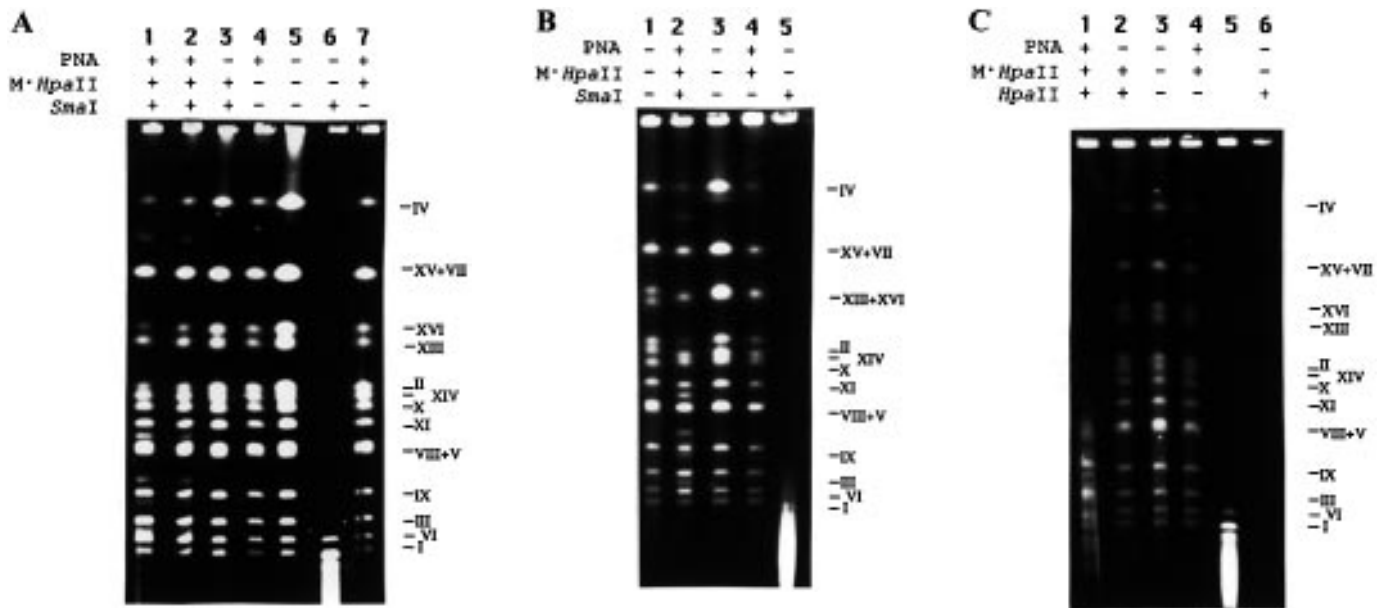
Binding of PNA 1 selectively protected the desired site from methylation by *M·ClaI* (data not shown), yielding the expected 7.6 kbp fragment upon subsequent cleavage by *ClaI* endonuclease. However, as the time of incubation with the methylase increased, a gradual decrease in PNA protection was observed. This clearly indicated that PNA 1 was gradually displaced from its binding sites on the duplex DNA. Because of the displacement effect we failed to achieve a quantitative and selective cleavage of agarose-embedded full-length  $\lambda$  DNA at a chosen site using this bis-PNA, which carried two positive charges, one at each end (data not shown). Thus, to achieve quantitative cutting, further stabilization of the PNA–DNA complex was needed. An obvious way of so doing by increasing PNA length was unacceptable for our purposes (see Introduction).

We therefore used another approach to stabilize PNA–dsDNA complexes. We enhanced the electrostatic attraction of PNAs to the negatively charged DNA targets by tagging PNAs with positively charged oligolysine ‘tails’, which is an efficient way to increase its binding efficiency (V.V.Demidov and P.E.Nielsen, unpublished results). Hence, in the next series of experiments we used bis-PNA H-Lys<sub>3</sub>-T<sub>2</sub>J<sub>2</sub>T<sub>3</sub>-egl<sub>3</sub>-T<sub>3</sub>C<sub>2</sub>T<sub>2</sub>-LysNH<sub>2</sub> (PNA 2), with five positive charges, which was targeted to the complementary 7 bp homopurine tract at position 27 990 on  $\lambda$  DNA. We also tested bis-PNA H-Lys<sub>2</sub>-T<sub>4</sub>T<sub>2</sub>J-egl<sub>3</sub>-CT<sub>2</sub>C<sub>4</sub>T-LysNH<sub>2</sub> (PNA 3), with four positive charges, which was initially chosen to target chromosomes 2 and 3 of yeast (*vide infra*), but which also proved to target the adjacent 8 bp homopurine tract on  $\lambda$  DNA located around position 27 980. This is a unique site, **TTCCCTTCGAAGGAAA**, at positions 27 975–27 990 on  $\lambda$  DNA in which homopurine/homopyrimidine binding sites for PNA 2 and PNA 3 overlap with one of 121 *TaqI* sites (TCGA) and one of seven *SfuI* sites (TTCGAA) on  $\lambda$  DNA; both homopurine tracts overlap with *M·CviBIII* site *TCGA* (here and below, the methylation sites are italicized). Figure 2A (lanes 2 and 3) shows that with PNA 2 as a shield protecting against *CviBIII* methylase

within the PARC approach, agarose-embedded full-length  $\lambda$  DNA is quantitatively cleaved by the *SfuI* enzyme. This cleavage yields only two fragments with the expected lengths instead of eight fragments, the two largest of which (18 050 and 8310 bp) are clearly seen after complete *SfuI* digestion. Figure 2B (lane 6) shows that with PNA 3 within the same approach,  $\lambda$  DNA is cleaved by the *TaqI* enzyme virtually completely and totally sequence specifically, also yielding only two large fragments with the expected lengths instead of 122 small fragments after complete digestion by *TaqI* enzyme. Under the chosen experimental conditions we did not observe any additional bands, even though  $\lambda$  DNA contains one binding site with a single mismatch for PNA 3 overlapping with the *TaqI* site at position 29 230. Thus, bis-PNAs carrying multiple positive charges proved to be remarkably good tools for sequence-specific cutting of  $\lambda$  DNA within the PARC strategy.

### Cutting of yeast chromosomes using different enzyme pairs

To check whether the PARC approach will work on long genomic DNAs we have applied PNA 3 to the whole yeast genome, as we have already reported (19). Here we present additional data on yeast genome cutting by this technique. We first tried the *M·HpaII*/*SmaI* pair of methylation/restriction enzymes. Figure 3A and B show the results. When PNA 3 and the *M·HpaII*/*SmaI* pair are applied to chromosomes from yeast strain PSY316 (Fig. 3A, lanes 1 and 2), several additional strong bands appear between chromosomes IV and XV/VII, below chromosome XI, above and below chromosome IX and above chromosome VI. Chromosomes IV, XVI and II are quantitatively cut. Figure 3B, lane 2 shows results similar to those in Figure 3A but obtained for another yeast strain (BP1). It is observed that the cleavage patterns are very similar for the two strains, which have clear differences in the lengths of some of their chromosomes (compare, for example, chromosomes XIII and XVI in lanes 1 and 3 in Fig. 3B). Another



**Figure 3.** Application of the PARC technique to yeast genomes (the chromosome order numbers are shown at the right of the pattern) using the PNA3/M:HpaII/SmaI combination. (A) PFGE separation of chromosomes from yeast *S.cerevisiae* strain PSY316 (28). Lanes 1 and 2 show the PARC results for 0.4  $\mu$ M PNA with 8 and 12 U methylase, respectively. Other lanes present various control experiments: lane 3 gives results of methylation/digestion without PNA; lane 4 presents results of incubation with PNA but without enzymes; lane 5 gives the intact genome; lane 6 presents digestion of the yeast genome with the *SmaI* enzyme; lane 7 gives results for PNA pre-incubation and methylation but without restriction enzyme treatment. (B) Results of experiments similar to the ones presented in (A) with 8 U methylase but for another *S.cerevisiae* yeast strain, BP1 (28). Lane 1, untreated PSY316 genome. Lanes 2–5 correspond to the BP1 strain. (C) Results of PFGE separation of the yeast genome (strain PSY316) treated with the M:HpaII/HpaII enzyme pair (8 U methylase) in the PARC strategy. Lane 5, digestion by the *SmaI* restriction enzyme.

pair of enzymes, M:HhaI/HaeII, also yielded additional, although quite different, bands when combined with PNA 3. Specifically, we could clearly see several new very distinct bands, below chromosome XIII, above chromosome IX and below chromosome I. Furthermore, chromosomes XVI, III and II are quantitatively cut while the rest of the chromosomes were uncut (19).

In the above experiments with the yeast genome we used restriction enzymes recognizing 6 bp. As anticipated, in combination with bis-PNA recognizing 8 bp sites such enzymes produced very limited cutting in the entire yeast genome. We expected more frequent cutting of the genome when using a restriction enzyme recognizing 4 bp. Figure 3C, lane 1, shows that experiments fully confirmed this expectation. The M:HpaII/HpaII pair combined with PNA 3 yielded a cleavage pattern for all yeast chromosomes that is most similar to one of a restriction endonuclease recognizing 8 bp.

Our cleavage data are consistent with available sequence information about yeast genomes. The sequence data (*Saccharomyces* Genome Database) for chromosomes II and III show that each carries a unique binding site for PNA 3 overlapping with the *HaeII* restriction site and *HhaI* methylation site: GGCGCTTCCCCT. We assign four fragments of the yeast genome cut by the PNA 3/M:HhaI/HaeII combination to cutting of chromosome II nearly into two halves and chromosome III into one third and two thirds of its length. It should be emphasized that because of variability of yeast chromosome lengths from strain to strain, one cannot expect full agreement between fragment lengths observed in our experiments and those predicted from analysis of the *Saccharomyces* Genome Database. We did not find any potential cutting sites for this combination in the rest of the sequenced yeast chromosomes (I, V, VIII, IX and XI) and these chromosomes were uncut in our experiments with the

PNA 3/M:HhaI/HaeII system. Among the sequenced yeast chromosomes, we found only one potential cutting site CTTCCCCGGG on chromosome II for the PNA 3/M:HpaII/SmaI system. The PNA binding site here is 1 bp shorter than the full binding site for PNA 3, so the complex of PNA 3 with this binding site should be weaker than with the full site. This is probably the reason why chromosome II in lanes 1 and 2 of Figure 3A is only partially cut, in contrast to the majority of other cases, where virtually complete digestion was observed. However, this site still behaves as a cleavage site, probably because of extensive overlap between the PNA 3 binding site and the *HpaII* methylation site. The two fragments produced from this cutting are most probably located just above chromosome IX and just below chromosome III (19). In addition to cutting chromosome II, the PNA 3/M:HpaII/SmaI system also cuts chromosomes IV and XVI. We believe that the two other short fragments clearly seen at higher resolution (19), as well as the two larger fragments seen in Figure 3A, appear due to cutting of chromosomes IV and XVI.

The main conclusion from these experiments is that the employment of positively charged short bis-PNAs in combination with an appropriate methylase permits the con- version of very frequent cutting restriction enzymes into extremely rare DNA cutters. Application of our method to the yeast genome yields characteristic, fully reproducible and, in most cases, virtually complete cleavage patterns which depend on the PNA/methylation/restriction enzyme combination. In contrast to RARE (4,14,15,17,18), the PNA–DNA complex does not require any bivalent cations and therefore the methylation reaction can be performed under conditions disfavoring activation of contaminating nucleases.

It should be emphasized that the requirements for orientation of the PNA strands relative to the DNA strand in the PNA–DNA complex is far less stringent than in the case of oligonucleotide–DNA complexes. The available data indicate that in the PNA–DNA duplex the parallel complex (N-terminus of the PNA strand facing the 5'-end of the DNA strand) is only slightly less stable than the antiparallel one (30). Thus, although the proper choice of bis-PNA strand orientation in the P-loop (antiparallel orientation of the 'Watson–Crick', cytosine-containing half of the bis-PNA relative to the DNA strand and parallel orientation of the 'Hoogsteen' half of the bis-PNA with J bases) results in a more stable P-loop (25), our PARC data demonstrate that bis-PNAs work quite well on the target sites in both orientations. Indeed, in experiments with  $\lambda$  DNA, PNA 2 had the 'correct' orientation with respect to its target site and ensured full protection of the overlapping methylation site (see Fig. 2A). PNA 3 had the 'wrong' orientation with respect to its target site on  $\lambda$  DNA. Nevertheless, the complex was sufficiently stable to ensure full protection of the overlapping methylation site (see Fig. 2B). In the case of the potential site on yeast chromosome III for the PNA 3/M-HhaI/HaeII combination, the site orientation with respect to PNA 3 is 'correct' and the protection effect is evidently again strong (19). These data show that in 'blind' cutting of unknown genomes either of the two equally possible orientations of the bis-PNA relative to the DNA target will probably be efficient in the PARC approach. This question requires further study.

### A new class of genome cutters

The presented data demonstrate that by combining short bis-PNAs with an appropriate pair of methylation and frequently cutting restriction enzymes a new class of genome rare cutters can be created. Although only pyrimidine sequences of PNAs can be used, even pyrimidine 7- and 8mers provide a large pool of hundreds of different sequences.

Our data show that virtually complete digestion occurs when the bis-PNA binding site overlaps the methylation site by at least 1 nt. Incomplete digestion may occur when the PNA binding site does not overlap the restriction site but they are located very close to each other. Our preliminary data (not shown) indicate that if the PNA binding site does not overlap the restriction site and these two sites are adjacent to each other or separated by 1 nt, incomplete protection against methylation is observed. The protection effect drops dramatically with increasing distance between the two sites and is unnoticeable when the sites are separated by 2 nt. It should be noted, however, that in cases of no overlap not only should protection be much weaker, but the total recognition sequences are also longer and, therefore, these sites are found much more rarely than the overlapping situation.

Combining the pool of specific homopyrimidine bis-PNAs with a large pool of known methylation/restriction enzyme pairs one obtains a huge variety of efficient rare cutters. The most frequent of them are expected to produce, theoretically, fragments in the Mbp range (because the shortest recognition sites of 9–10 bp in total correspond to 7–8 bp recognized by PNA overlapping with 2–3 bp of a 4–6 bp restriction site). In practice, they may cut more often if the number of restriction sites is much larger than theoretically expected, as is apparently the case for the HpaII restriction site CCGG in the yeast genome (see Fig. 3C). The proposed strategy provides a solution to the problem of how

to quantitatively cleave an unknown genome into a limited number of pieces with lengths of from several hundred kbp to several Mbp, thus operating in the range of recognition specificities where very few, if any, cutters are now available.

### ACKNOWLEDGEMENTS

We thank Dr N.Silverman for yeast strains and Drs C.R.Cantor and C.L.Smith for sharing with us some of their equipment during the early stage of the project. This work was supported in part by the NIH, PerSeptive Biosystems and the Danish National Research Foundation.

### REFERENCES

- Schwartz,D.C. and Cantor,C.R. (1984) *Cell*, **37**, 67–75.
- Carle,G.F., Frank,M. and Olson,M.V. (1986) *Science*, **232**, 65–68.
- Schwartz,D.C., Li,X., Hernandez,L.I., Rammaraín,S.P., Huff,E.J. and Wang,Y.-K. (1993) *Science*, **262**, 110–114.
- Wang,Y.-K., Huff,E.J. and Schwartz,D.C. (1995) *Proc. Natl. Acad. Sci. USA*, **92**, 165–169.
- Murray,W.M. and Szostak,J.W. (1983) *Nature (Lond.)*, **305**, 189–193.
- Burke,D.T., Carle,G.F. and Olson,M.V. (1987) *Science*, **236**, 806–812.
- Bhagwat,A.S. (1992) *Methods Enzymol.*, **216**, 199–224.
- Perler,F.B., Davis,E.O., Dean,G.E., Gimble,F.S., Jack,W.E., Neff,N., Noren,C.J., Thorner,J. and Belfort,M. (1994) *Nucleic Acids Res.*, **22**, 1125–1127.
- Dujon,B., Belfort,M., Butow,R.A., Jacq,C., Lemieux,C., Perlman,P.C. and Vogt,V.M. (1989) *Gene*, **82**, 115–118.
- Koob,M. (1992) *Methods Enzymol.*, **216**, 321–329.
- Koob,M. (1995) In Meyers,R.A. (ed.), *Molecular Biology and Biotechnology: A Comprehensive Desk Reference*. VCH, New York, NY, pp. 1–3.
- Koob,M. and Szybalski,W. (1990) *Science*, **250**, 271–273.
- Strobel,S.A. and Dervan,P.B. (1991) *Nature*, **350**, 172–174.
- Ferrin,L.J. and Camerini-Otero,R.D. (1991) *Science*, **254**, 1494–1497.
- Koob,M., Burkiewicz,A., Kur,J. and Szybalski,W. (1992) *Nucleic Acids Res.*, **20**, 5831–5836.
- Kur,J., Koob,M., Burkiewicz,A. and Szybalski,W. (1992) *Gene*, **110**, 1–7.
- Gnirke,A., Iadonato,S., Kwok,P. and Olson,M. (1994) *Genomics*, **24**, 199–210.
- Ferrin,L.J. and Camerini-Otero,R.D. (1994) *Nature Genet.*, **6**, 379–383.
- Veselkov,A.V., Demidov,V.V., Frank-Kamenetskii,M.D. and Nielsen,P.E. (1996) *Nature*, **379**, 214.
- Nielsen,P.E., Egholm,M., Berg,R.H., Buchardt,O. (1991) *Science*, **254**, 1497–1500.
- Cherny,D.Y., Belotserkovskii,B.P., Frank-Kamenetskii,M.D., Egholm,M., Buchardt,O., Berg,R.H. and Nielsen,P.E. (1993) *Proc. Natl. Acad. Sci. USA*, **90**, 1667–1670.
- Demidov,V.V., Yavnilovich,M.V., Belotserkovskii,B.P., Frank-Kamenetskii,M.D. and Nielsen,P.E. (1995) *Proc. Natl. Acad. Sci. USA*, **92**, 2637–2641.
- Nielsen,P.E., Egholm,M., Berg,R.H. and Buchardt,O. (1993) *Nucleic Acids Res.*, **21**, 197–200.
- Betts,L., Josey,J.A., Veal,J.M. and Jordan,S.R. (1995) *Science*, **270**, 1838–1841.
- Egholm,M., Christensen,L., Dueholm,K.L., Buchardt,O., Coull,J. and Nielsen,P.E. (1995) *Nucleic Acids Res.*, **23**, 217–222.
- Griffith,M.C., Risen,L.M., Greig,M.J., Lesnik,E.A., Sprankle,K.G., Griffey,R.H., Kiely,J.S. and Freier,S.M. (1995) *J. Am. Chem. Soc.*, **117**, 831–832.
- Christensen,L., Fitzpatrick,R., Gildea,B., Petersen,K.H., Hansen,H.F., Koch,T., Egholm,M., Buchardt,O., Nielsen,P.E., Coull,J. and Berg,R.H. (1995) *J. Peptide Sci.*, **3**, 175–183.
- Pina,B., Berger,S., Marcus,G.A., Silverman,N., Agapite,J. and Guarente,L. (1993) *Mol. Cell. Biol.*, **13**, 5981–5989.
- Smith,C.L., Klco,S.R. and Cantor,C.R. (1988) In Davies,K. (ed.), *Genome Analysis: A Practical Approach*. IRL Press, Oxford, UK, pp. 41–72.
- Egholm,M., Buchardt,O., Christensen,L., Behrens,C., Freier,S.M., Driver,D.A., Berg,R.H., Kim,S.K., Norden,B. and Nielsen,P.E. (1993) *Nature*, **365**, 556–568.