Extension of a pUC18-like polylinker by the octanucleotide recognition sites of *Not*I, *Fse*I, *Sfi*I and *Pac*I

In anticipation that the recently described octanucleotide-recognizing restriction endonuclease *Fse*I[1] will soon be commercialized and thereby be as easily available as the other new rare-cutter enzyme *Pac*I (New England Biolabs), the vectors pTZ19U/pTZ19R[2] were engineered to contain the pUC18/19 polylinker plus the sites for the four known octanucleotide restriction enzymes – *Not*I, *Fse*I, *Sfi*I and *Pac*I – as well as unique hexanucleotide sites for *Sac*II, *Nde*I and *Bal*I (Fig. 1). Although the sites of *Sfi*I and *Fse*I do overlap, cloning into either site does not affect the other recognition sequence. The expanded plasmids (pHD plasmids) still allow blue/white selection of recombinants, the creation of a single-stranded form via their f1 origin of replication, and the translation of cloned DNA from the promoter for T7 RNA polymerase, situated directly adjacent to the polylinker. For the extension, a small DNA cassette was inserted (Fig. 1) similar to the one cloned into the plasmids of the pJDH series[3], which already bear the *Sfi*I and *Not*I sites plus five unique 6 bp restriction sites in addition to the pUC18 polylinker[4], but the *Apa*I site was replaced by the *Fse*I site. This change also introduced a second *Xma*III site. Plasmids pHD19U and pHD19R were isolated and characterized as described[3]. The presence of the *Fse*I site was confirmed by the ability to cut the polylinker with *Nae*I, which has another site in the f1 origin.

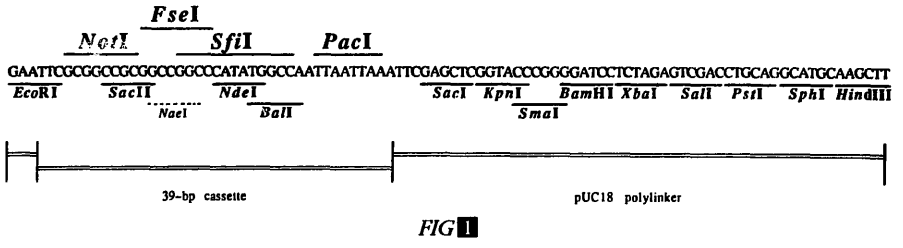

FIG 1

Polylinker sequence of the plasmids pHD19U and pHD19R. As well as all unique restriction sites, the *Nae*I site is also indicated; it is used to confirm the presence of the *Fse*I site.

The cassette could be inserted into any *Eco*RI site, thereby increasing the usefulness of many vector systems, and could be used, for example, for blunt-end cloning[5], chromosome walking and jumping[6], or as additional cloning sites in pYAC4 (Ref. 7), for instance.

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Casting multiple aliquots of agarose-embedded cells for PFGE analysis

We routinely cast multiple aliquots of cells embedded in agarose for pulsed-field gel electrophoresis (PFGE) analysis and have found that the following method offers several advantages. The well bottoms of a standard rigid polystyrene flat-bottomed 96-well microtitre plate (e.g. Flow Laboratories, catalogue number 76-307-05) are drilled out or removed by milling and the base is sealed with Titretek™ sealing strip (Flow Laboratories, catalogue number 77-420-00). Cells suspended in molten low melting point agarose (e.g. BRL ultraPURE™, final concentration of 0.5%) are dispensed into individual wells (typically in 100 µl aliquots) from a reservoir (e.g. Flow Laboratories, catalogue number 77-824-01) using an 8-channel or 12-channel pipette. Bubbles are avoided by dispensing only to the first 'stop' on the pipette. The agarose plugs are allowed to set at approximately 4°C, and the sealing strip is then peeled back and the plugs transferred using a PTFE pusher, blunt-ended plastic pastette, or similar, to appropriate solutions for bulk processing before analysis by conventional electrophoresis or PFGE. The casting plate is recycled for further use by rinsing in 0.1 M HCl then distilled water. The plates can be sterilized by spraying with 70% ethanol. Air-dried plates are resealed with a fresh sealing strip and the casting process repeated.

The advantage of the method is its simplicity, as it involves cheap and readily available materials. Large numbers of plugs can be cast and set very quickly, ensuring that cells are evenly distributed within and between plugs. The cylindrical plugs formed by our method are more robust than cuboid plugs, which tend to lose their corners during processing. Our plugs can be picked up individually using standard sterile plastic bacterial inoculation loops. These 100 µl plugs squeeze neatly into the slots formed by conventional gel slot combs. They can be easily sliced to reduce loading or to suit small comb sizes. The system is also suitable for simultaneous preparation of multiple plugs from several different samples within one plate, using the microtitre plate grid numbering system for unequivocal identification of each sample.

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