## RFLP analysis in *Brassica napus*, *B. oleracea* and *B. rapa*

*A G Sharpe, K Lawler, D J Lydiate (Cambridge Laboratory)*

We intend to construct saturated RFLP-based genetic linkage maps of *B. napus* and its two diploid parental species. As a source of RFLP probes, we have generated genomic libraries of small (0.8 to 1.6kb) *Pst*I fragments from *B. napus*, *B. oleracea* and *B. rapa* inserted into a plasmid vector related to pUC18 and we have isolated over 200 clones from each library. We have used over 200 of these cloned genomic fragments as probes on Southern blots of DNA from 14 diverse oilseed rape cultivars, one resynthesized *B. napus* line, three *B. oleracea* subspecies and two *B. rapa* subspecies (Fig. 11).

Only those probes which recognize one or a few polymorphic loci are useful for RFLP mapping. Of the probes tested: 15% hybridize to repeated sequences; 5% hybridize to 15-25 fragments, most of which are polymorphic (these probes will be useful for genetic fingerprinting); 31% hybridize to five or more fragments, some of which are polymorphic; 32% hybridize to four or fewer fragments at least one of which is polymorphic (however, approximately half of these probes reveal alleles in *B. napus* which are of obvious *B. oleracea* and *B. rapa* origin) and 17% hybridize to five or fewer fragments none of which reveal polymorphisms in any of the 14 oilseed rape cultivars (over half of these "non-polymorphic" probes reveal loci which are polymorphic when comparing the resynthesized *B. napus* line with an oilseed rape variety).

For an RFLP-defined locus to be followed in a particular genetic cross there must be polymorphism between the parental lines used; in *B. napus* crosses most often involve a pair of oilseed rape cultivars. Sixty per cent of the probes are polymorphic in comparisons between any pair of the 14 oilseed rape cultivars screened (although, only one third of these are considered ideal for RFLP mapping). In comparisons between a winter cultivar and a spring cultivar 80-90% of probes reveal at least one polymorphism. In comparisons between pairs of spring cultivars 50-80% of probes reveal at least one polymorphism and in comparisons between the slightly more uniform winter cultivars 50-70% of probes still revealed at least one polymorphism. Ninety six to ninety eight per cent of probes detect at least one polymorphism in comparisons between the resynthesized line and any of the oilseed rape cultivars. All of the above results are based on hybridization to *Eco*RI digested genomic DNA. Experiments suggest that approximately half of the loci which yield monomorphic *Eco*RI fragments will give polymorphic fragments when the DNA is digested with another restriction enzyme. These results demonstrate that a high proportion of RFLP-defined loci will be informative in the segregating progeny of most crosses between oilseed rape varieties.

The high level of polymorphism between oilseed rape cultivars and the resynthesized *B. napus* was expected because the diploid parents of the resynthesized line are phylogenetically distinct from the diploid ancestors of oilseed rape. An RFLP mapping cross between an oilseed rape line and the resynthesized *B. napus* will utilize this high degree of polymorphism and allow a very high proportion of the loci which hybridize to our RFLP probes to be mapped in a single cross. In addition, because much of the new genetic diversity available for future crop improvement in oilseed rape will be introduced into *B. napus* from one or other of the diploid parental species by resynthesis (see page XXX), an understanding of the segregation of markers in a cross between a resynthesized line and a standard oilseed rape cultivar will be very useful. However, we are using this cross with some caution because it is possible that such diverse parents may have regions of their chromosomes which are translocated with respect to one another.

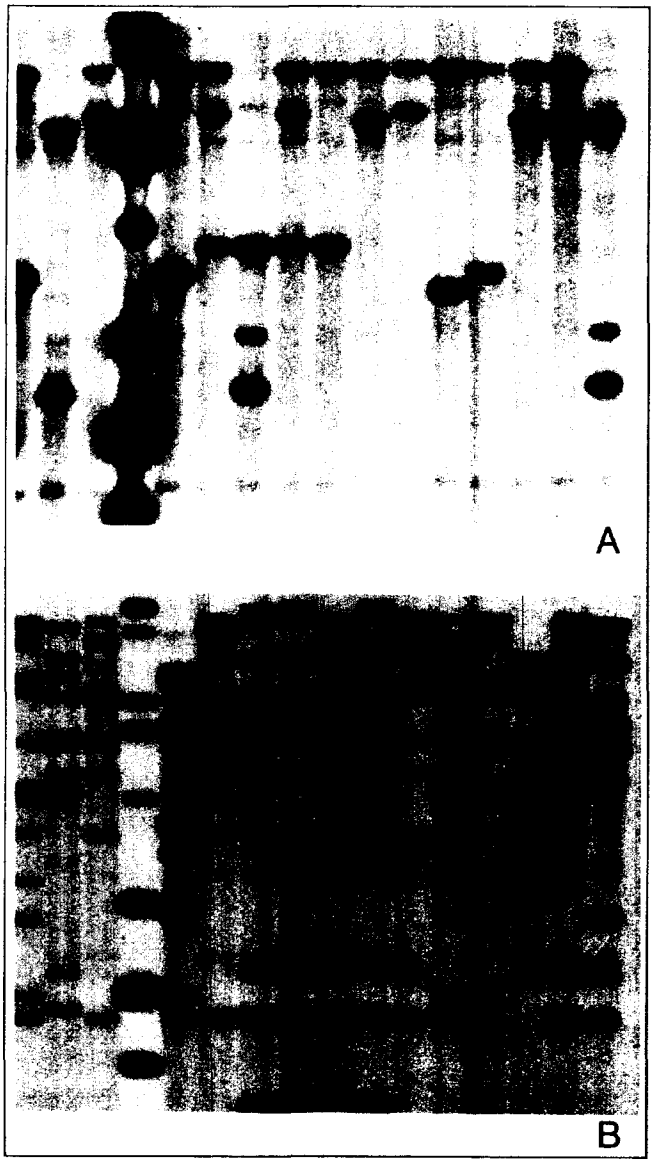


**Fig. 11** Southern hybridizations of *Eco*RI digested *Brassica* DNA probed with small, cloned *Pst*I fragments of the *B. napus* genome: A, pN22, a probe which recognizes a small number of highly polymorphic loci in *B. napus*; B, pN107, a 'fingerprinting' probe which recognizes a large number of highly polymorphic loci.