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Introduction *Lolium perenne*, as one of the most important forage grasses of temperate regions, combines a number of very useful characteristics, e.g., good seedling establishment, with a low resistance to drought and limited winter hardiness. Trait selection and introgression can be greatly enhanced by the use of molecular markers in a genetic linkage map. The aim of this project was the generation of a genomic microsatellite library which when combined with microsatellites developed from a Genethresher database would give good genome coverage coupled to high levels of marker polymorphism.

Materials and methods Six microsatellite enriched libraries of a *L. perenne* genotype (Liprior) were constructed using the procedure of Edwards *et al*. (1996). The libraries were based on three enzymes (*Rsa* I, *Alu* I and *Hae* III) with filter selection for two dinucleotide repeats (CA and CT). DNA fragments were cloned and transformed and microsatellite containing clones selected by P_{32} labelling. After sequencing with M13 forward and reverse primers, microsatellite containing sequences were entered into a local database to screen for redundancy. Non redundant primers were used to PCR genomic DNA from two mapping populations based on a forage/amenity cross:

Forage x Amenity = Hybrid

Hybrid x Forage = Forage BC2
Hybrid x Amenity = Amenity BC2

Products were run on a denaturing polyacrylamide gel with bands scored as present or absent. Mapping was performed using JOINMAP 2.0. The two populations were also screened with selected microsatellite primers from the Vialactia Genethresher database. Primers selected were those already mapped to the Vialactia genetic linkage map of *L. perenne* and to the IGER WSC map (Winz *et al*., 2003).

Results After microsatellite selection and redundancy screening, primers to 230 microsatellites were produced. One hundred (45%) proved polymorphic in one or both mapping populations. Maps of seven linkage groups were produced with most microsatellites mapping to the same linkage group in both mapping populations. Screening of the mapping populations for a variety of traits has shown association for heading date to some of the markers on linkage group 4.

Conclusions The genomic microsatellites, whilst quite complex to use because of complicated banding patterns, proved consistent by mapping to the same linkage groups in both mapping populations. Genome coverage varied between chromosomes with, for example, group 7 having very good coverage but linkage group 5 possibly having regions marker free. The combination of the 100 genomic microsatellites produced at IGER with the 400 produced and mapped from the Genethresher database at Vialactia should prove a valuable resource for the analysis of traits throughout the *L perenne* genome. For example, the microsatellites shown to associate with the QTL for heading date on linkage group 4 will prove useful for the selection of this trait.

References

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