Integration of perennial ryegrass (*L. perenne*) genetic maps using gene-associated SNPs

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Introduction The reference genetic map of perennial ryegrass was developed by the International *Lolium* Genome Initiative (ILGI), using the p150/112 one-way pseudo-testcross population. A selection of public domain genetic markers including RFLPs, detected by wheat, barley, oat and rice cDNA probes, and AFLPs were mapped, allowing studies of comparative relationships between perennial ryegrass and other Poaceae species. The map was enhanced through the addition of unique perennial ryegrass genomic DNA-derived SSR (LPSSR) markers, providing the basis of framework genetic mapping in other populations. In addition, a small number of RFLP loci detected by candidate genes involved in herbage quality traits were added to the map. A second-generation reference genetic mapping family was developed based on the $F_1(NA_6 \times AU_6)$ two-way pseudo-testcross family, generating two parental genetic maps. These maps were populated by genomic SSR loci, EST-RFLP loci and EST-SSR loci (corresponding to multiple functional categories of agronomic importance). A third genetic mapping population based on an interspecific cross between perennial and annual ryegrass genotypes [$F_1(Andrea_{1246} \times Lincoln_{1133})$] generated a map based on LPSSR and EST-SSR markers. Linkage groups in the two latter maps were inferred using common LPSSR loci with the p150/112 genetic map.

Materials and methods Highly efficient molecular markers based on single nucleotide polymorphism (SNP) variation in selected candidate genes were discovered and validated in the $F_1(NA_6 \times AU_6)$ mapping family using the single nucleotide primer extension (SNuPe) technique. The presence of these specific SNP variants in other mapping populations was empirically determined. Common gene-associated SNPs were mapped using a minimal selection of genotypes from the three mapping populations chosen for maximal recombination.

Results and conclusions The *Lp*CCR1 SNP locus gene was assigned to a central location on LG7 of the AU_6 parental genetic map and the *Lp*CCR1 gene-associated RFLP locus was assigned a similar position on LG 7 in the p150/112 reference mapping population (Fig 1). The ideogram shown is a representation of 16 genotypes of the p150/112 mapping population possessing maximal non-overlapping recombination for LG7. This approach permits enhanced genome coverage of current maps and provides a more structured reference framework between maps based on common marker positions. These enhanced maps will provide a robust basis for QTL comparisons and validation as well as comparative genomics.

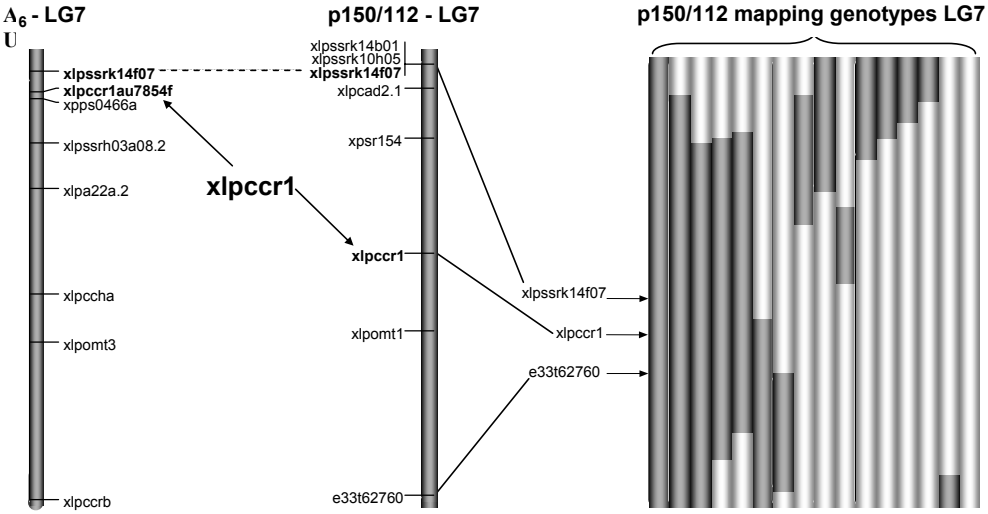

Figure 1 Linkage groups derived from analysis of the mapping populations p150/112 and $F_1(NA_6xAU_6)$, with common markers identified.