Construction and Exploitation of a Bacterial Artificial Chromosome (BAC) Library for *Lolium Perenne* (Perennial Ryegrass)

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Introduction BAC libraries are an important tool in genomics, enabling physical maps, genome sequencing, marker development and map based cloning strategies. A BAC library has therefore been generated for the temperate grass species *Lolium perenne* (perennial ryegrass) which compliments an existing BAC library of the closely related species, *Festuca pratensis* also generated by IGER. Moreover the *L. perenne* BAC library will provide a useful tool for grass comparative genomics to compliment the existing BAC libraries of cereal crops including rice, wheat, barley, *Sorghum* and maize. In particular it will allow a comparison of micro-synteny between this large genome forage crop species and the model small genome monocot species *Orzya sativa*.

Materials and methods High molecular weight (HMW) DNA was isolated from 20 g of leaf material from a diploid *L. perenne* (2n=2x=14) genotype. Nuclei were isolated according to the method of Zhang *et al.* (1995) and the nuclei embedded in agarose plugs. The *L. perenne* HMW DNA was partially digested using *Hind*III and then separated by pulse field gel electrophoresis before cloning into vector pIndigoBAC-5 (Epicentre) following the method adopted by O'Sullivan *et al.* (2001). In addition to filters for hybridisation based screening, DNA pools have been generated to enable a rapid PCR based screen of the library.

Results The library currently consists of > 50,000 clones, picked into 96 well plates, with an average insert size of 100 kb. Additional clones are being generated to ensure that the final library comprises 5 genome equivalents of *L. perenne*. The first half of the library (i.e. comprising 2.5 genome equivalents) has been screened using PCR primers for candidate genes for traits of interest including forage quality, nitrogen stability, control of flowering time, and sugar metabolism. Upstream regions of these genes are being sequenced and from this new PCR primers designed. These primers will be used to amplify the respective alleles from 20 *L. perenne* genotypes which will be sequenced and aligned to derive allele-specific single nucleotide polymorphism (SNP) markers.

Conclusions A BAC library has been generated for the large genome (haploid genome size is estimated at 2034 Mbp; Bennett and Smith, 1976) species *L. perenne*, and this has provided a rapid method for obtaining 5' upstream and other non-coding regions for candidate genes of forage quality traits in this important forage and turf grass species. This *L. perenne* BAC library has been produced as part of the EU Framework 5 project, GRASP (http://www.grasp-euv.dk/). As part of this project, SNPs derived from these BAC sequences will be associated to relevant QTL and the same molecular markers will be tracked in genetically diverse *L. perenne* populations undergoing a range of selection pressures.

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