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ANALYSIS OF WHOLE-GENOME GENETIC VARIATION USING NEXT-GENERATION SEQUENCING TO IMPROVE BREEDING PROGRAMS IN POPULUS TREES

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Next-generation sequencing technologies, genetic variation, Marker-Assisted-Selection

Till few years ago, progress in applying new methods to forest tree breeding has understandably been slower because of the difficulty to acquire genomic data in the major commercial tree species. The situation has improved rapidly through whole genome sequencing in *Populus trichocarpa*, and the construction of large EST databases in poplar.

In the framework of a joint effort undertaken by the EU funded projects Evoltree, Noveltree and EnergyPoplar, genetic variability has been investigated in *Populus nigra* in order to develop additional genomic resources to be used in novel breeding programs. Mainly we are interested in obtaining a large amount of single nucleotide polymorphisms (SNPs) spread across the whole black poplar genome and the next-generation sequencing technologies allow us to easily reach this objective.

In the first phase of the joint work, by using Illumina/Solexa technology we resequenced three different European genotypes at a high coverage (> 20X) in order to obtain not only SNPs but also and especially a *consensus* sequence for the black poplar genome. Reference-guided assembly showed the feasibility of using *P. trichocarpa* genome sequence as reference for *P. nigra*, given that 75% of *P. nigra* reads were uniquely mapped on the *P. trichocarpa* sequence. Then, in order to rescue the missing *P. nigra* sequences which might characterize only the black poplar genome, we performed also a *de novo* assembly. A comparison of the results achieved using the two approaches, as well as an attempt of their combination, will be provided.

After the reference-guided assembly, we detected more than six millions SNPs: data on types and frequency of sequence variants at the whole genome level will be presented. Such SNP detection has been validated by *P. nigra* sequences of several gene fragments, which were resequenced in different natural European populations by using the traditional Sanger method.

In the second phase of the resequencing work, to maximize informative and rare SNP discovery, sequence reads obtained from fifty additional European clones, which have been resequenced at low coverage (2X), will be aligned to the black poplar draft *consensus* sequence. The final SNPs data will be used for high throughput genotyping of an association population made of 1100 poplar clones established in two experimental sites, in order to monitor genetic variation

during the selection process and develop Marker-Assisted-Selection and Gene-Assisted-Selection breeding programs.