

基于简化基因组测序的落叶松高效 KASP 标记的开发和应用

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摘要:【目的】: 建立高世代育种群体, 加速落叶松的遗传改良。【方法】: 通过简化基因组测序获得20245个SNP (单核苷酸多态性), 并采用一般线性模型 (GLM)、混合线性模型 (MLM) 及固定和随机模型循环概率统一 (FarmCPU) 方法, 将落叶松的基因型与表型数据关联, 筛选出23个与生长、材性及结实相关的SNP位点。接着, 提取各位点上下游150bp的序列, 剔除包含较多其他SNP或Indel的序列, 最终保留16个SNP进行KASP标记的开发与引物设计。从268株杂种落叶松中提取DNA, 利用设计好的引物在CFX荧光定量仪中进行PCR扩增。PCR结束后, 使用荧光定量PCR仪进行分型分析, 显示红色、绿色和蓝色的分簇, 并且红色和蓝色的分簇分别靠近 X 轴和 Y 轴的为有效引物。【结果】: 根据分型结果与分型图, 成功筛选出10个KASP标记。数据分析显示, 与胸径相关的KASP标记为sca235-1015388, 与结实相关的为sca1151-1431418, 与纤维素含量相关的为sca3332-283854。构建了268份落叶松材料的指纹图谱, 为后续的育种和鉴定提供了基础数据。【结论】: 本研究基于简化基因组测序成功开发了KASP分子标记, 获得了与落叶松早实性、纤维素含量及生长速度相关的标记。这些标记将有助于加速落叶松的选育进程, 并为后续的科学研究和育种提供重要依据。

Development and Application of Efficient KASP Markers for Larch Based on Simplified Genome Sequencing

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Abstract: Objective: To establish high-generation breeding populations and accelerate the genetic improvement of larch. Methods: A total of 20,245 SNPs (single nucleotide polymorphisms) were obtained through simplified genome sequencing. General linear models (GLM), mixed linear models (MLM), and Fixed and Random Model Circulating Probability Unified (FarmCPU) methods were employed to associate the genotypes of larch with phenotypic data, resulting in the identification of 23 SNP loci related to growth, wood properties, and seed production. Subsequently, sequences of 150 bp upstream and downstream of each locus were extracted, and those containing a high number of other SNPs or Indels were discarded, ultimately retaining 16 SNPs for KASP marker development and primer design. DNA was extracted from 268 hybrid larch individuals, and PCR amplification was performed using the designed primers on a CFX fluorescence quantitative instrument. After PCR, genotyping analysis was conducted using a quantitative PCR instrument, with red, green, and blue clusters displayed; effective primers were indicated by red and blue clusters located near the X and Y axes, respectively. Results: Based on the genotyping results and genotyping plots, 10 KASP markers were successfully screened. Data analysis revealed that the KASP marker associated with diameter at breast height (DBH) was sca235-1015388, the marker related to seed

production was sca1151-1431418, and the marker associated with cellulose content was sca3332-283854. A fingerprint map of 268 larch materials was constructed, providing foundational data for subsequent breeding and identification efforts. Conclusion: This study successfully developed KASP molecular markers based on simplified genome sequencing, resulting in markers related to early seed production, cellulose content, and growth rate in larch. These markers will aid in accelerating the breeding process of larch and provide important references for future scientific research and breeding efforts.