Title: SNP Genotyping

Abstract:

Single nucleotide polymorphisms, or SNPs, are the most common form of genetic variation in humans. These differences at individual bases in the DNA often do not directly affect gene expression, but in many cases can still be useful for locating disease-associated genes or for diagnosing patients. Numerous methodologies have been established to identify, or “genotype”, SNPs.

JoVE’s introduction to SNP Genotyping begins by discussing what SNPs are and how they can be used to identify disease-associated genes. Several common SNP genotyping methods are then examined, including direct hybridization, PCR-based methods, fragment analysis, and sequencing. Finally, we present several examples of how these techniques are applied to genetic research today.

Application videos:

1. A PCR-based Genotyping Method to Distinguish Between Wild-type and Ornamental Varieties of *Imperata cylindrica* **(3265 Thumbnail @ 9:12 – Visualizing gel bands under UV light)**

Description: Phenotypically similar varieties of organisms might in fact be genetically distinct, which could have important ecological or agricultural consequences. Authors of this article used PCR-based genotyping to distinguish between wild type cogongrass, a highly invasive weed, and individuals from a cultivated ornamental strain of the same species that had reverted to a non-sterile state. This data is important in assessing whether these two varieties co-occur at a locale and thus have the possibility of hybridizing to form hardier strains.

2. Demonstrating a Multi-drug Resistant Mycobacterium tuberculosis Amplification Microarray **(51256 Thumbnail @ 2:05 loading the microarray)**

Description: Early detection and treatment are important for control of tuberculosis transmission. Here, researchers demonstrate a microarray workflow for detection and characterization of multi-drug resistant *Mycobacterium tuberculosis*, which tests for the presence of genetic variants known to confer antibiotic resistance. By combining the steps for sample amplification and microarray hybridization, this simplified process is more amenable for use in low-resource settings.

3. An Allele-specific Gene Expression Assay to Test the Functional Basis of Genetic Associations **(2279 Thumbnail @ 5:17– Experimental workflow schematic)**

Description: In this video, an experiment is presented to study the effect of SNPs on gene expression. By a clever selection of cell lines as well as SNPs that are located both in transcribed and untranscribed sequences, the researchers were able to use primer extension assays to generate SNP-containing DNA fragments. These were then quantitated using MALDI-TOF mass spectrometry, a technique capable of differentiating the fragments based on the small mass changes caused by the single nucleotide sequence differences. Results indicated whether a disease-associated SNP altered the expression level of a target gene.

4. Rapid and Efficient Zebrafish Genotyping Using PCR with High-resolution Melt Analysis **(51138 Thumbnail @ 6:00 – Graph of melting curves)**

Description: Here, scientists demonstrate a protocol for using high-resolution melt analysis (HRMA) to identify variation in targeted genomic regions. Zebrafish genomic DNA is first amplified with primers designed against a region of interest. Because the dissociation, or “melting”, of the two strands of a DNA molecule is affected by its sequence, researchers can heat the amplified DNA and then examine their “melting curves” to identify sequence differences as small as one nucleotide.

5. DNA-based Fish Species Identification Protocol **(1871 Thumbnail @ 8:20 – RFLP results screen)**

Description: Seafood fraud, or the intentional mislabeling of seafood products, is a common occurrence. This article presents a protocol for fish species identification using restriction length fragment polymorphism (RFLP) analysis, Restriction enzyme digestion of individual DNA samples generated different fragment patterns based on the sequence-dependent presence and location of restriction sites. These fragments were analyzed using a software called RFLP Decoder, which matched the test patterns with those from known fish species.

Related Videos

5056 – PCR: The Polymerase Chain Reaction

5070 – Restriction Enzyme Digests

5160 – Mouse Genotyping

5543 – Genetics and Disease