Using real-time (Taqman®) PCR to genotype Cftr\textsuperscript{im1UNC} mice – a core facility of the UK Cystic Fibrosis Gene Therapy Consortium

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Introduction

- Cystic fibrosis (CF) is a common and fatal recessive disease caused by dysfunction of a chloride ion channel, the CF transmembrane conductance regulator (CFTR).
- Several transgenic mouse models have been created to assess the effectiveness of a variety of gene transfer vectors for CF in the respiratory epithelium of nose and lung (1).
- Using these animal models requires a sensitive, fast and accurate method for genotyping neonatal mice in order that individual animals may be selected for experiments.
- We have established a Core Testing Facility using the ABI 7700 Sequence Detector (Taqman\textsuperscript{®}) to differentiate wild type, homozygous Cftr\textsuperscript{im1UNC} and heterozygous littermates by using a novel assay that simultaneously detects mCFTR exon 10 and the TK promoter region of the vector that replaces exon 10 in the transgenic mice.
- Genotyping can be performed on a range of samples, including ear clips, tail clips and blood, from immediately after birth.

Methods

- Samples are collected and the DNA extracted using the DNEasy mini kit (Qiagen).
- DNA is amplified using the Taqman\textsuperscript{®} instrument with custom probes and primers and standard PCR reagents (Applied Biosystems).
- Each sample is analysed in triplicate.
- Fluorescent detection avoids the use of gel electrophoresis and potential sample contamination and observer error.
- Controls include mice of known genotype and no template controls (NTC).
- Only 20ng DNA is necessary for reproducible genotyping.
- Up to 28 mice can be assayed at once.
- Results are typically available less than two hours after DNA extraction.

Results

Raw results from the Taqman\textsuperscript{®} instrument of DNA from CF-null (blue), heterozygous (green) and wild type (red) mice. Samples with higher levels of target sequence cross the threshold earlier than those with lower levels. The NTC negative controls, shown in white, never cross the threshold at all. Low levels of wild type CFTR were detected in CF-null mice - this may reflect non-specific hydrolysis of the reverse Taqman probe for mutant CFTR

Other Methods of Genotyping

- Genotyping by observation of phenotype characteristics is generally less accurate than the Taqman assay described.
- Heterozygous mice appear similar in phenotype to their wild type littermates, and although many methods may be used to identify CF-null mice, heterozygous individuals are more difficult to differentiate.

Conclusions

- Use of PCR in real time is as reliable and sensitive as either weight or nasal PD, and is capable of identifying heterozygotes.
- It can be used to measure both wild type and mutant CFTR simultaneously.
- It is recommended as a timely and reliable method of genotyping from immediately after birth.

References


Schematic diagram of probe and primer sequences and locations on the Cftr\textsuperscript{im1UNC} genome. Primers and probes for wild type CFTR were previously published (2).

Mutant / wild type cycle threshold (Ct) ratios. White bars show group medians. Groups are statistically different by Kruskal-Wallis analysis P<0.001.

CFTR exon 10

TK promoter

Forward primer  probe  Reverse primer

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