

Introduction

Single nucleotide polymorphisms (SNPs) are the most common type of genetic variation in the human genome. According to dbSNP (128 Build) there are over 30 million SNPs in the human genome, more than 10% of which have been found in the gene sequence. Any two individuals are predicted to vary at more than a million different SNPs scattered throughout the genome. However, only a small fraction of this genetic variation is likely to explain the majority of the differences between individuals, including their predisposition to development of the many common human diseases, such as cardiovascular disease, hypertension, diabetes, asthma, Alzheimer's disease, and cancer. SNPs are of great value for genetic mapping studies, as well as unlocking the relationship of genotypes and phenotypes. Some SNPs reside in open reading frames, leading to changes in amino acid sequences, while others reside in regulatory regions that may affect the control of gene expression and ultimately affect the level of protein production and its functions.

Other forms of genetic variations include base deletions or insertions that may lead to protein reading frame shifts resulting in altered or nonfunctional proteins. In some individuals, the gene copy number may be completely deleted or multiplied. A good example of gene deletion is the CYP2D6*5 allele, in which the CYP2D6 gene is deleted, leading to a poor metabolizer (PM) phenotype. Conversely, individuals with CYP2D6*2XN genotype possess an additional set of the CYP2D6 gene, which causes an ultrarapid metabolizer (UM) phenotype for the drug of interest.

As laid out in the 2006 Critical Path Initiative, the US Food and Drug Administration was instrumental in driving the use of pharmacogenomics (PGx) and biomarkers in drug development. While the drug industry is moving forward with the use of these new tools for assessing drug efficacy and safety, the application of genetic biomarker assays is increasingly utilized for pharmacokinetic (PK) and pharmacodynamic (PD) profile assessment, toxicity prediction, risk assessment, dosing determination, treatment decision making, and drug response evaluation.

Overview of QPS Genotyping Services

There is an increasing demand from the pharmaceutical industry for genetic analysis of clinical trial populations to help understand the individual variation in drug pharmacokinetics and pharmacodynamics. To meet these needs, QPS offers a range of genetic analysis services to enable the delivery of safe and effective medicines. The QPS genotyping facility is equipped with high performance instrumentations, including an automated sample processing system, Qiagen BioRobot®, as well as the genotyping assay platforms of Biotage® Pyrosequencing HS96 and the Applied Biosystems' Real-Time PCR TaqMan® 7900HT system. To serve our clients better, we provide support for drug development and often perform assays designed specifically to meet each individual client's needs in a highly efficient, timely manner. To support patient stratification and randomization for clinical studies, we also offer a 48-72 hour turnaround.

Our genetic analysis services fall into three general categories:

- (1) DNA Extraction and DNA Banking
- (2) Clinical Genotyping Analysis
- (3) Allele Quantification Analysis

(1) DNA Extraction and DNA Banking

For DNA extraction, we accept samples from whole blood in K₂EDTA or K₃EDTA anticoagulant, saliva samples in Oragene self-collecting tubes, tissues, cell lines, and formalin fixed paraffin embedded (FFPE) biopsy tissue sections. Since samples are tracked based on the barcode numbers labeled on the original sample tubes throughout the study, the sample ID and traceability are ensured.

For genotyping studies, in most cases approximately 1.0-mL EDTA whole blood sample from each clinical subject is collected and shipped frozen on dry ice. Typically, only a 200 µL blood sample is used for genomic DNA isolation, and on average, 3 - 5 µg of high quality genomic DNA is obtained. The quantity of the genomic DNA prepared from a 1.0-mL blood sample will be sufficient for running 500 – 1,000 genetic mutation assays or allele quantification assays.

Recently, it has become a routine practice to preserve DNA samples from the clinical study populations and bank them for potential retrospective study in the future. For this purpose, DNA samples may be supplied in tubes or deep-well plates. Daughter dilution plates can be made for direct use by the client. All samples can be normalized to a defined volume or concentration and ready for use. A secure storage facility is available at QPS, where the samples are stored at either -80°C or -20°C with REES environment monitoring system for long-term storage and DNA archiving.

(2) Clinical Genotyping Analysis

Clinical genotyping analysis provides a significant value in cost cutting for clinical development, allowing pre-selection of patients most likely to benefit from the investigational therapy, reducing the size of clinical trial by using a defined population with an improved chance of a positive clinical outcome, and shortening the time needed for clinical development. By analyzing the results of the trial and understanding the individual differences of the trial population, sponsors may be able to develop “personalized medicine” with improved therapeutic profiles more cost effectively.

QPS offers the following three types of clinical genotyping assays:

- »» Drug Metabolism Genes – An extensive range of validated tests are available to help drug sponsors better understand the metabolism, efficacy, and safety of new medicines. These tests include CYP2C9, CYP2C19, CYP2D6, UGT1A1, NAT1, NAT2, and others.
- »» Drug Specific Target Genes – Assays to detect a known gene variant that will predict a patient’s risk factor to a

specific disease and response to a specific drug, for example assays for ApoE, CCR5, and Perilipin.

- »» Custom Assays – QPS can provide custom developed tests for sponsor’s specific drug or genetic variant of interest. We offer assay design, assay validation, and test service, in which the genetic assay of interest will be developed for a specific customer, validated in line with regulatory guidelines on known samples and applied to the clinical sample analysis. Expert consultation is also available if you are not sure which SNPs are of significant importance for your drug.

(3) Allele Quantification Analysis

In the oncology area, genetic mutations detected in the cancer biopsy samples can predict a response to oncology drugs. Tumor mutation analysis is technically challenging because of the heterogeneity of the sample. In practice, this means that tumor mutation tests must be able to detect mutations even when the majority of the sample is not mutated.

Using the Pyrosequencing® Technology platform, QPS can develop sensitive assays that can detect mutations in the background of normal cells. Mutations can be detected at a ratio of 5:100 mutant:normal DNA, and this allows researchers to detect genetic variations that could not be seen using Dideoxy DNA sequencing methods. This is particularly valuable in an oncology clinical trial since it can improve the accuracy of detection and allow a better correlation between mutation, prognosis, and drug response, for example, the EGFR mutation in colon cancers.

The allele quantification mode available in the Pyrosequencing® platform also allows quantification of the CpG methylation levels in the target gene of interest. It has been well documented that DNA methylation patterns at the CpG islands of the target gene often serve as one of the major epigenetic regulators for gene expression. In various cancers, certain tumor suppressor genes or specific tumor-growth related genes may be over-methylated or under-methylated, resulting in uncontrolled cell growth and cancer formation. To help assess the significance of DNA methylation pattern change in disease and therapies, QPS offers the CpG methylation quant services using DNA bisulfite treatment and pyrosequencing approach. The client may send us either the tumor biopsy samples or patient’s serum samples. Of these clinical research efforts, analyzing cancer patient’s serum samples at the epigenetic level may potentially lead to novel cancer biomarker assay development as a non-invasive cancer diagnostic.

CASE STUDY 2:

ApoE Genotyping and Patient Stratification for Alzheimer Disease Clinical Trials

In humans, 3 apolipoprotein E (ApoE) isoforms (E2, E3, and E4) are encoded by 3 ApoE alleles with 2 single nucleotide polymorphisms at Codon 112 (SNP112) and Codon 158 (SNP158). The ApoE3 isoform was found in more than half of the population and was considered to be the normal form. E2 and E4 isoforms have been associated with increased risk of cardiovascular disease, Alzheimer’s disease (AD), and other disease states. A genetic epidemiology study has shown that approximately 2% of the population carries the worst combination, i.e. two copies of the deleterious E4 allele, which has been postulated to confer a roughly 15 times higher risk of developing a late-onset of Alzheimer’s disease.

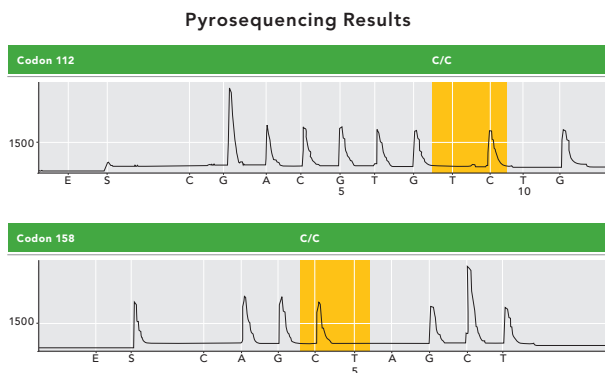
ApoE genotyping is sometimes prescribed by a doctor as an adjunct test when patients have symptoms of progressive dementia, such as decreasing intellectual ability and speech skills, memory loss, and behavioral changes in daily living. To rule out the root of causes, ApoE genotyping may help to determine whether the dementia is caused by AD or another disease, such as strokes or vascular dementia.

For clinical trials, ApoE genotyping allows for the pre-selection of patients as ApoE4 non-carriers or carriers and determines the significance of genetic contribution to the disease progression and response to the investigational drug in clinic.

QPS is experienced in ApoE genotyping and data management to support Phase I, II, and III trials. We also offer 48-72 hour turnaround for patient stratification and randomization for global clinical trials in the Alzheimer’s disease therapeutic area.

The following table and figures represent the raw data from some of our ApoE genotyping services:

Sample PD0040 Genotyping Results



ApoE Genotyping Analysis Data

Sample ID	Ethnicity	Codon 112	Codon 158	PyroSeq Results	Dideoxy Seq Results
PD0029	Multi	T/T	T/T	E2/E2	E2/E2
QPS0047	European Caucasian	T/T	C/T	E2/E3	E2/E3
QPS0046	European Caucasian	T/C	C/T	E2/E4	E2/E4
QPS0062	African American	T/T	C/C	E3/E3	E3/E3
QPS0051	European Caucasian	T/C	C/C	E3/E4	E3/E4
PD0040	Multi	C/C	C/C	E4/E4	E4/E4