

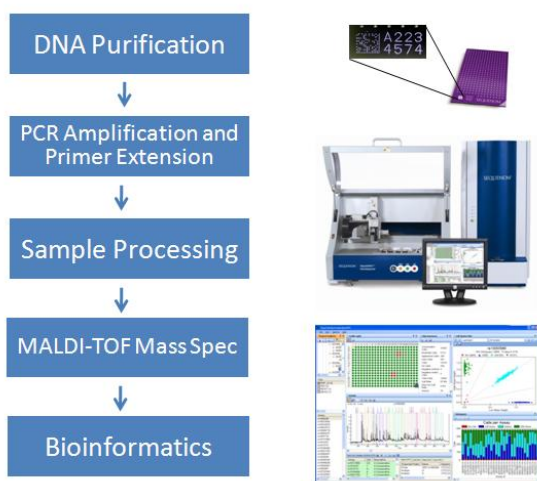
Low Abundant Mutation Profiling in Tumor Samples using the Sequenom OncoCarta™ Panel

Mathias Ehrich, Grant Hogg, and Marisa Pearce*
*Corresponding author: mpearce@sequenom.com

Introduction

The aims of profiling cancer mutations are to provide a molecular snapshot of the mutations in each individual tumor sample for understanding the pathways involved in driving the cancer's growth and ultimately tailor the best therapeutic strategy to each patient [1]. The cost, complexity, and relative lack of sensitivity in identifying mutations that contribute to each cancer have hampered the development of translational medicine strategies. However, several recent studies describe using Sequenom's primer extension and MALDI-TOF mass spectrometry-based methods to resolve many of these issues^{1, 2}.

Figure 1—Oncogene mutation profiling with the Sequenom MassARRAY® System and OncoCarta™ Panel



Oncogene mutations do not usually occur randomly, but are more frequent in certain regions of the genome. Instead of scanning the entire genome of cancer cells, Roman Thomas and colleagues from the Dana Farber Cancer

Institute focused on these regions with highly prevalent mutations. Using Sequenom's high-throughput genotyping technique (Figure 1), the team analyzed the frequency and distribution of 238 known mutations — affecting 17 oncogenes — in 1,000 samples derived from 17 different tumor types. The Sequenom genotyping approach enabled generation of mutation profiles that were more sensitive and cost-effective compared with Sanger sequencing methods¹.

Overview

Sequenom has developed an oncogene panel based on the mutations analyzed in the Roman Thomas publication. The OncoCarta™ Panel v1.0 offers rapid, parallel analysis of over 230 simple and complex mutations across 19 common oncogenes (Table 1). The panel consists of a set of pre-designed and pre-validated mutations for sensitive and efficient screening. Tumor samples from fresh, frozen or formalin-fixed paraffin embedded (FFPE) tissue as well as cell lines can be used. The OncoCarta™ Panel assay consists of 24 wells per sample, and requires no more than 20 ng of DNA per well.

Table 1—List of Oncogenes in OncoCarta™ Panel

Gene	No. Mutations	Gene	No. Mutations
ABL1	14	JAK2	1
AKT1	7	KIT	27
AKT2	2	MET	5
BRAF	24	HRAS	6
CDK	2	KRAS	12
EGFR	43	NRAS	8
ERBB2	7	PDGFRα	11
FGFR1	2	PIK3CA	13
FGFR3	5	RET	6
FLT3	2		

The panel is suited for simultaneous identification of both wild type and up to 3 different mutant alleles are included. Only non-synonymous coding mutations previously reported to occur as somatic mutations in human cancers were selected for the OncoCarta™ Panel v1.0.

Assay Design and Data Analysis

While standard genotyping is fairly straightforward in design and interpretation, low abundant mutation analysis is more challenging due to the higher level of sensitivity required, coupled with the desire to keep the number of wells as low as possible. The pre-designed content in the OncoCarta™ Panel v1.0 eliminates the time required to design assays. Assay design and specially designed software reduces downstream data analysis. The panel takes advantage of several new features in order to achieve these objectives including:

- Assays designed with low mass mutant alleles and high mass wild-type alleles for easier interpretation
- Adduct correction algorithm to automatically compensate for salt and matrix adducts that obscure C to A and G to T mutations
- New SpectroCHIP™ II for less likelihood of adduct formation and higher signal to noise
- Complex mutations are resolved over multiple wells for increased sensitivity
- TypePLEX® chemistry for reduced primer extension in the absence of substrate

Analyzing data obtained from the OncoCarta™ Panel is performed using a new version of Typer Analyzer. A new menu command called “Oncomutation Report” will initiate oncomutation recall and create mutation frequency reports. The software includes a

number of customizable parameters which can be adjusted based on the probabilities desired (table 2). However, the software does provide default settings which are the suggested parameters from validation experiments that have been performed (see Results section). Of particular note, a mutation frequency cutoff of 0.1 (10%) is recommended as this provides the easiest interpretation with fewer false positives. If a lower mutation frequency cutoff is desired, Sequenom recommends running controls and manually assessing the spectra (see figure 2) to identify and distinguish false positives from true low abundant mutations.

Table 2—List of OncoCarta™ software parameters

Parameter	Range/Default	Description
Primer extension rate cutoff	0.1 - 0.5; Default 0.2	Cutoff for rate of extension for extend primer call.
Typar peak probability minimum	0.05 - 0.75; Default 0.25	Minimum peak probability for valid analyte peaks.
Typar peak probability cutoff	0.25 - 0.95; Default 0.85	Cutoff for peak probability for high confidence calls.
Concordant mutation frequency variation	0.05 - 0.5; Default 0.85	Allowed composite assay frequency variation for concordant calls.
Mutation frequency cutoff	0.0 - 0.25; Default 0.1	Cutoff for reported mutation frequency.
Show low confidence call status	Default on	Show/Hide all mutations and status for low confidence data in mutation table and composite assay details table.

Figure 2—TyperAnalyzer for data interpretation

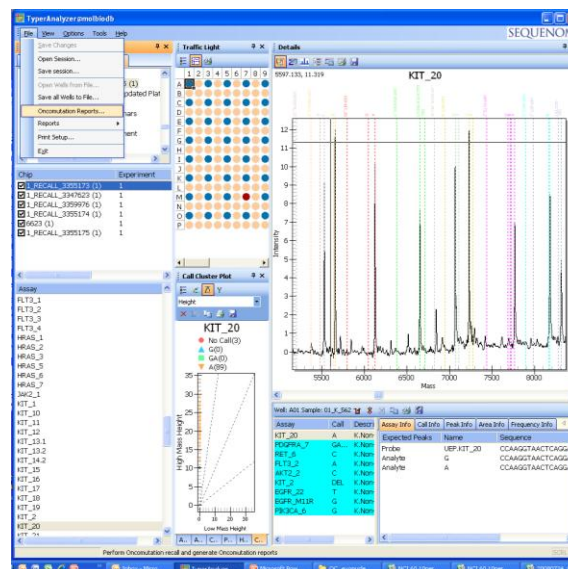


Figure 2 depicts an example of one assay, KIT_20, where the individual peaks for the different alleles can be visually inspected.

Results

Formalin-fixed paraffin tissue

To test the validity and sensitivity of the OncoCarta™ Panel, we validated the assays in two biologically relevant model systems. The first model system for validating the OncoCarta™ Panel was a set of 96 paraffin embedded cancer samples that confirmed the results of the NCI60 samples. For this set of samples, 74 mutations were previously identified using conventional Sanger sequencing. The OncoCarta™ Panel identified 70 out of the 74 mutations. The OncoCarta™ Panel also revealed the presence of previously unknown mutations. A total of 31 new mutations were identified, indicating that more than 40% new mutations were detected with frequencies ranging from 10-40%.

Figure 3—Oncogene Mutations across Tumor Types

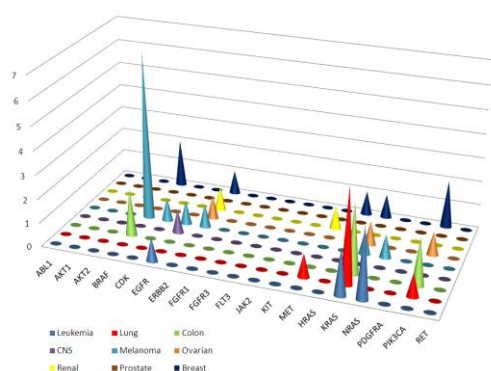


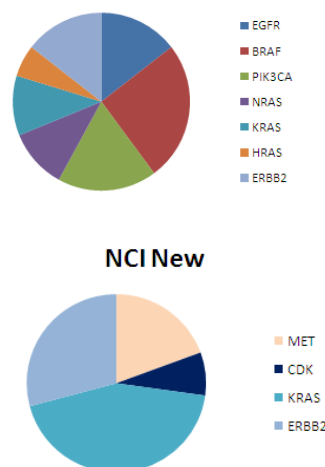
Figure 3 shows the distribution of mutations across tumor types. The genes are listed against the types of tumor tissue, and the numbers of mutations per gene and tumor tissue are depicted in the peak heights. Interestingly, in the melanoma samples tested, we saw a high number of *BRAF* mutations as well as *CDK*, *EGFR*, *ERBB2*, *KRAS*, and *NRAS* mutations present. For colon cancer tissue, however, there were a few mutations in *BRAF*, *KRAS*, and *PIK3CA* while leukemias had *EGFR*, *KRAS*, and *NRAS* mutations. Mutations in *FGFR*, *JAK2*, and *FLT* were uncommon in the samples we tested.

Taken together, these results show that we have been able to detect known and unknown mutations as well as co-occurring mutations using the OncoCarta™ Panel v1.0.

NCI60 Cell Line DNA

In the second model system, DNA from a set of 60 cell lines (NCI-60 panel) was used for mutation detection. The NCI-60 cells are supplied by the NCI/NIH Developmental Therapeutics Program and are thoroughly characterized using gene expression, DNA methylation and karyotype information. The NCI-60 cell lines have also been analyzed by the Sanger Center using sequencing, and these results are summarized on the Catalogue of Somatic Mutations in Cancer (COSMIC) website³. The OncoCarta™ Panel contains a subset of 37 mutations. For more than 86% of these same mutations, the data from OncoCarta™ Panel was in concordance with the COSMIC database. In addition, the OncoCarta™ Panel detected seven previously unknown mutations. These results show an increase of detected mutations by approximately 20%. The novel mutations detected ranged in frequency between 10 and 50%, and may not have been as easily discernable with standard Sanger sequencing methods.

Figure 4—Known and New Mutations in NCI60 DNA



Discussion

While the OncoCarta™ Panel has been validated using samples against known mutations, it has also been used to identify and quantify previously unreported mutations. By design, the panel covers a large number of regions that would normally require at least sixty sequencing runs per sample. The panel provides a comprehensive set of pre-designed assays to simultaneously investigate not only commonly occurring mutations such as those in *EGFR* and/or *K-RAS*, but also those that may be at low abundance or infrequent in the majority of samples.

Summary

Sequenom's MassARRAY® system enables analysis of mutation profiles in tumor cells or archival samples that are more sensitive and cost-effective than the commonly used Sanger sequencing methods. Internal validation studies revealed mutations in most of the analyzed oncogenes, and the mutation frequency was consistent with previous reports. In addition, new mutations were also found in tumor types that had not been previously reported, indicating that rare and potentially targetable mutations can be identified with this approach¹. The implication of these findings, of course, is that rare mutations present in only a small percentage of the tumor mass may actually be important in the natural selection process that takes place during tumorigenesis or once a patient undergoes treatment. Thus, *a priori* knowledge of the presence of a clinically relevant mutation can provide clinicians with important information by which to classify the tumor and to select rational intervention.

References

1. Thomas, R.K., *et al.* High-throughput oncogene mutation profiling in human cancer, *Nature Genetics* (2007), 39(3):283-4.
2. Vivante, A., *et al.* High-throughput, sensitive and quantitative assay for the detection of BCR-ABL kinase domain mutations, *Leukemia* (2007) 21, 1318–1321.
3. COSMIC database
<http://www.sanger.ac.uk/genetics/CGP/cosmic/>

Notes: