Application Note

DNA isolation with the MagDEA Dx SV kit for macroarray-based detection of liver enzyme mutations

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Introduction

Drug clearance and toxicity rely on the activity of metabolic liver enzymes. Genomic tests have been developed to identify mutations in liver enzymes that are known to influence metabolic turnover rates. Screening for these mutations before administering a drug can significantly improve therapeutic efficacy and safety.

Micro- and macroarrays are particularly useful, because a large set of mutations can be screened in a relatively short time using a single genomic DNA preparation. PharmGenomics has compiled a comprehensive set of 29 relevant mutations in the MutaCHIP® TOXO DNA Macroarray Kit to analyze metabolic configurations in patient samples. The data obtained from this evaluation can inform decisions on therapies that reduce side effects and drug incompatibilities.

The PharmGenomics MutaCHIP TOXO DNA Macroarray Kit has been established using genomic DNA isolated with a column-based kit from Supplier Q. The goal of this study is to evaluate whether other commercially available methods that enable automated, magnetic bead-based DNA purification are suitable alternatives.

Materials & Methods

Blood samples from three patients were collected into EDTA tubes. DNA was purified from each sample using three different protocols: A manual, spin column-based protocol using a kit from Supplier Q, and two automated, bead-based protocols using the MagDEA Dx SV kit on the magLEAD 6gC instrument (Precision System Sciences), or a comparable kit/instrument combination from Supplier R.

DNA quantity and quality of all nine samples were assessed by measuring absorbance at 280, 260, and 230 nm. DNA concentrations were normalized, and six end-point PCR reactions were set up using primers contained in the PharmGenomics MutaCHIP TOXO DNA Macroarray Kit, following the instructions in the handbook (1).
The amplified PCR products were hybridized with a MutaCHIP TOXO DNA Macroarray according to the instructions in the handbook (1), and evaluated using the software provided by the manufacturer.

The resulting signals were classified into three categories:
- Homozygous wild type: Both alleles carry the wild type variant (green)
- Heterozygous mutated: One allele carries the wild type variant; the other allele is mutated (yellow)
- Homozygous mutated: Both alleles carry the mutated variant (red)

Results

DNA was successfully isolated using all three methods. DNA quantity and quality in all samples were comparable. Macroarray analyses were performed successfully (Figure 1).

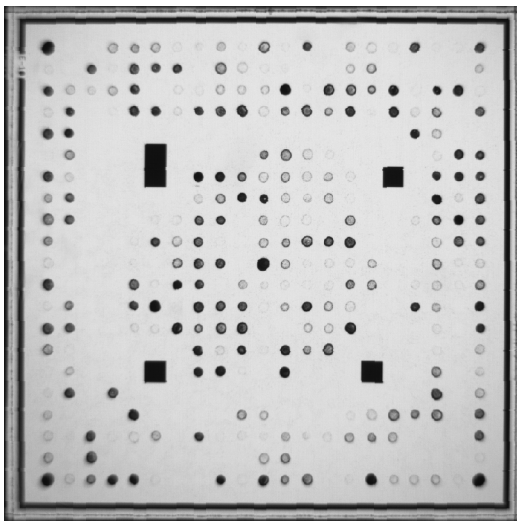
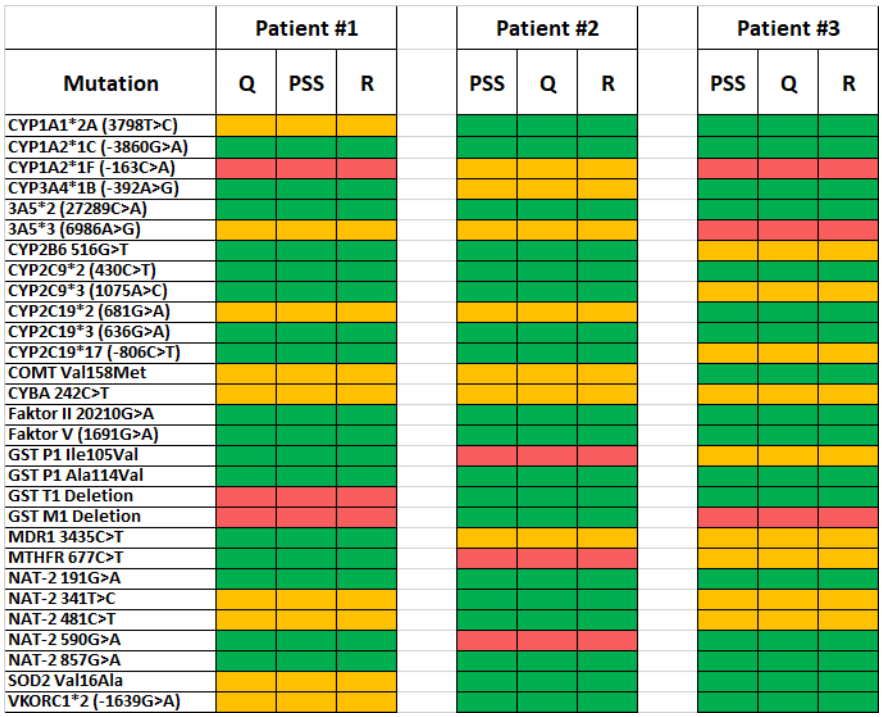


Figure 1: Successful PharmGenomics MutaCHIP TOXO DNA Macroarray Imaging.
Sample: Genomic DNA isolated from patient #1 using the MagDEA Dx SV Reagent kit on the magLEAD 6gC device.



Mutation	Patient #1			Patient #2			Patient #3		
	Q	PSS	R	PSS	Q	R	PSS	Q	R
CYP1A1*2A (3798T>C)									
CYP1A2*1C (-3860G>A)									
CYP1A2*1F (-163C>A)									
CYP3A4*1B (-392A>G)									
3A5*2 (27289C>A)									
3A5*3 (6986A>G)									
CYP2B6 516G>T									
CYP2C9*2 (430C>T)									
CYP2C9*3 (1075A>C)									
CYP2C19*2 (681G>A)									
CYP2C19*3 (636G>A)									
CYP2C19*17 (-806C>T)									
COMT Val158Met									
CYBA 242C>T									
Faktor II 20210G>A									
Faktor V (1691G>A)									
GST P1 Ile105Val									
GST P1 Ala114Val									
GST T1 Deletion									
GST M1 Deletion									
MDR1 3435C>T									
MTHFR 677C>T									
NAT-2 191G>A									
NAT-2 341T>C									
NAT-2 481C>T									
NAT-2 590G>A									
NAT-2 857G>A									
SOD2 Val16Ala									
VKORC1*2 (-1639G>A)									

Figure 2: Samples purified by all three DNA purification methods provide reliable results when applied to the MutaCHIP®TOXO DNA Macroarray.
Left column: List of screened mutations. All other columns: Data obtained for DNA from the three patients.
Q: DNA isolated with a kit from supplier Q. PSS: DNA isolated with the MagDEA Dx SV kit on the PSS MagLEAD 6gC instrument. R: DNA isolated with a kit from supplier R on the recommended instrument from the same supplier.
Red: homozygous mutation, yellow: heterozygous mutation, green: wild type genotype.

In the set of 29 mutations, results obtained with all three methods showed 100% identity within each patient, but clear differences between the three patients (Figure 2).
These differences were confirmed by orthogonal methods including real-time Förster resonance energy transfer (FRET) assays and restriction fragment length polymorphism (RFLP) analyses.

Conclusions

All three DNA isolation methods, the PSS MagDEA Dx SV kit run on the PSS MagLEAD 6gC instrument, a comparable bead-based, automated procedure provided by Supplier R, and a manual, column-based procedure provided by Supplier Q, yielded comparable, reliable results and can be recommended for use with the PharmGenomics MutaCHIP TOXO DNA Macroarray Kit.
Automated, magnetic bead-based DNA isolation procedures using pre-filled reagent cartridges such as provided in the PSS MagDEA Dx SV kit reduce hands-on time and error rates, and therefore provide excellent alternatives to manual DNA purification methods.

Product information

Product name	Product No.	Quantity
MagDEA Dx SV Reagent kit	E1300	48 tests
magLEAD Consumable kit	F4430	50 tests
magLEAD 6gC Instrument	A1060	1 device
magLEAD 12gC Instrument	A1120	1 device

Reference:
(1) PharmGenomics MutaCHIP®TOXO DNA Macroarray Kit, version 3.3, February 2018

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