Welcome!

Drug Metabolism and Toxicity

Contact Technical Support

support@SABiosciences.com
1-888-503-3187

International customers:
SABio@Qiagen.com

Webinar related questions:
Qiawebinars@qiagen.com

Wei Cao, Ph.D.
Wei.Cao@Qiagen.com

The products described in this webinar are intended for molecular biology applications. These products are not intended for the diagnosis, prevention or treatment of disease.
Topics will be covered

- **Overview**
  - Drug metabolism & metabolism-based toxicity
  - Current approaches

- **Research solutions @ QIAGEN**
  - Detect the drug metabolism abnormality
  - Identify the mechanism of toxicity
  - Monitor genotoxicity

- **Application examples**

- **What’s next**
**Drug toxicity: why is it concerned?**

- Cost of developing a drug is high: average $800m-1b
- Toxicity is a major barrier for new drugs
  - Responsible for the failure of 1/3 drug candidates
  - A major contributor to the high cost of drug development

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*F. Peter Guengerich, Drug Metab. Pharmacokinet, 2011, 26(1): 3*

*F. Peter Guengerich, The AAPS Journal, 2006, 8(1), E101*
Current hot spots in toxicity risk assessment

Sites for toxicology attrition - target organs or tissues

- Hepatotoxicity: a major issue in both pre-clinical and clinical development; Many drugs withdrawn due to hepatotoxicity.

- Genotoxicity: not limited to a specific organ or tissue type; Compounds causing genotoxicity are the most dangerous ones as classified by FDA.

F. Peter Guengerich, Drug Metab. Pharmacokinet, 2011, 26(1): 3
F. Peter Guengerich, The AAPS Journal, 2006, 8(1), E101
Drug metabolism induced toxicity

Drug metabolism phases:
**Phase I**: Oxidation/reduction/hydrolysis
**Phase II**: Conjugation

**Outcome of drug metabolism**

Drug metabolism phases:
- **Phase I**: Oxidation/reduction/hydrolysis
- **Phase II**: Conjugation

Drug
- Toxic metabolite
- Active metabolite
- Inactive metabolite
- Reversible metabolite

Toxicity
- Altered activity
- Enhanced activity
- Loss of activity
- Prolonged activity

**Drug metabolism phases**:
- Nucleic acid
- Enzyme
- Transport proteins
- Signaling proteins
- Receptors
- Autologous proteins

**Side effects**:
- Carcinogenicity
- Genotoxicity
- Necrosis
- Apoptosis
- Hypersensitivity
Prediction of human drug toxicity is difficult:

- Lack of mechanistic understanding
- Species differences
- Other factors affecting drug metabolism, such as age, gender, genetic polymorphism, disease state, and environmental factors

A systematic evaluation of the roles of drug metabolism in drug toxicity in pre-clinical stage is needed

FAIL IT EARLIER, FAIL IT CHEAPER!
Drug metabolism induced toxicity

FDA guidance on metabolism-based drug-drug interaction

Evaluation of drug induced toxicity

*in vitro* approaches

**Specificity**
- IC50
- EC50

**Metabolic Stability**
- Stability in Human (M, D, R, Monkey) liver microsome & Human Hepatocyte

**Inhibition**
- Inhibition of drug metabolizing enzymes in Human Hepatocytes

**Induction**
- Induction of drug metabolizing enzymes in Human Hepatocytes

**Permeability**
- Substrate of Pg-P
- Inhibitor of Pg-P
- Inducer of Pg-P

Feed information to *in vivo* studies and the types of clinical trials needed to assess potential DDIs

Research solutions @ QIAGEN

- Detect the drug metabolism abnormality
- Identify the mechanism of toxicity
- Monitor genotoxicity

Application examples

What’s next
Gene expression regulates biology

All require molecular signaling for action
Gene expression and drug toxicity

- Gene expression profiles should be able to discriminate compounds that have different mechanisms.

- The toxicity of unknown compounds can be predicted by comparing their molecular fingerprints with those obtained with compounds of known toxicity.

- **Specific to drug metabolism:**
  - Induction of drug metabolizing enzymes usually results from overexpression of the respective genes
  - The major mechanisms for enzyme inhibition is due to the down-regulation of gene expression
Q: How to assess the expression of different mRNAs in a sample involved in a process and compare it across multiple conditions?

A:
Principles of qRT-PCR: overview

- **Real-Time PCR**
  - Amplify and simultaneously quantify target DNA

- **RT² PCR (Reverse transcription teal-time PCR)**
  - Amplify and simultaneously quantify mRNA

- **Ct Values: Threshold Cycle**
Pathway- or disease-focused PCR Arrays

- 84 Pathway-Specific Genes of Interest
- 5 Housekeeping Genes
- Genomic DNA Contamination Control
- Reverse Transcription Controls (RTC) n=3
- Positive PCR Controls (PPC) n=3

96-well plate format

100-well rotor gene ring
384-well plate
96x96 chips
Customizable
How RT² Profiler PCR Arrays work?

### Isolate Total RNA

1. Convert Total RNA to cDNA.

   - **Control**
   - **Hot Sauce**

2. Add cDNA to RT² qPCR Master Mix & Aliquot Mixture Across PCR Array.


4. Data Analysis.

### cDNA Synthesis
- Genomic DNA Removal Step (5 min.)
- Reverse Transcription Step (20 min.)

### Load Plates
- 1 Sample per PCR Array
- 2 minutes with multi-channel pipet

### Run 40 cycle qPCR Program
- Standard cycling conditions for all Real Time PCR Instruments
- 2 hours

### Upload and Analyze Data (FREE)
- 15 minutes from Raw Ct to Fold Change Data
Profile gene expression of 84 drug-metabolizing enzymes in primary human hepatocytes
Drug metabolism RT2 Profiler PCR Array

- Profile gene expression of drug metabolizing enzymes
- Detect drug metabolism abnormality
- Human, mouse and rat
- Expandable to other species

Drug Transporters:
Metallothioneins: MT2A, MT3.
P-Glycoprotein family: ABCB1 (MDR1), ABCC1 (MRP1), GPi.

Phase I Metabolizing Enzymes:
CYP11B2, CYP17A1, CYP19A1, CYP1A1, CYP2B6, CYP2C19, CYP2C8, CYP2C9, CYP2D6, CYP2E1, CYP2F1, CYP2J2, CYP3A4, CYP3A5.

Phase II Metabolizing Enzymes:
Carboxylesterases: CES1, CES2, CES3.
Decarboxylases: GAD1, GAD2.
Dehydrogenases: ADH1B, ADH1C, ADH4, ADH5, ADH6, ALAD, ALDH1A1, HSD17B1, HSD17B2, HSD17B3.
Glutathione Peroxidases: GPX1, GPX2, GPX3, GPX4, GPX5, GSTA3, GSTA4, GSTM2, GSTM3, GSTM5, GSTP1, GSTT1, GSTZ1, LPO, MPO.
Lipoxygenases: ALOX12, ALOX15, ALOX5, APOE.
Hydrolases: ASNA1, EPHX1, FAAH, FBP1.
Kinases: HK2, PKLR, PKM2.
Oxidoreductases: ABP1, BLVRA, BLVRC, CYB5R3 (DIA1), GPX1, GPX2, GSR, MTHFR, NOS3 (eNOS), NQO1, SRD5A1, SRD5A2.
Paraoxonases: PON1, PON2, PON3.
Glutathione S-Transferases: GSTA3, GSTA4, GSTM2, GSTM3, GSTM5, GSTP1, GSTT1, MGST1, MGST2, MGST3.
Other Transferases: CHST1, NAT1, NAT2, COMT.

Other Genes: AHR, ARNT, GCKR, SNN.

Application 1 – Induction & inhibition

**3 compounds:** 20uM Rifampicin, 50uM Omeprazole, 8uM 3-MC (3-methylcholanthrene)

**Experimental workflow**

**Control**
- Incubate with primary human hepatocytes, 3 male donors

**Sample**
- Isolate total RNA using RNeasy system

**Reverse transcription**
- cDNA synthesis

**Data Analysis**
- Upload raw data (Ct) and analyze data

**RT² PCR Array**
Application 1 - Interpretation of $C_t$ Values

Fold Change Calculations: PCR Array Results / Data

- **Raw $C_t$ Value:**
  Relative expression level of that gene in the sample

- **Data normalization $\Delta C_t$ per Gene:**
  $C_t^{\text{Gene X}} - C_t^{\text{HKGs (Avg)}}$

- **Up or Down-Regulation**
  $\Delta \Delta C_t$ per Gene = $\Delta C_t^{\text{Sample}} - \Delta C_t^{\text{Control}}$

  \[
  \text{Fold change result per gene} = 2^{-\Delta \Delta C_t}
  \]

The gene is present 4x more in the treated sample than in control sample.

FREE Complete & easy data analysis with web-based software
Application 1 - Results

Up-regulation of metabolizing enzymes observed

Rifampicin

- CYP2B6 8-20 fold
- CYP2C8 4-5.5 fold
- CYP2C9 (2.5-7 fold)

Omeprazole

- CYP1A1 >60 fold

3-MC

- CYP1A1 >40 fold

Rifampicin: CYP2B6 8-20 fold, CYP2C8 4-5.5 fold, CYP2C9 (2.5-7 fold)

Omeprazole: CYP1A1 >60 fold

3-MC: CYP1A1 >40 fold

Baitang Ning, etc. J Biomol Screen, 2008; 13; 194
Application 1 - Results

Down-regulation of metabolizing enzymes observed

- Rifampicin
- 3-MC

Baitang Ning, etc. J Biomol Screen, 2008; 13; 194
Gene expression profiling provides a systematic, efficient and relatively reliable high-throughput method to assess drug metabolizing enzymes expression after drug treatments.
Gene expression profiling applications in drug toxicity

Application 2

Identify the mechanism of toxicity
IRE1α activation protects mice against acetaminophen-induced hepatotoxicity

Kyu Yeon Hur,1 Jae-Seon So,1 Vera Ruda,2 Maria Frank-Kamenetsky,3 Kevin Fitzgerald,3 Victor Koteliansky,3 Takao Iwawaki,4,5,6 Laurie H. Glimcher,1,7,8 and Ann-Hwee Lee1,7

1Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, MA 02115
2Cardiovascular Research Center and Center for Human Genetic Research, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114
3Alnylam Pharmaceuticals, Cambridge, MA 02142
4Iwawaki Initiative Research Unit, Institute of Physical and Chemical Research, Wako, Saitama 351-0198, Japan
5PRESTO, Japan Science and Technology Agency, Kawaguchi, Saitama 332--0012, Japan
6Advanced Scientific Research Leaders Development Unit, Gunma University, Gunma 371-8511, Japan
7Dept of Medicine, Harvard Medical School, Boston, MA 02115
8Ragon Institute of MGH, MIT, and Harvard, Boston, MA 02115
Background:

- **Acetaminophen (APAP)** is an endoplasmic reticulum (ER) inducer
- APAP overdose causes acute liver failure
- APAP hepatotoxicity is mainly mediated by its metabolite, NAPQI (N-acetyl-p-benzoquinone imine) generated by APAP oxidation by CYP450 enzymes
- NAPQI is detoxified primarily by glutathione (GSH) conjugation
- Excessive amount of NAPQI depletes GSH and covalently binds to macromolecules, resulting in cytotoxicity

The mechanism of APAP caused hepatotoxicity?
**In vivo application**

- Mice model: XBP1 is deleted
- High dose APAP treatment
- RNA isolation
- qRT-PCR
- Data analysis

- Genetic ablation of XBP1 activates IRE1a in liver, leads to the degradation of CYP1A2 and CYP2E1 mRNAs, and protect mice from APAP-induced hepatotoxicity;

- **APAP metabolism in liver:** upon ER stress, APAP activate IRE1a suppressed expression of CYP1A2 and CYP2E1, which drive the conversion of APAP to hepatotoxic metabolites.

- CYP1A2 and CYP2E1, crucial for APAP oxidation to toxic NAPQI were significantly decreased in XBP1Δ mice
- Gene involved in GSH synthesis were not affected
Drug Metabolism: Phase I Enzymes PCR Array


**Cytochrome P450:** CYP11A1, CYP11B1, CYP11B2, CYP17A1, CYP19A1, CYP1A1, CYP1A2, CYP1B1, CYP21A2, CYP24A1, CYP26A1, CYP26B1, CYP26C1, CYP27A1, CYP27B1, CYP2A13, CYP2R1, CYP2S1, CYP2B6, CYP2C18, CYP2C19, CYP2C8, CYP2C9, CYP2D6, CYP2E1, CYP2F1, CYP2W1, CYP3A4, CYP3A43, CYP3A5, CYP3A7, CYP4A11, CYP4A22, CYP4B1, CYP4F11, CYP4F12, CYP4F2, CYP4F3, CYP4F8, CYP7A1, CYP7B1, CYP8B1.

**Alcohol Dehydrogenase:** ADH1A, ADH1B, ADH1C, ADH4, ADH5, ADH6, ADH7, DHRS2, HSD17B10 (HADH2).

**Esterase:** AADAC, CEL, ESD, GZMA, GZMB, UCHL1, UCHL3.

**Aldehyde Dehydrogenase:** ALDH1A1, ALDH1A2, ALDH1A3, ALDH1B1, ALDH2, ALDH3A1, ALDH3A2, ALDH3B1, ALDH3B2, ALDH4A1, ALDH5A1, ALDH6A1, ALDH7A1, ALDH8A1, ALDH9A1.

**Flavin containing Monooxygenase:** FMO1, FMO2, FMO3, FMO4, FMO5.

**Monoamine Oxidase:** MAOA, MAOB.

**Prostaglandin-endoperoxide Synthase:** PTGS1, PTGS2.

**Xanthine Dehydrogenase:** XDH.

**Dihydropyrimidine Dehydrogenase:** DPYD.
7 major classes of phase II drug metabolism enzymes catalyzing such reactions as glutathione conjugation, glucuronidation, sulfation, methylation, amino acid conjugation, epoxidation, and esterification.

**Amino Acid Transferases**: AGXT, BAAT, CCBL1, GLYAT.

**Dehydrogenases**: NQO1, NQO2, XDH.

**Epoxidases**: EPHX1, EPHX2.

**Esterases**: CES1, CES2, CES3, CES5A (CES7).

**Glucuronosyltransferases**: DDOST, UGT1A1, UGT1A4, UGT1A9, UGT2A1, UGT2A3, UGT2B10, UGT2B17, UGT2B28, UGT2B4, UGT2B7, UGT3A1, UGT8.

**Glutathione Transferases**: GSTA1, GSTA3, GSTA4, GSTA5 (YC2), GSTK1, GSTM2, GSTM3, GSTM4, GSTM5, GSTO1, GSTO2, GSTP1, GSTT1, HNMT, INMT, MGST1, MGST2, MGST3, PTGES.

**Methyltransferases**: AS3MT, ASMT, COMT, GAMT, GNMT, NNMT, PNMT, TPMT.

**N-Acetyltransferases**: AANAT, ACSL1, ACSL3, ACSL4, ACSM1, ACSM2B, ACSM3, GALNT1, GALNT4, GCNT1, MGAT1, MGAT2, NAT1, NAT2, NAA20 (NAT5), POMGNT1, SAT1, UGCG.

**Sulfotransferases**: CHST7, SULT1A1, SULT1A2, SULT1B1, SULT1C4, SULT1C2, SULT1C3, SULT1E1 (STE), SULT2A1, SULT2B1, SULT4A1, SULT6B1, TST.
Gene expression profiling applications in drug toxicity

Application 3

Discover unique gene expression profiles associated with liver toxicity
**Oxidative Stress:** FTH1, GCLC, GCLM, GSR, GSTP1, HMOX1, NQO1, PRDX1, SQSTM1, TXN, TXNRD1.

**Hypoxia:** ADM, ARNT, BNIP3L, CA9, EPO, HMOX1, LDHA, MMP9 (Gelatinase B), SERPINE1 (PAI-1), SLC2A1, VEGFA.

**Osmotic Stress:** AKR1B1, AQP1, AQP2, AQP4, CFTR, EDN1, HSPA4L, NFAT5, SLC5A3.

**Cell Death:**
- **Apoptosis:** CASP1 (ICE), FAS, MCL1, TNFRSF10A, TNFRSF10B (DR5), TNFRSF1A.
- **Autophagy:** ATG5, ATG7, ATG12, BECN1, FAS, ULK1.
- **Necrosis:** FAS (TNFRSF6), GRB2, PARP1 (ADPRT1), PVR, RIPK1, TNFRSF10A, TNFRSF1A, TXNL4B.

**Inflammatory Response:** CCL2 (MCP-1), CD40LG (TNFSF5), CRP, IFNG, IL1A, IL1B, IL6, IL8, TLR4, TNF.

**DNA Damage Signaling:**
- **Cell Cycle Checkpoint/Arrest:** CDKN1A (p21CIP1/WAF1), CHEK1, CHEK2 (RAD53), DDIT3 (GADD153/CHOP), HUS1, MRE11A, NBN (NBS1), RAD17, RAD9A.
- **Other Responses:** ATM, ATR, DDB2, GADD45A, GADD45G, RAD51, TP53, XPC.

**Heat Shock Proteins/Unfolded Protein Response:** ATF4, ATF6, ATF6B, BBC3, BID, CALR, DDIT3, DNAJC3, HSP90AA1, HSP90B1 (TRA1), HSPA4 (HSP70), HSPA5 (GRP78).
In vitro: HepG2

- Treat cells with 3 diabetes drugs
- RNA isolation
- qRT-PCR
- Data analysis

Uncovered distinct gene expression profiles associated with liver toxicity caused by 3 PPARγ agonists (Actos, Avandia, and Rezulin).

3 anti-diabetic drugs:
Rezulin (Troglitazone): withdrawn due to idiosyncratic liver toxicity
Actos (Pioglitazone) and Avandia (Rosiglitazone): safe
Monitor genotoxicity via gene expression profiling
Genotoxicity tests are defined as *in vitro* and *in vivo* tests designed to detect compounds that induce genetic damage directly or indirectly by various mechanisms.

- Test for gene mutation in bacteria (60% sensitivity)
- *In vitro* test with cytogenetic evaluation of chromosomal damage with mammalian cells
- *In vitro* mouse lymphoma tk assay
- *In vivo* test for chromosomal damage using rodent hematopoietic cells

**Application 4- Monitor Genotoxicity via gene expression**

Selected 20 compounds for genotoxicity testing

<table>
<thead>
<tr>
<th>Genotoxic Compounds</th>
<th>CAS #</th>
<th>Mode of Action</th>
<th>Profiling Dose</th>
<th>Ames Test</th>
<th>Mammalian Cell Mutation</th>
<th>Chromosomal Aberration</th>
<th>Micronucleus Tests</th>
<th>Carcinogenicity Tests (Rodent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzo(a)pyrene (BAP)</td>
<td>50-32-8</td>
<td>DNA Adduct Formation</td>
<td>5 µM</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Benzo(k)fluoranthene (BKF)</td>
<td>207-08-9</td>
<td>DNA Adduct Formation</td>
<td>5 µM</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>not tested</td>
<td>+</td>
</tr>
<tr>
<td>Camptothecin (CPT)</td>
<td>7689-034</td>
<td>Topoisomerase Inhibitors</td>
<td>2 µM</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>not tested</td>
</tr>
<tr>
<td>Cisplatin (CIS)</td>
<td>15663-27-1</td>
<td>DNA Cross-linking</td>
<td>10 µM</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Doxorubicin hydrochloride (DOX)</td>
<td>25316-40-9</td>
<td>Topoisomerase Inhibitors</td>
<td>2 µM</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>not tested</td>
<td>+</td>
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<tr>
<td>5-Fluorouracil (5FU)</td>
<td>207291-81-4</td>
<td>DNA Synthesis Inhibitors</td>
<td>1 µM</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Mitomycin (MMC)</td>
<td>50-07-7</td>
<td>DNA Cross-linking</td>
<td>5 µM</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Mitoxantrone dihydrochloride (MXT)</td>
<td>70476-82-3</td>
<td>Topoisomerase Inhibitors</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>not tested</td>
<td>not tested</td>
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<tr>
<td>Methyl Methanesulfonate (MMS)</td>
<td>66-27-3</td>
<td>Methylation</td>
<td>400 µM</td>
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<td>N-Nitrosodimethylamine (NDMA)</td>
<td>62-75-9</td>
<td>Methylation</td>
<td>2 mM</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td><strong>Non-genotoxic Compounds</strong></td>
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<td>Acetone (ACT)</td>
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<td>2 mM</td>
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<td>Ampicillin (AMP)</td>
<td>67-64-1</td>
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<td>2 mM</td>
<td>-</td>
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<td>Diflunisal (DFN)</td>
<td>22494-42-4</td>
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<td>250 µM</td>
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<td>Dimethyl sulfoxide (DMSO)</td>
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<td>2 mM</td>
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<td>Ethanol (ETOH)</td>
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<td>530-78-9</td>
<td></td>
<td>200 µM</td>
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<td>-</td>
<td>-</td>
<td>not tested</td>
<td>-</td>
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<tr>
<td>Pentachlorophenol (PCP)</td>
<td>67-96-5</td>
<td></td>
<td>50 µM</td>
<td>-</td>
<td>not tested</td>
<td>weak +</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Phorbol 12-myristate 13-acetate (TPA)</td>
<td>127-18-4</td>
<td></td>
<td>500 nM</td>
<td>not tested</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Tetrachloroethylene (TCE)</td>
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<td></td>
<td>2 mM</td>
<td>-</td>
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<tr>
<td>Sodium chloride (NaCl)</td>
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<td></td>
<td>2 mM</td>
<td>-</td>
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</tbody>
</table>
Application 4- Genotoxicity Experiment Design

**Cells:**
HepG2 cells: 2 X 10⁵ cells/ml at seeding.
96-well plate for cytotoxicity assays --- 3 to 5 biological replicates.
6-well plate for RNA isolation --- 4 biological replicates.

**Dose:**
IC20 - low dose but substantial to trigger gene expression changes.

**Time:**
24 hr – to avoid generic stress responses often observed at shorter incubation time (e.g. 3 hr or 6 hr).

**RNA Isolation and PCR Array Analysis:**
RT² PCR Array protocols.
Expression profiles of 11 signature genes
Application 4 - Results

Gene Signatures Define Compound Class

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BAP</th>
<th>BKF</th>
<th>CPT</th>
<th>CIS</th>
<th>DOX</th>
<th>5FU</th>
<th>MMC</th>
<th>MXT</th>
<th>MMS</th>
<th>NDMA</th>
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<td>Pearson r</td>
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<td>0.9502</td>
<td>0.9087</td>
<td>0.9242</td>
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<td>95% Confidence Interval</td>
<td>0.8573-0.9904</td>
<td>0.8145-0.9873</td>
<td>0.6789-0.9764</td>
<td>0.7278-0.9805</td>
<td>0.5697-0.9663</td>
<td>0.9147-0.9944</td>
<td>0.9570-0.9973</td>
<td>0.6584-0.9746</td>
<td>0.7025-0.9784</td>
<td>0.3737-0.7589</td>
</tr>
<tr>
<td>P Value (two-tailed)</td>
<td>P&lt;0.0001</td>
<td>P&lt;0.001</td>
<td>0.0001</td>
<td>P&lt;0.0001</td>
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<td>P Value Summary</td>
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Expression profiles from different modes of action

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We identified 11 genes in DNA damage repair and p53 pathways as a classifier for genotoxic and non-genotoxic compounds.

Genotoxic compounds with different modes of action elicit distinct gene expression profiles.
Many pathways important for drug discovery process

- Drug Transporters
- Cancer Drug Resistance
- DNA Damage Signaling Pathway PCR array
- Heat Shock Proteins PCR array
- Unfolded Protein Response PCR array
- Oxidative stress PCR Array
- Cardiotoxicity PCR Array
- Hepatotoxicity PCR Array
- Custom PCR Arrays
Toxicology and drug metabolism @ QIAGEN

QIAGEN offers many methods to analyze the toxicology and ADME profiles of drug candidates

- Gene Expression
  - RT² Profiler PCR Arrays & Assays

- Mutational analysis
  - qBiomarker Somatic Mutation PCR Arrays & Assays
  - qBiomarker Copy Number PCR Arrays & Assays

- Epigenetics
  - miScript miRNA PCR Arrays & Assays
  - EpiTect Methyl qPCR Arrays & Assays

- Functional Studies
  - Cignal Reporter Assays
  - siRNA and plasmid-based shRNA
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Whole Genome
- Illumina Gene Expression Profiling
- Illumina Genotyping

Pathway / Focused Panel
- Mutation Profiling
- Methylation
- PCR Array
- miRNA PCR Array

Individual Gene / Locus
- Mutation Detection
- Methylation
- qPCR

Sample Preparation – DNA, RNA Extraction and Purification
- Cells, Tissue or Biofluids
- Fixed Tissue
- Small Sample

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New Products for toxicology

**RT² Profiler PCR Array Plus**
- Upgrade from current RT² Profile PCR Array
- Same workflow as current RT² Profile PCR array
- Content
  - Signature genes (16)
  - Pathway relevant genes (68)
  - HK gene (5), GDC (1), RTC (3), PPC (3)
- Data analysis
  - Profiling
  - Pathway activity readout

**RT² Predictor PCR Array**
- New product of RT² PCR Array
- Signature gene only plate
- Process 4 samples per plate
- Same workflow as current RT² PCR array
- Content
  - Signature gene (16)
  - HK gene (5), GDC (1), RTC (1), PPC (1)
- Data analysis
  - Pathway activity readout

Thank you for attending!

**PCR Array Starter Pack - Promotion Code FDK-PAFAS12**

PCR Arrays of any **Pathway (FREE)**
- 2 96-well/100-well (2 samples) OR 1 384-well (4 samples)

Required Reagents **(w/ Purchase)**
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- RT² SYBR Green Mastermix (2-Pack)

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