Gene Expression Profiling with NanoString Technology to Identify Genetic Modulators of Aryl Hydrocarbon Receptor (AHR)-mediated Toxicity

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Genomics Core Seminar

Michigan State University

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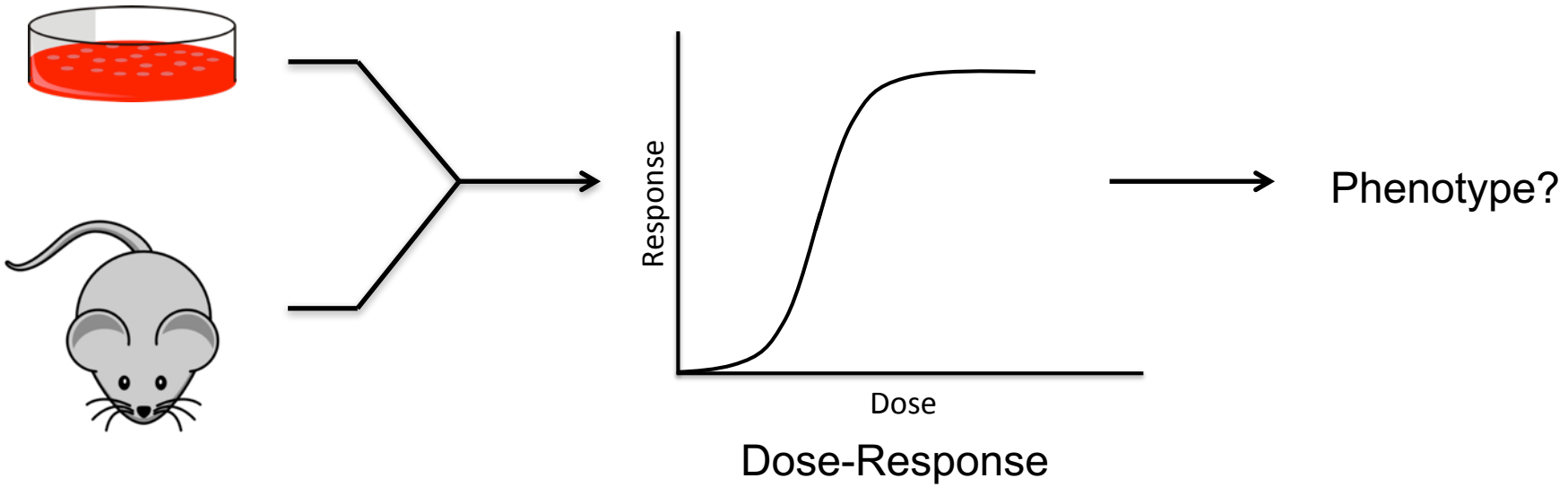
Characterizing Risk Associated with Chemical Exposure

Laboratory-based toxicology is primarily focused on altered phenotypes associated with increasing doses of a chemical of concern.



‘But remember throughout that no external cause is efficient without a predisposition of the body itself. Otherwise, external causes which affect one would affect all’

-Claudius Galenus (129-217 AD)

Assessing a Heterogeneous Population

Chemical Exposure

1 + 1 = 3

Concurrent Stressors

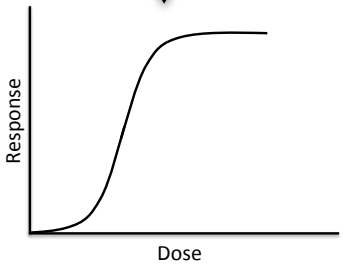
Disease State

Environment

Age

Sex

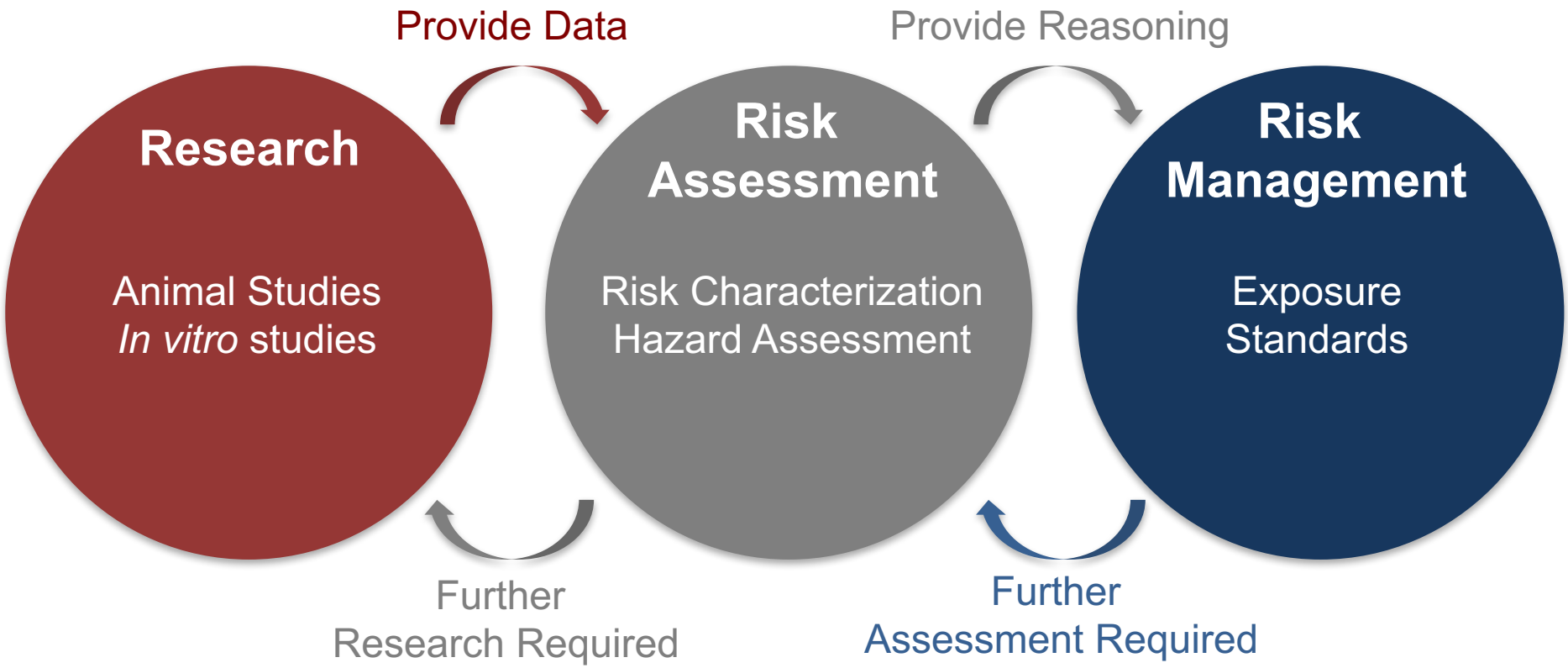
Genotype



Phenotypical Response

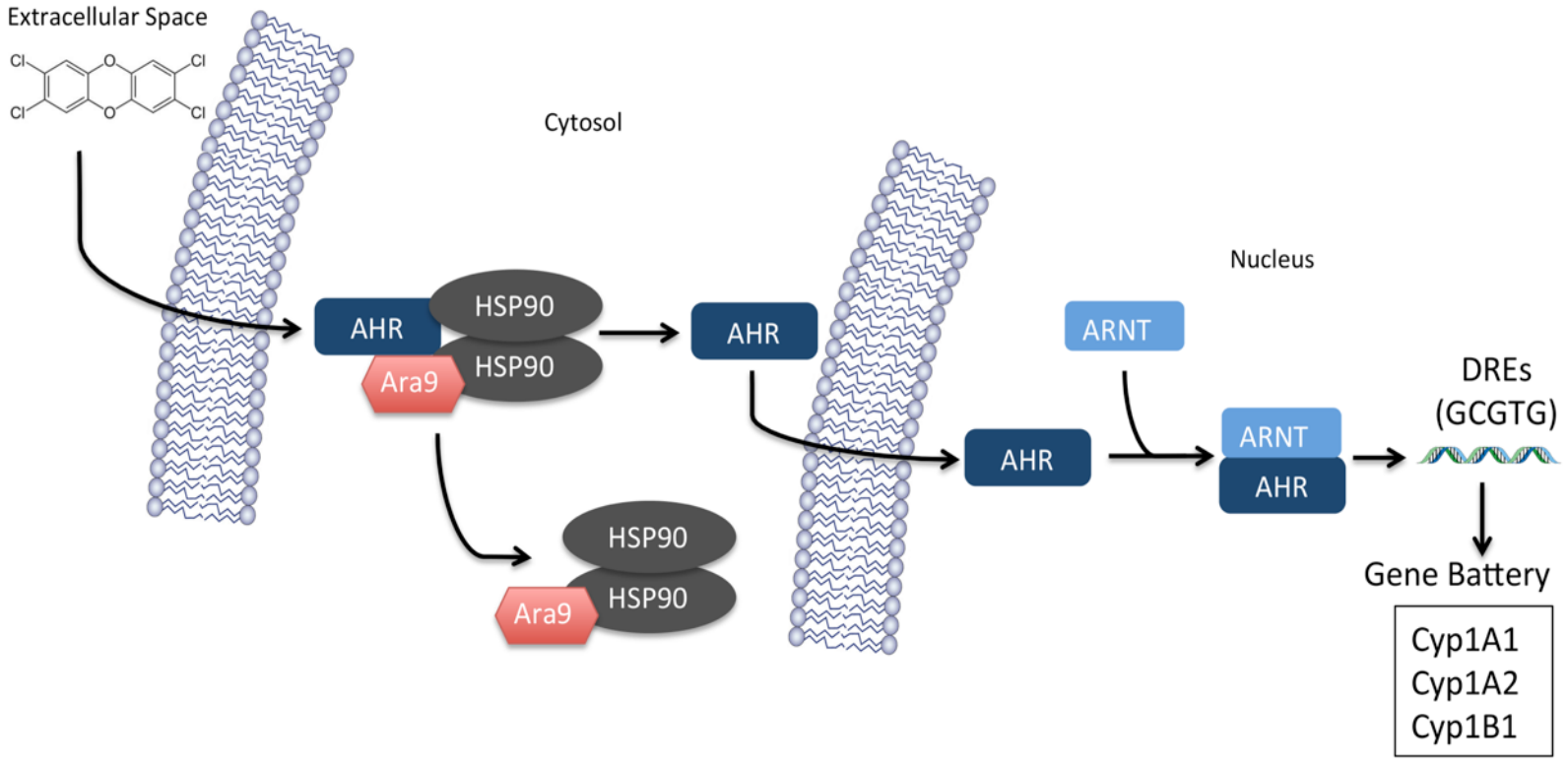
From the Laboratory to a Decision

The characterization of potential risks posed by chemicals is a dynamic, multifaceted process.



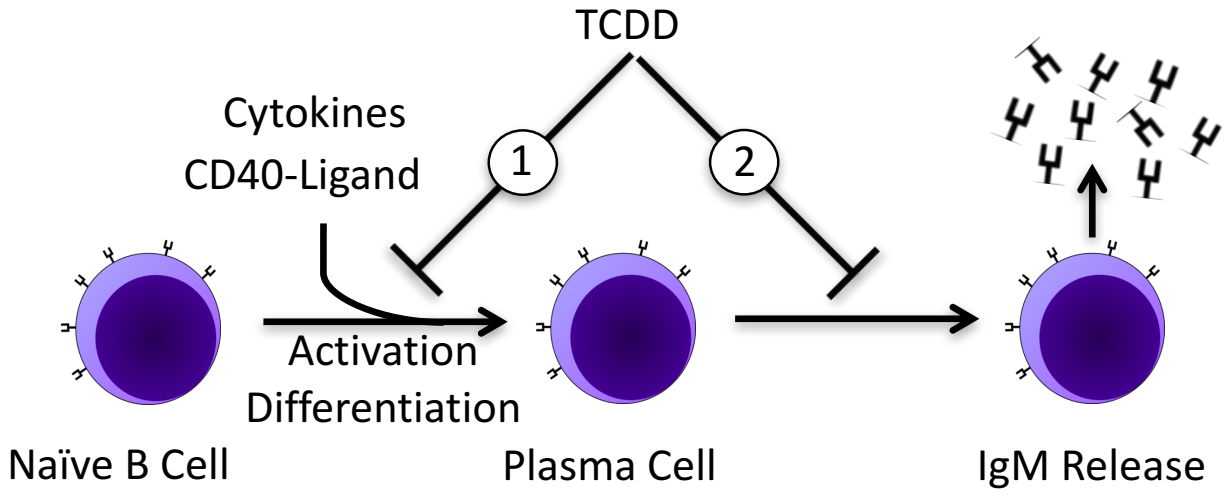
Aryl Hydrocarbon Receptor as a Case Study

- **Aryl Hydrocarbon Receptor (AHR) – transcription factor in the PAS superfamily of environmental sensors.**
- **Most potent ligand of the AHR is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD).**
- **TCDD is linked to several complex, chronic diseases in humans including chloracne, metabolic syndrome, potentially cancer, and immune suppression.**

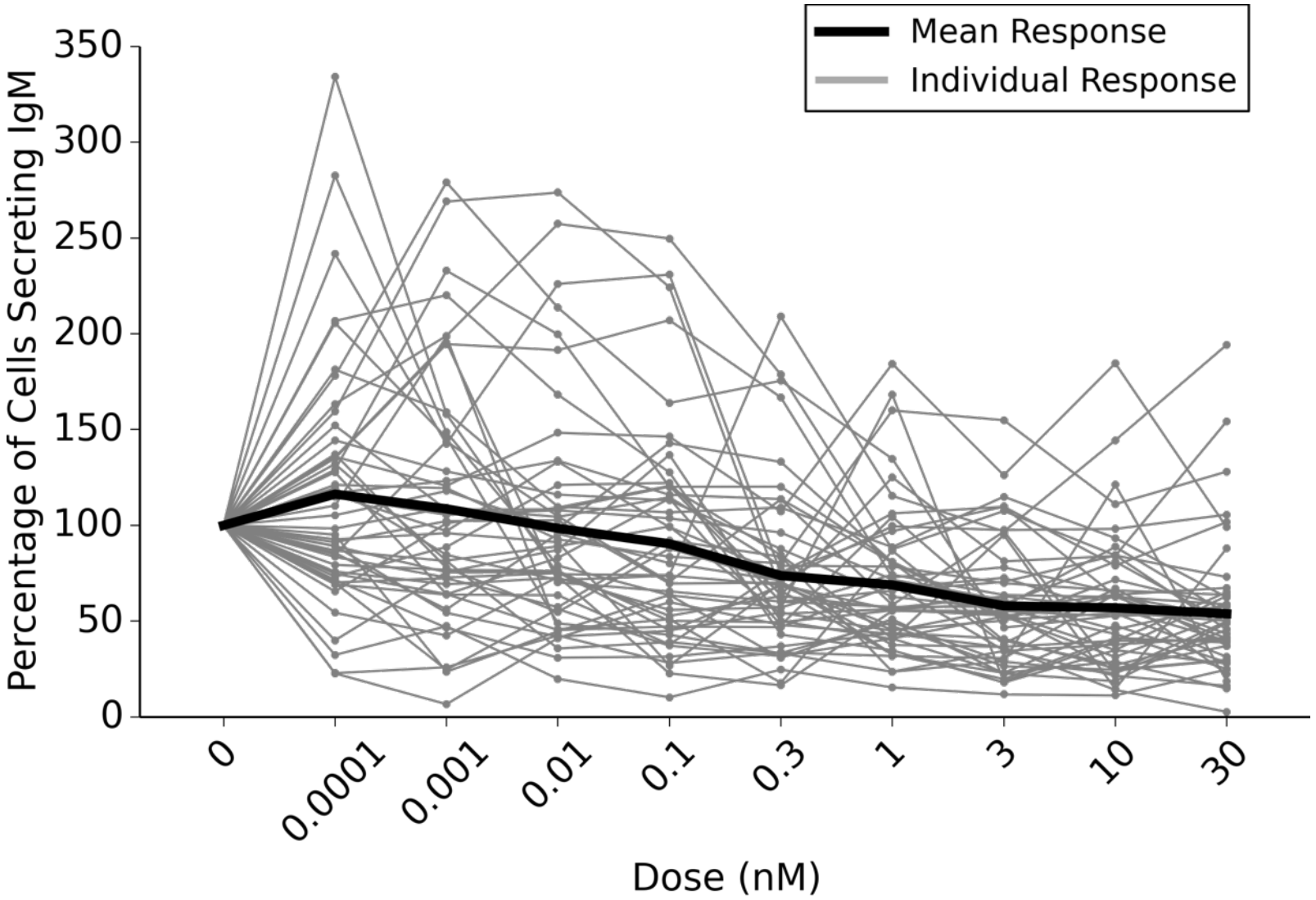


TCDD-induced Immunosuppression

- TCDD is a known suppressant of the human B cell function.
- While the mechanism is not completely understood, B cells exposed to TCDD release less antibody following activation.
- There are known variations in individual's immunosuppressive response to increasing doses of TCDD.



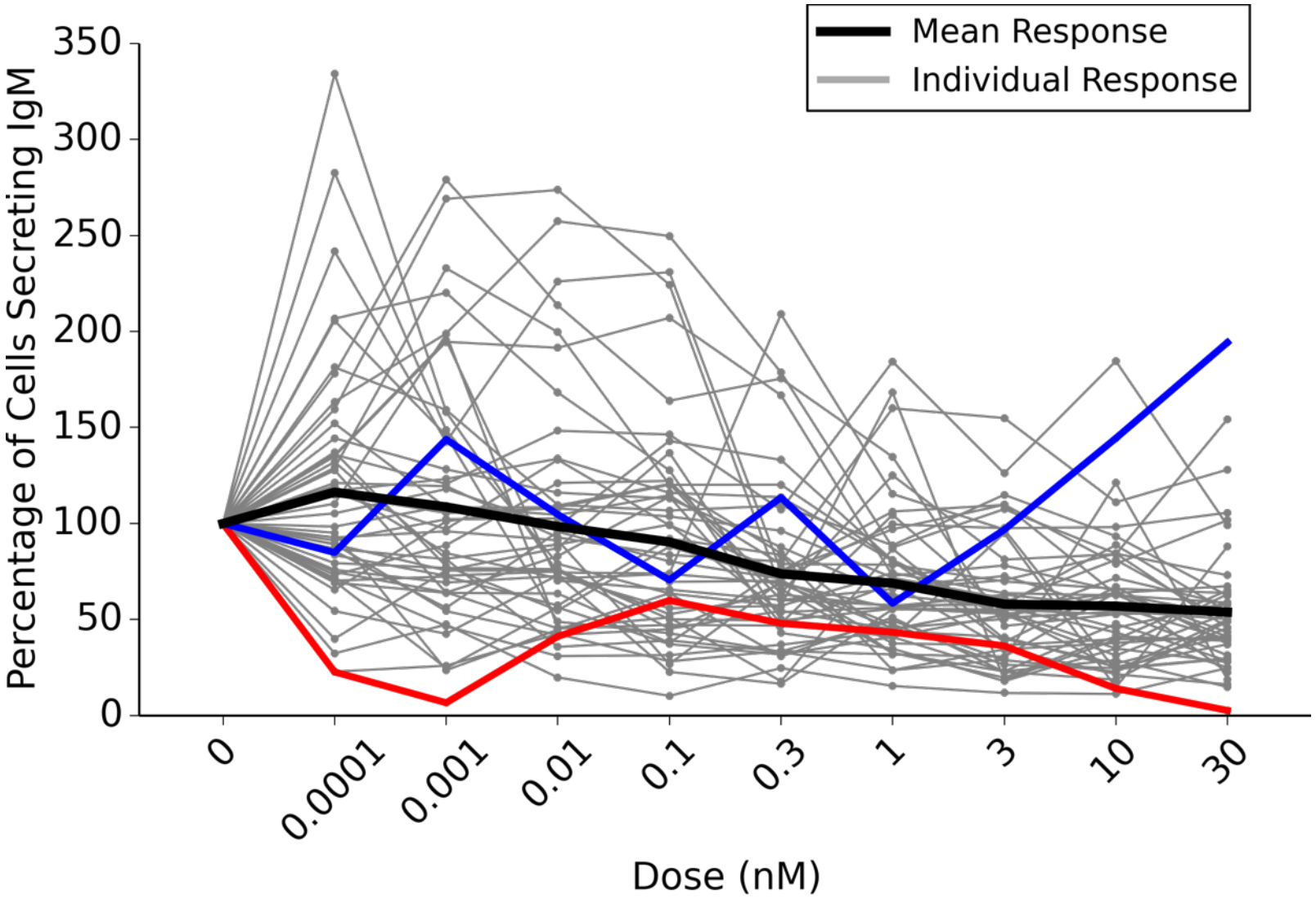
Interindividual Variability in Human Population Response to TCDD



There is a wide-array of responses found within the human population.

Dornbos *et al*., 2016

Interindividual Variability in Human Population Response to TCDD



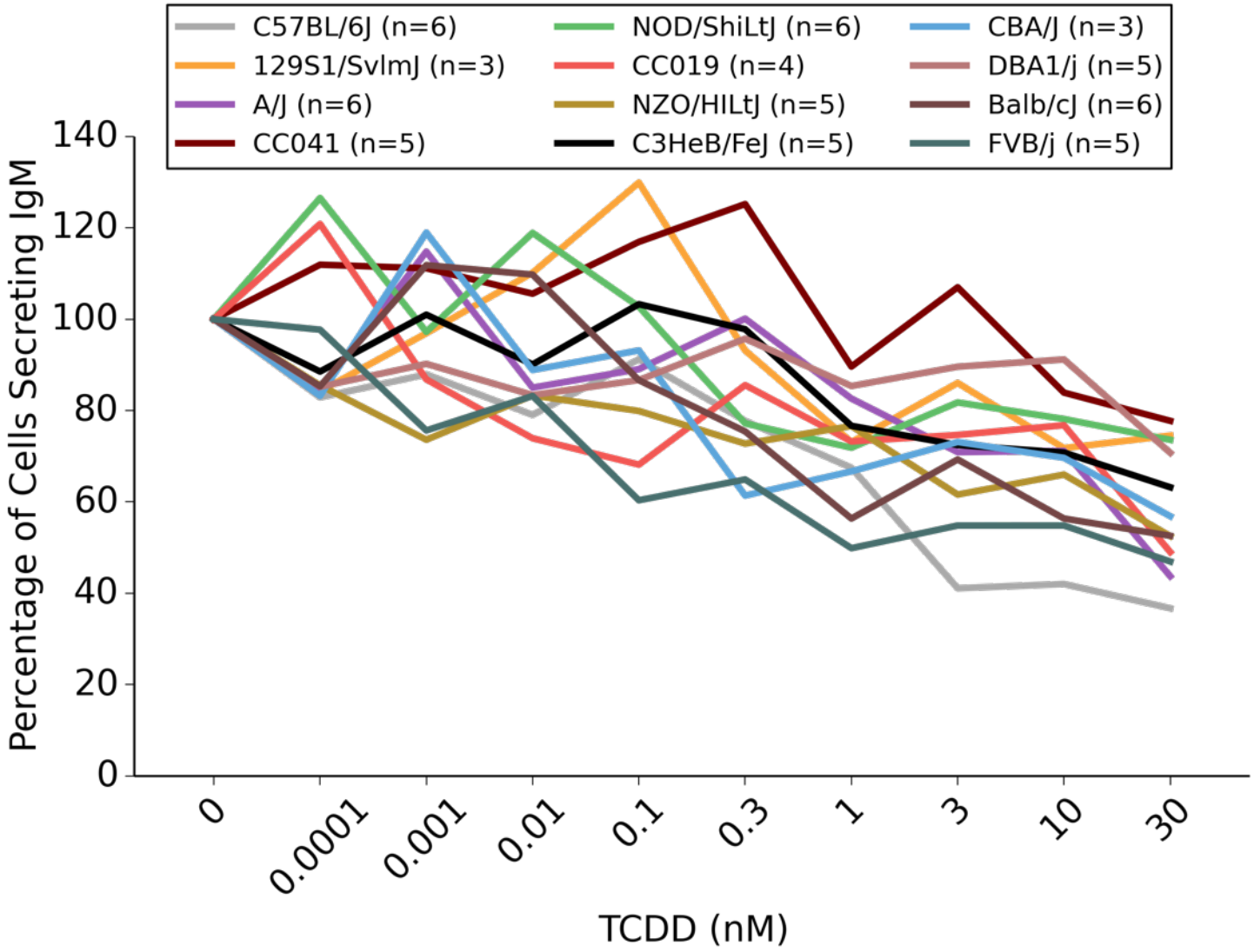
There is a wide-array of responses found within the human population.

Dornbos *et al*., 2016

Hypothesis

Use of a genetically diverse mouse panel will identify genetic variants that modulate TCDD-induced toxicity.

Proof of Principle: mice also respond differently



There is a wide-array of responses found within the a population of mice.

Dornbos *et al.*, *in prep*

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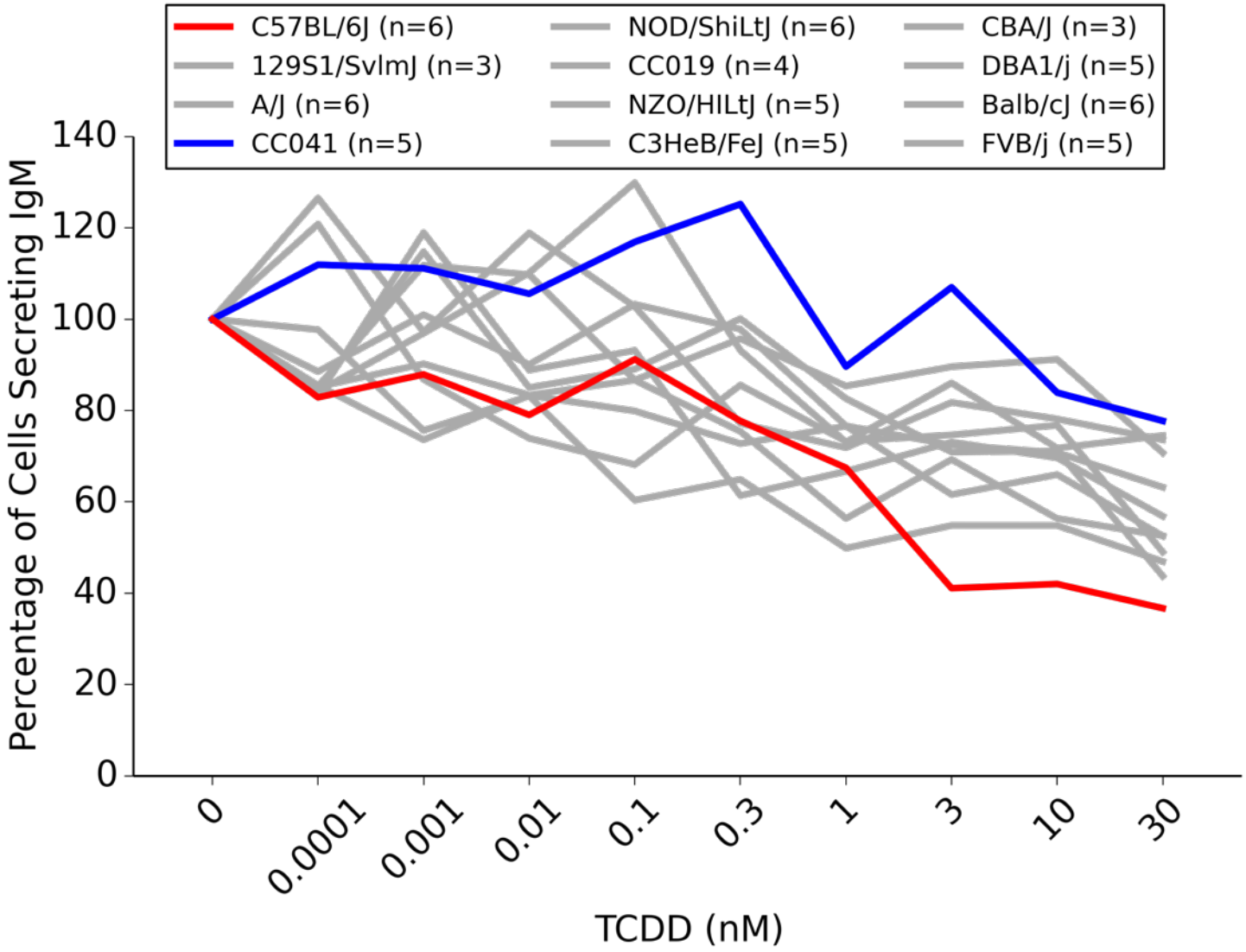

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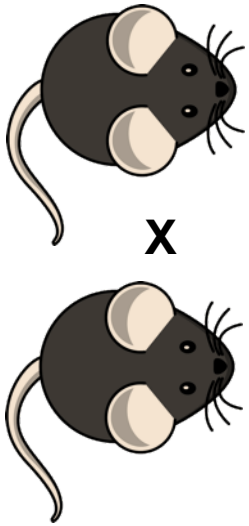
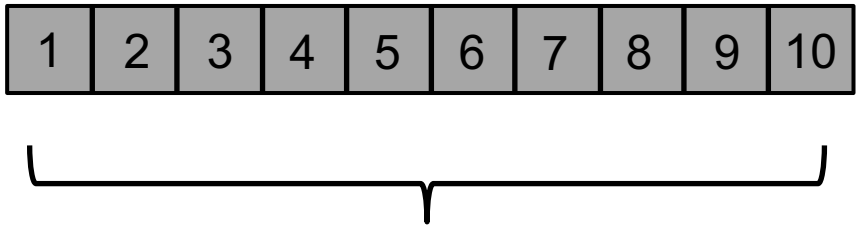
Experimental Pipeline

Biological Model



X

Overnight Mating of 14 genetically diverse strains



10 consecutive days of TCDD dosing

Data

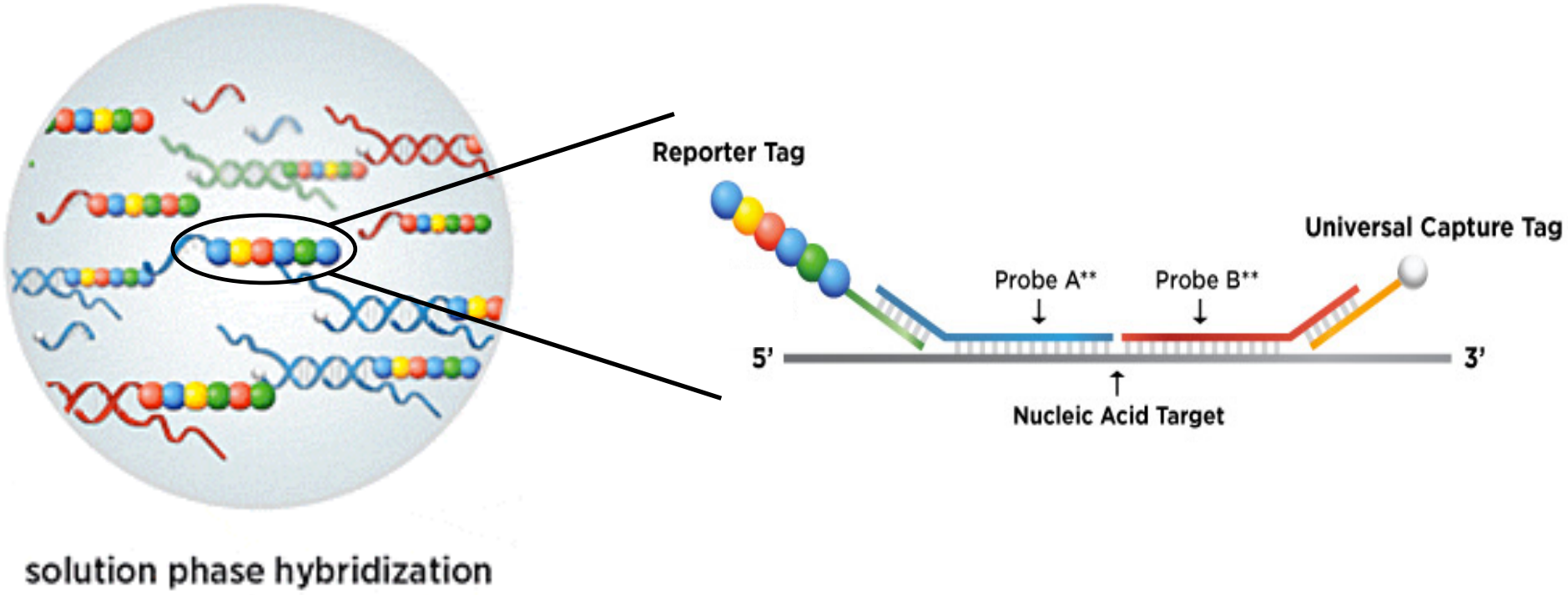
1. Gene expression in the liver
2. Gene expression in the uterus
3. Change in percent body fat

Analysis

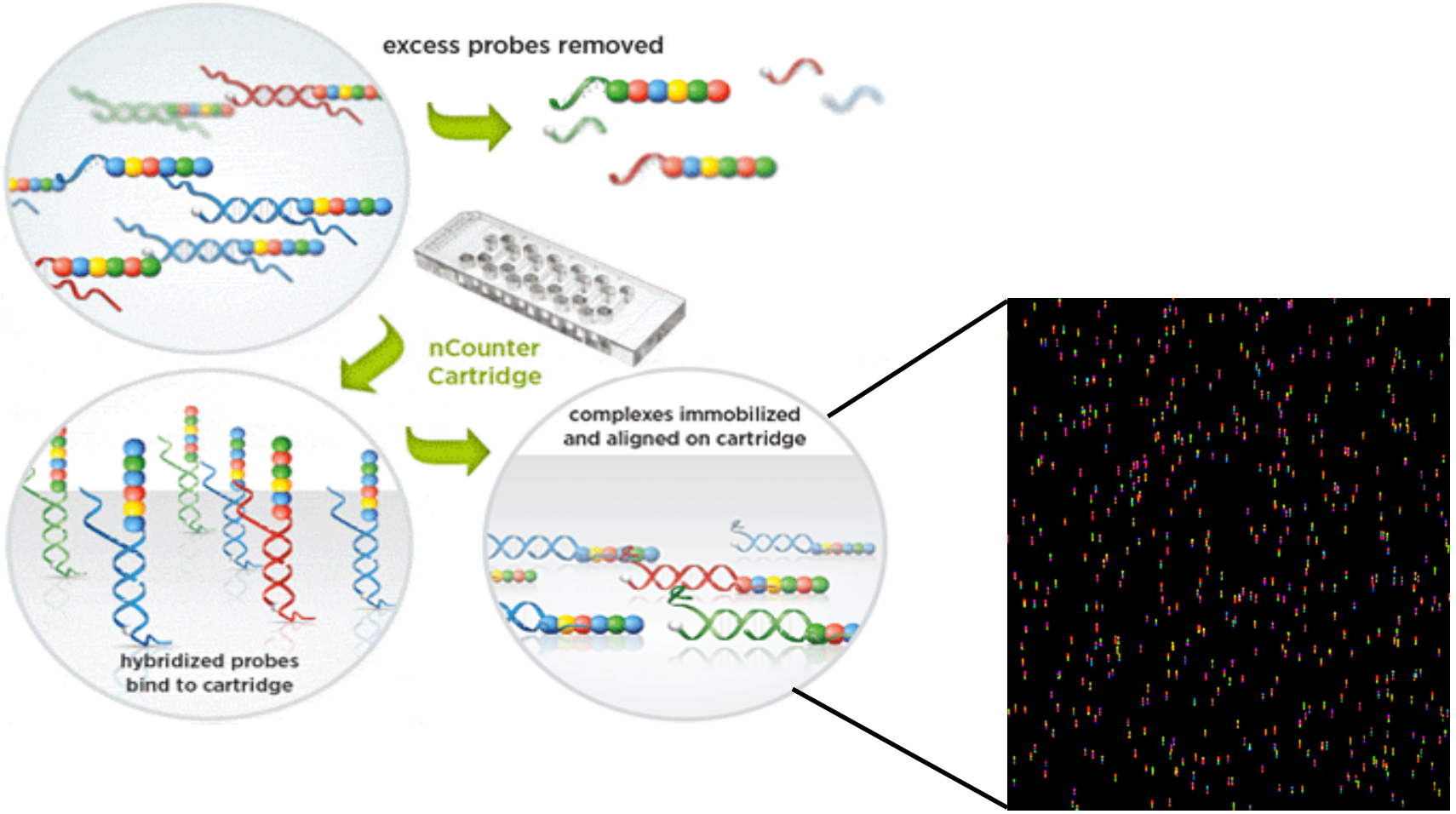
Quantitative Trait Loci Analysis

NanoString nCounter Technology

Step 1: Probe and Target Hybridization



Step 2: Hybridized Probes Bind to Cartridge



Step 3: nCounter Digitizes the Field of Vision and Counts Molecular Barcodes

Barcode	Counts	Identity
	3	XLSA
	2	FOX5
	1	INSULIN

Step 4: Data Analysis with nSolver Software

NanoString nCounter Technology

- Advantages:
  - No enzymes necessary
    - No Reverse Transcriptase
    - No Taq Polymerase
  - High Sensitivity --- < 1 copy of the target DNA/RNA per cell
  - Ability to multiplex up to 800 genes per assay
  - Ability to measure DNA, RNA, and protein in the same, single reaction*
  - Great customer service

- Disadvantages:
  - Closed platform
  - Assay optimization required for each tissue/species of interest
  - May not be cost-effective for lower-numbers of samples
  - High-expressing genes can dwarf low-expressing gene counts
  - Polymorphic target may result in false-negative(s)
    - Nanostring will prepare probes for you*

Cost Differential for ~650 Samples

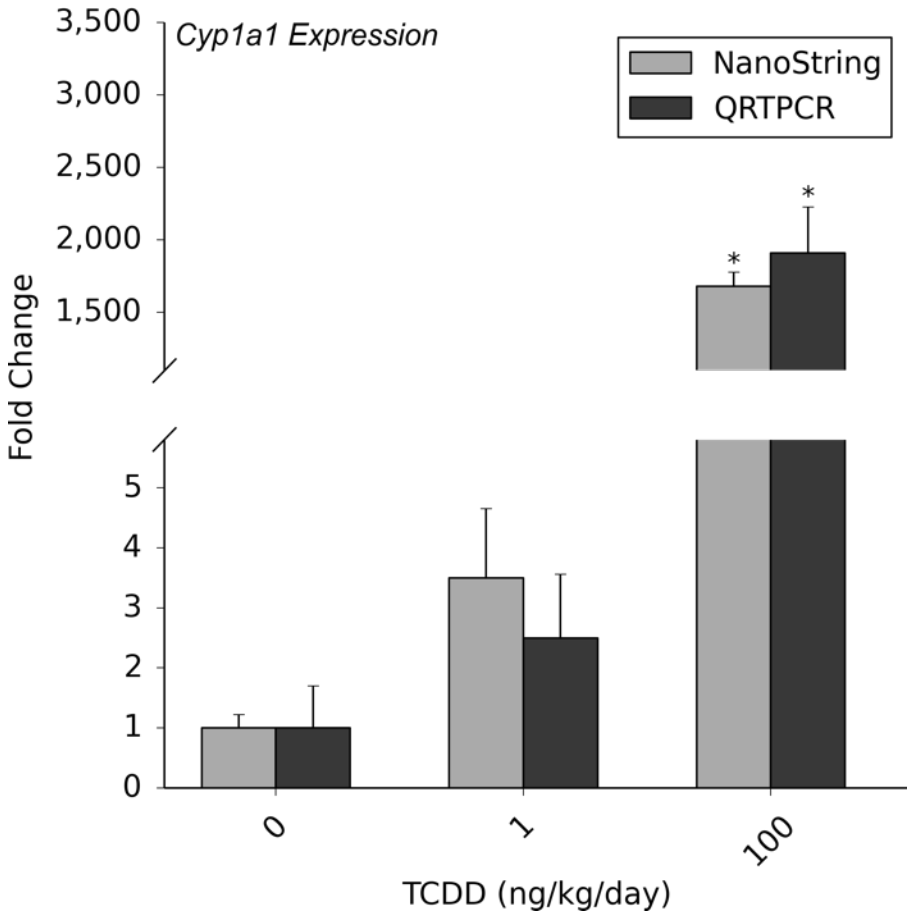
Assay	Component Cost	Consumable Cost	Time	Total Price
TaqMan QRTPCR	$0.74 18s + $0.66 probe + $0.75 Taqman Mastermix =$2.15 / rxn + $0.075 Reverse Transcriptase / rxn	2.25 per plates + 0.65 sealing tape	**140** hours in thermocycler	**$13,192.97**
SYBR Green QRTPCR	Primer is negligible + $0.56 SYBR Mastermix =$1.55/rxn + $0.075 Reverse Transcriptase / rxn	2.25 per plates + 0.65 sealing tape	**285** hours in thermocycler	**$5188.62**
NanoString nCounter (w/ quote)	Included in Final	Included in Final	**~24** hours / plate (TOTAL)	**$4,284.00**
NanoString nCounter (full price)	Included in Final	Included in Final	**~24** hours / plate (TOTAL)	**$8,874.00**

*Not Included: time + cost to quantify quantity and quality of RNA; pipette tip cost, core costs, and time to set-up assays (QRTPCR and nCounter)

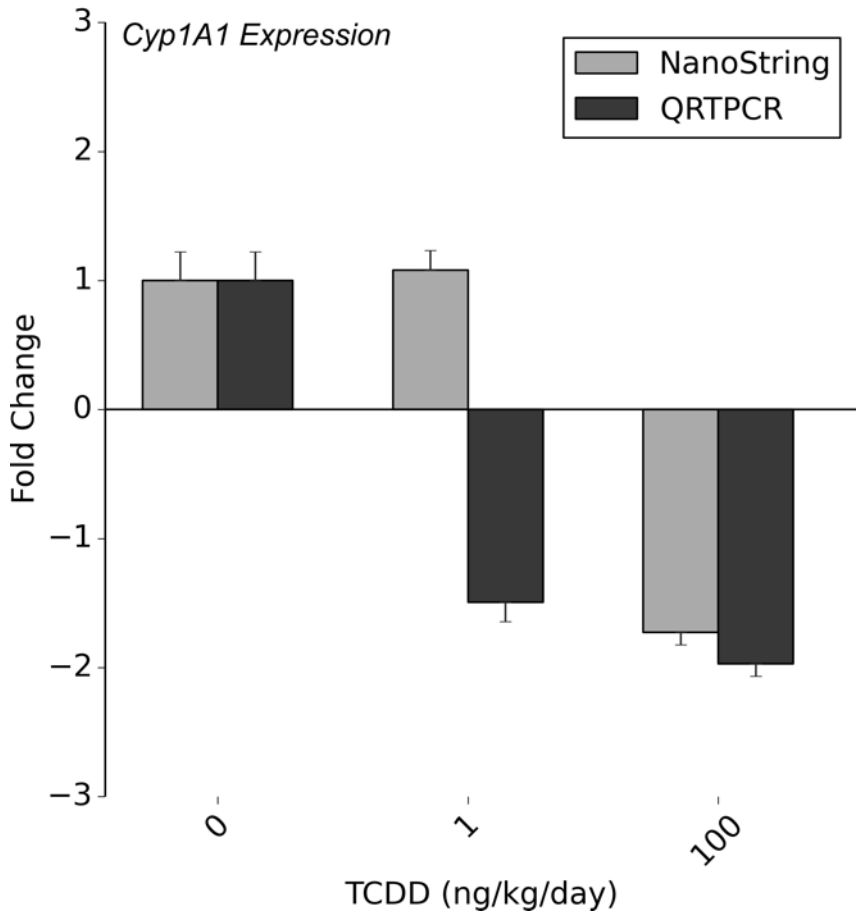
Expression Profiling in the Liver

NanoString nCounter Confirmation with QRTPCR - Liver

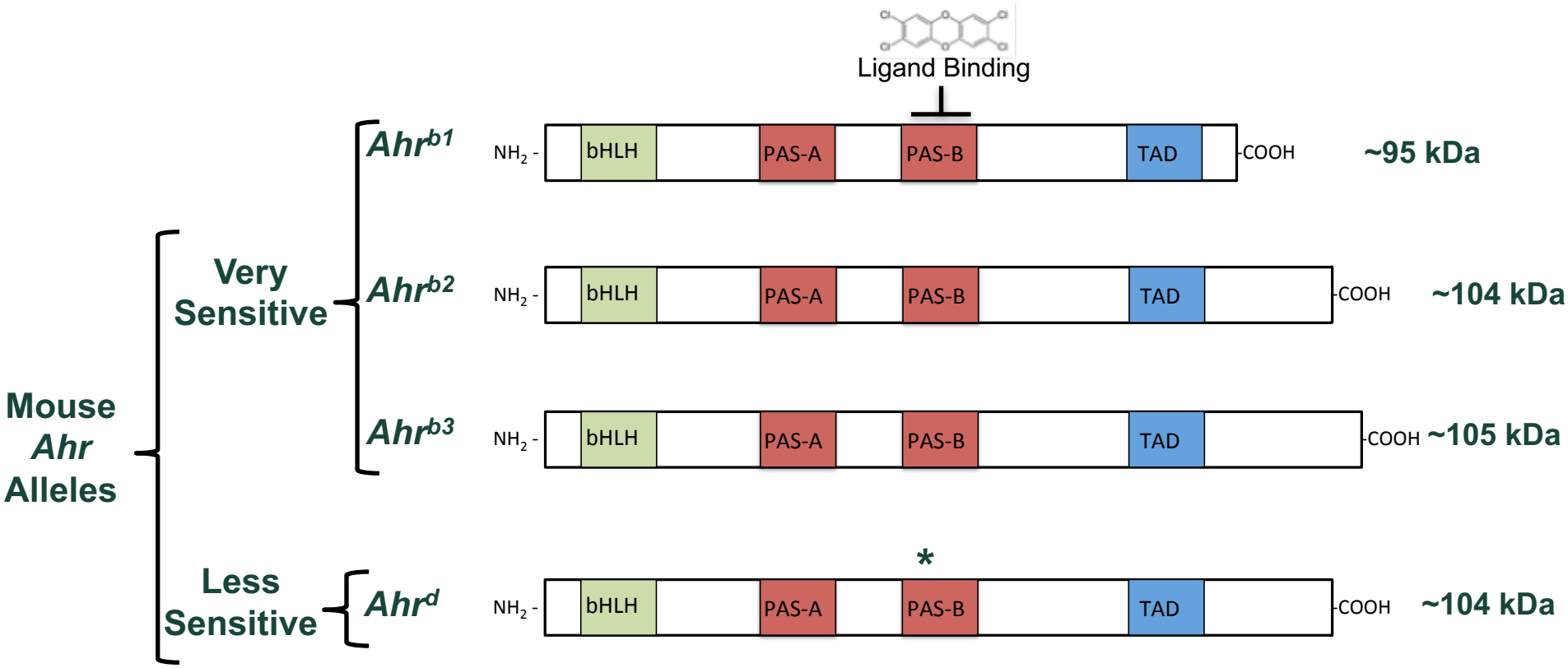
BXD100



NOD/ShilTJ

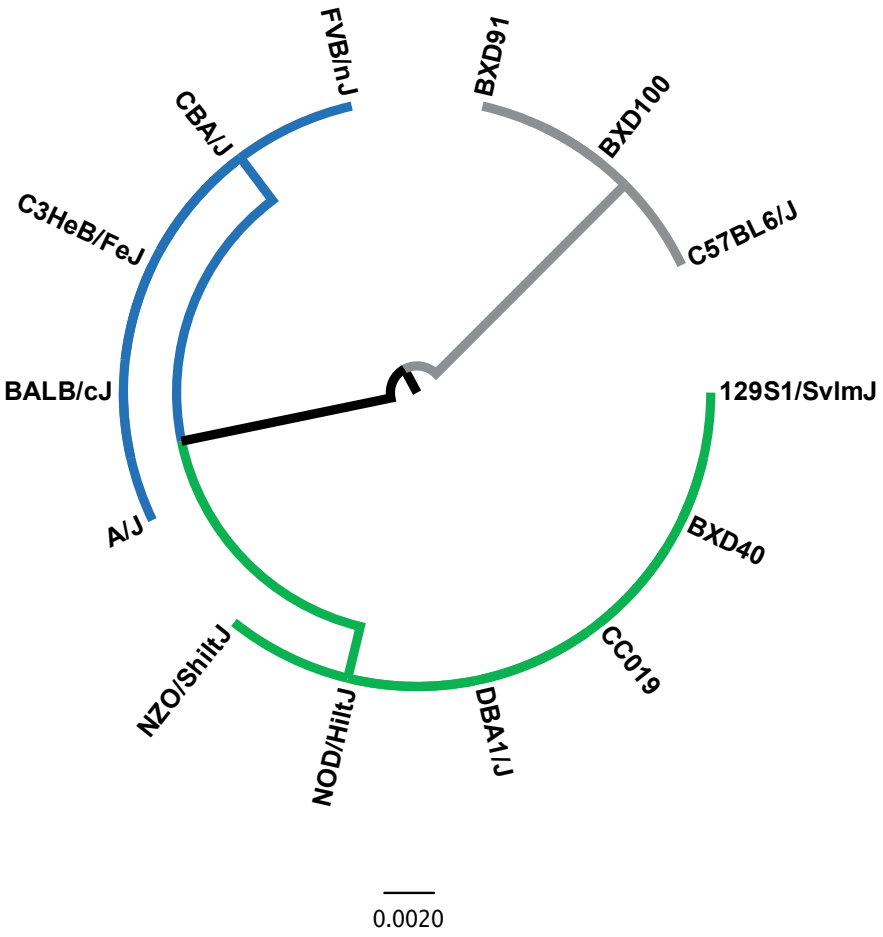


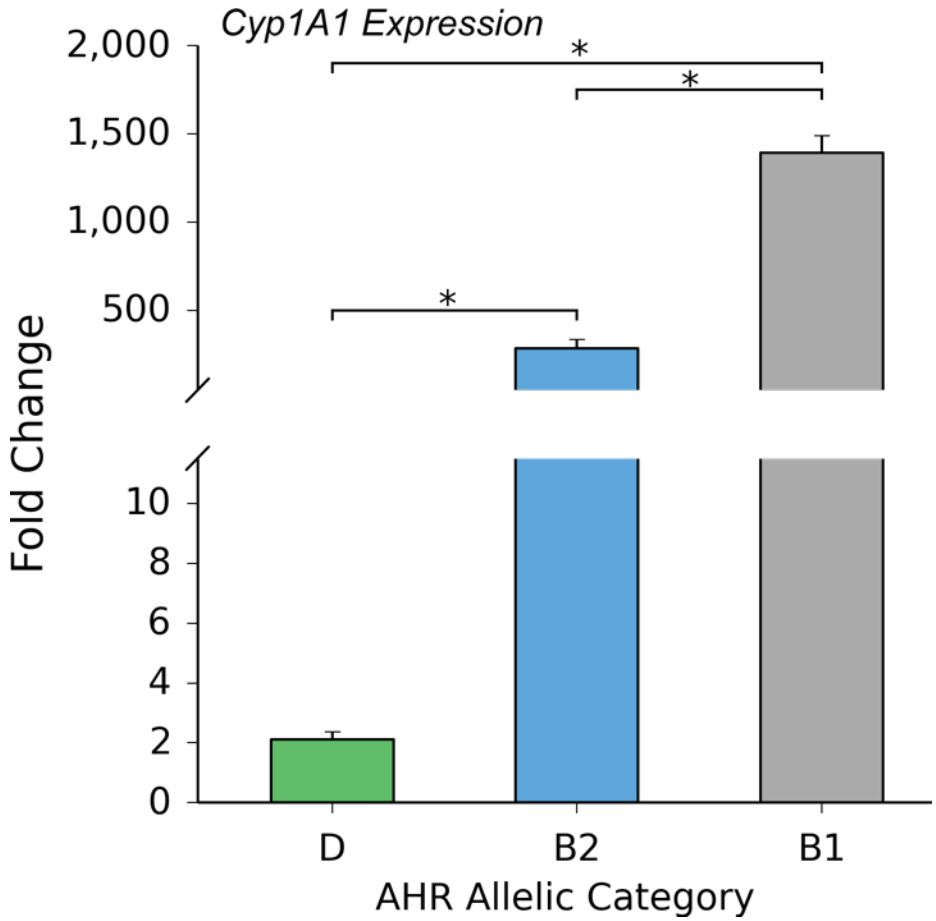
Ahr-dependent Variability in Response to TCDD



Poland et al., 1994, Harper et al., 2002

AHR-mediated *Cyp1a1* Expression in the Liver





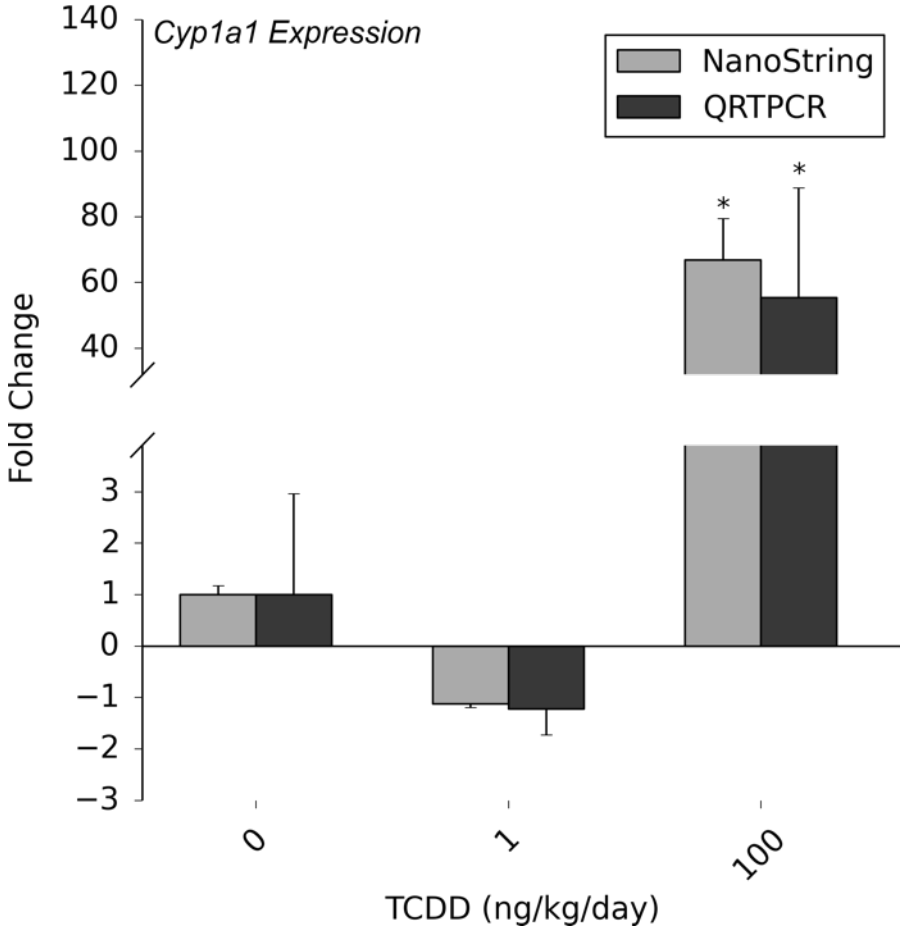
Heat Map of AHR-mediated Liver Expression

AHR Allele	Strain	Dose	*Ahrr*	*Cyp1a1*	*Cyp1a2*	*Cyp1b1*	*Gpnmb*	*Mt2*	*Pmm1*	*SerpinB2*	*Slc15a2*
AHR b1	BXD91	100 ng/kg/day									
	C57Bl6/J	1 ng/kg/day									
		100 ng/kg/day									
	BXD100	1 ng/kg/day									
		100 ng/kg/day									
AHR b2	A/J	1 ng/kg/day									
		100 ng/kg/day									
	Balb/cJ	1 ng/kg/day									
		100 ng/kg/day									
	FVB/nJ	1 ng/kg/day									
		100 ng/kg/day									
	C3Heb/FeJ	1 ng/kg/day									
		100 ng/kg/day									
	CBA/J	1 ng/kg/day									
		100 ng/kg/day									
AHR d	CC019	1 ng/kg/day									
		100 ng/kg/day									
	DBA/1J	1 ng/kg/day									
		100 ng/kg/day									
	BXD40	1 ng/kg/day									
		100 ng/kg/day									
	NOD/ShiLtJ	1 ng/kg/day									
		10 ng/kg/day0									
	NZO/HilTJ	1 ng/kg/day									
		10 ng/kg/day0									
	129S1/SvlmJ	1 ng/kg/day									
		100 ng/kg/day									

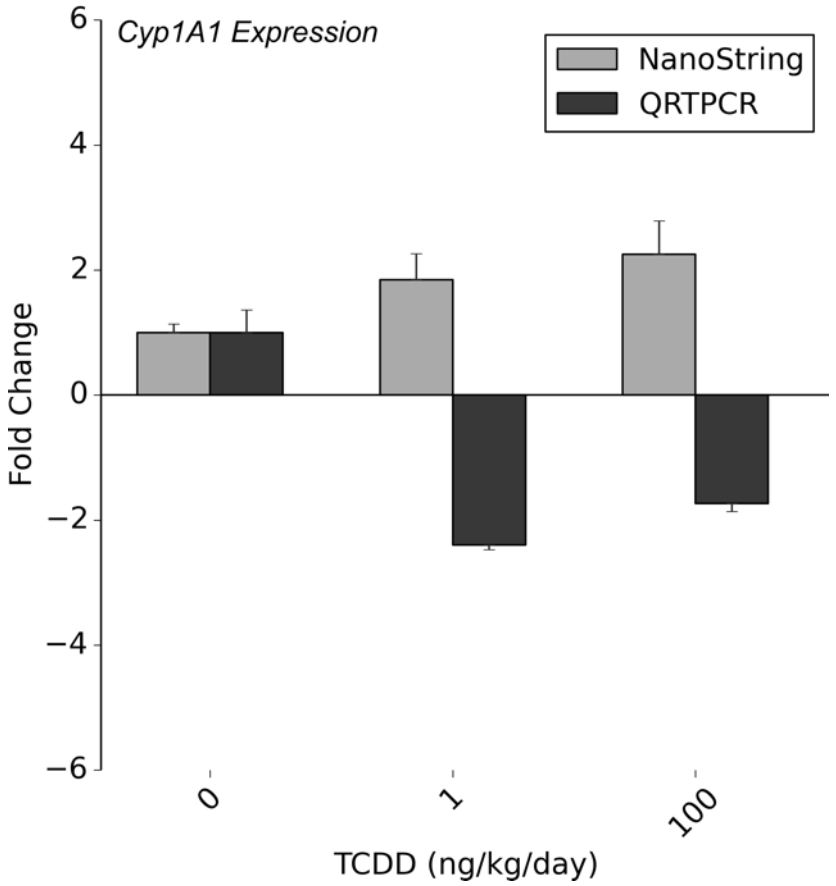
Expression Profiling in the Uterus

NanoString nCounter Confirmation with QRTPCR - Uterus

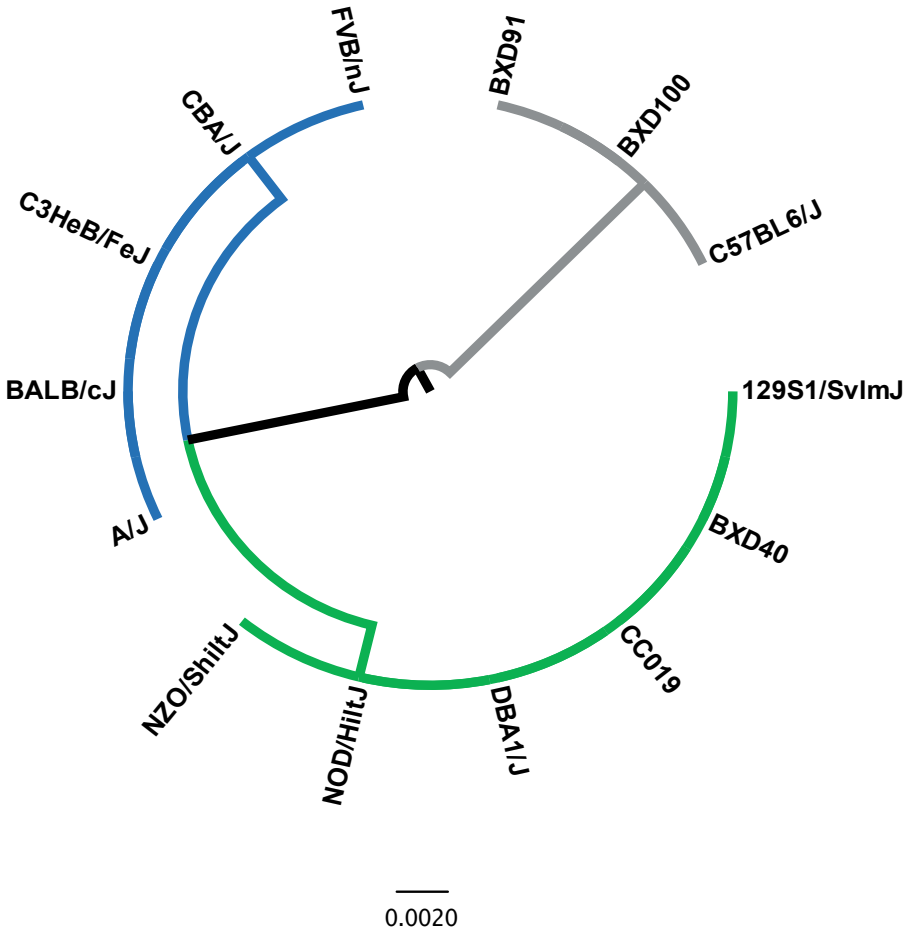
BXD100

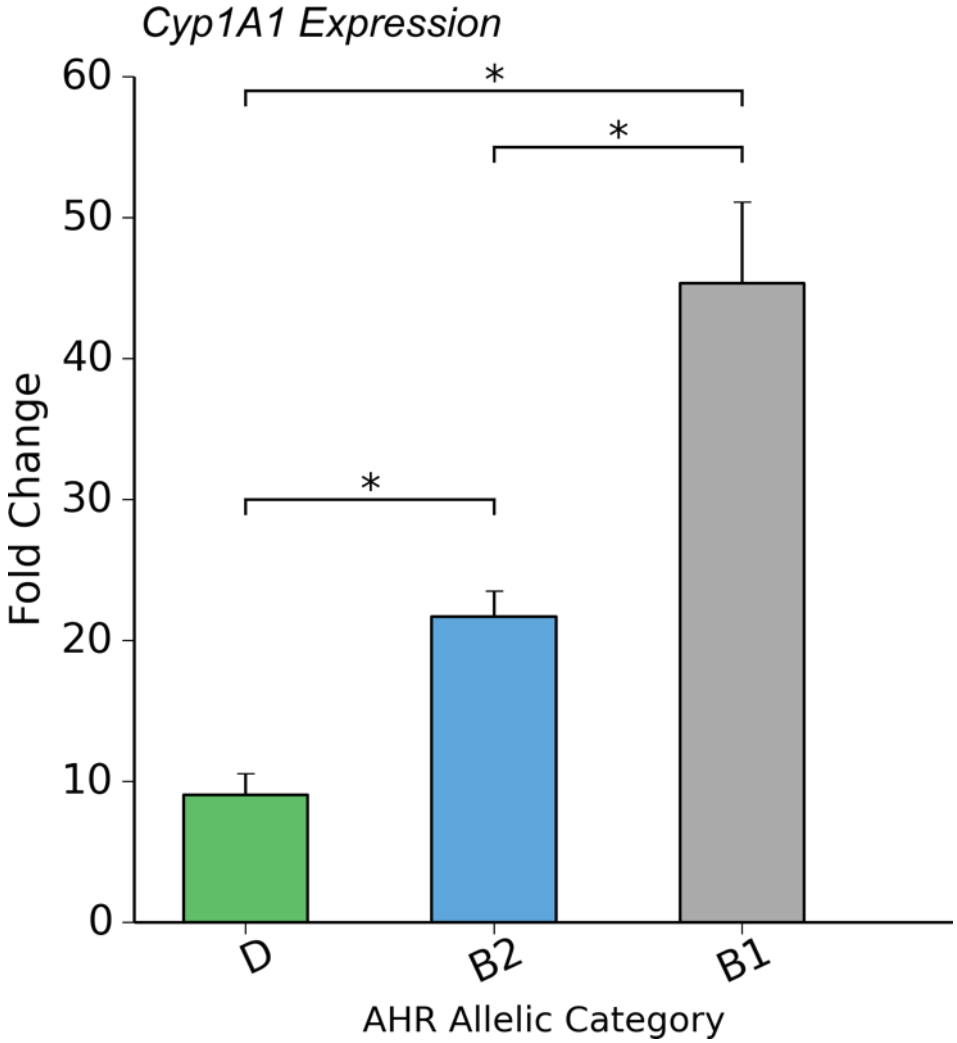


NOD/ShilTJ



AHR-mediated *Cyp1a1* Expression in the Uterus





Heat Map of AHR-mediated Uterus Expression

AHR Allele	Strain	Dose	*Ahrr*	*Cyp1a1*	*Cyp1a2*	*Cyp1b1*	*Gpnmb*	*Mt2*	*Pmm1*	*SerpinB2*	*Slc15a2*
AHR b1	BXD91	100 ng/kg/day									
	C57Bl6/J	1 ng/kg/day									
		100 ng/kg/day									
	BXD100	1 ng/kg/day									
		100 ng/kg/day									
AHR b2	A/J	1 ng/kg/day									
		100 ng/kg/day									
	Balb/cJ	1 ng/kg/day									
		100 ng/kg/day									
	FVB/nJ	1 ng/kg/day									
		100 ng/kg/day									
	C3Heb/FeJ	1 ng/kg/day									
		100 ng/kg/day									
	CBA/J	1 ng/kg/day									
		100 ng/kg/day									
AHR d	CC019	1 ng/kg/day									
		100 ng/kg/day									
	DBA/1J	1 ng/kg/day									
		100 ng/kg/day									
	BXD40	1 ng/kg/day									
		100 ng/kg/day									
	NOD/ShiLtJ	1 ng/kg/day									
		10 ng/kg/day0									
	NZO/HilTJ	1 ng/kg/day									
		10 ng/kg/day0									
	129S1/SvlmJ	1 ng/kg/day									
		100 ng/kg/day									

Questions?