

Prediction of *liver toxicity* in the  
animal study using the mechanistically  
relevant *in vitro* screening assay data

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# Drug Induced Liver Injury (DILI)

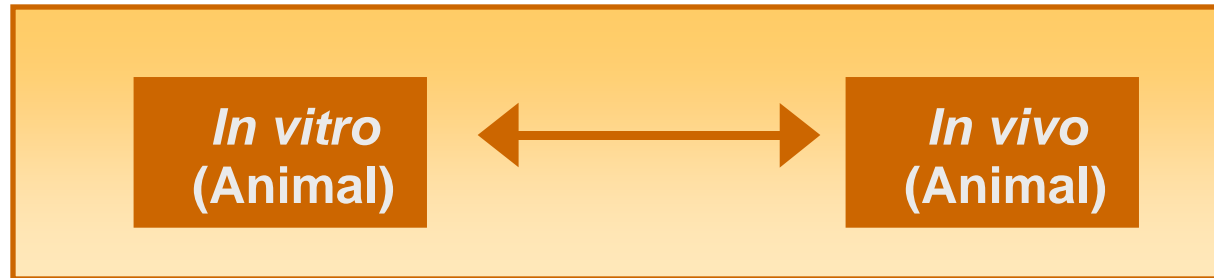
- Only 20% of patients with acute liver failure because of DILI survive with supportive care
- 1% patients develops DILI during the course of hospitalization
- DILI is the single most frequent reason for drug withdraw and “black-box” warning
  - DILI has been linked to nearly 1000 drugs
- Acetaminophen accounts for 46% incidences of acute liver failure
  - Trend: 28% in 1998 to 51% in 2003
- DILI is more common in females for reason unclear
  - Women accounts for 79% of APAP-related DILI and 73% of idiosyncratic drug reaction

Larson et al. Hepatology 2005, 42:1364-1372

Ostapowicz and Lee, J. Gast – Roenterol Hepatol 2000, 15: 480-488

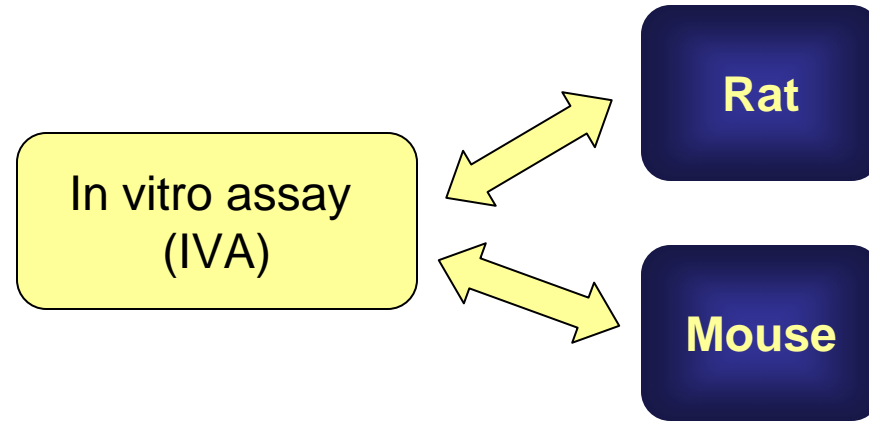
Ostapowicz et al. JMAM 1998: 279:1200-1205

# ToxCast Data



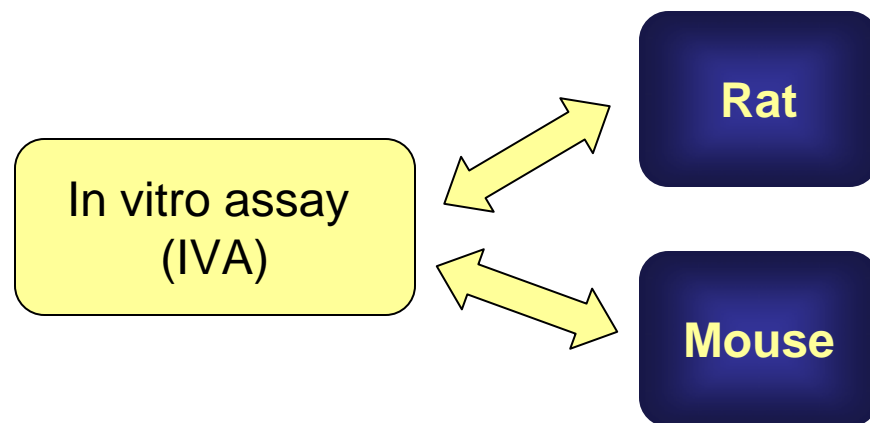
- Two types of data
  - *In vitro* assay (IVA) data
  - Calculated descriptors
- Liver tox endpoints for both rat and mouse
  - Hypertrophy
  - Necrosis
  - Proliferation Lesions
  - Tumors

# Study Design and Objectives



- Comparative analysis between the rat and mouse models
  - The performance of the rat models vs mouse models for each endpoints
  - Which IVAs contribute most to these models
  - Are the same features used in both species

# The Choice of Machine Learning



- Soft Independent Modeling of Chemical Analogy (SIMCA)
- Artificial Neural Network (ANN)
- K-Nearest Neighbor (KNN)
- Decision Tree
- Support Vector Machines (SVMs)
- Fisher's Linear Discriminant (FLD)

# The FDA MicroArray Quality Control (MAQC) Project

Feb 2005

MAQC-I

17 months

- MAQC-I: Technical Performance
  - Reliability of microarray technology
  - Cross-platform consistency
  - Reproducibility of microarray results
  - 137 participants from 51 organizations

Sept 2006

MAQC-II

30 months

- MAQC-II: Practical Application
  - Reliability and robustness of gene expression based molecular signatures for risk assessment and clinical application
  - Cross-platform consistency and reproducibility
  - >200 participants from >50 organizations
  - 21 manuscripts were submitted to Nat Biotech

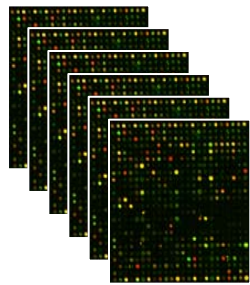


Mar 2009

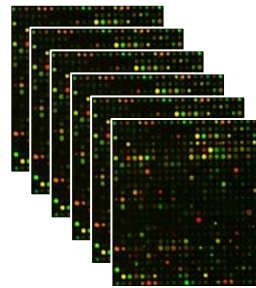
# MAQC Strategy

## Training

Patients

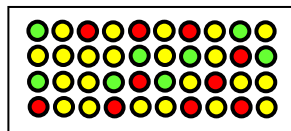


Healthy individuals



Machine Learning

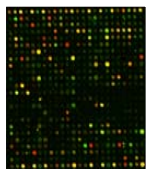
$$Y = f(X)$$



Disease signature

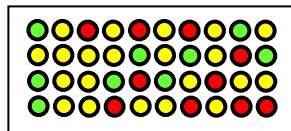
36 teams worked on the MAQC datasets with freedom of choice of machine learning methods

## Validation



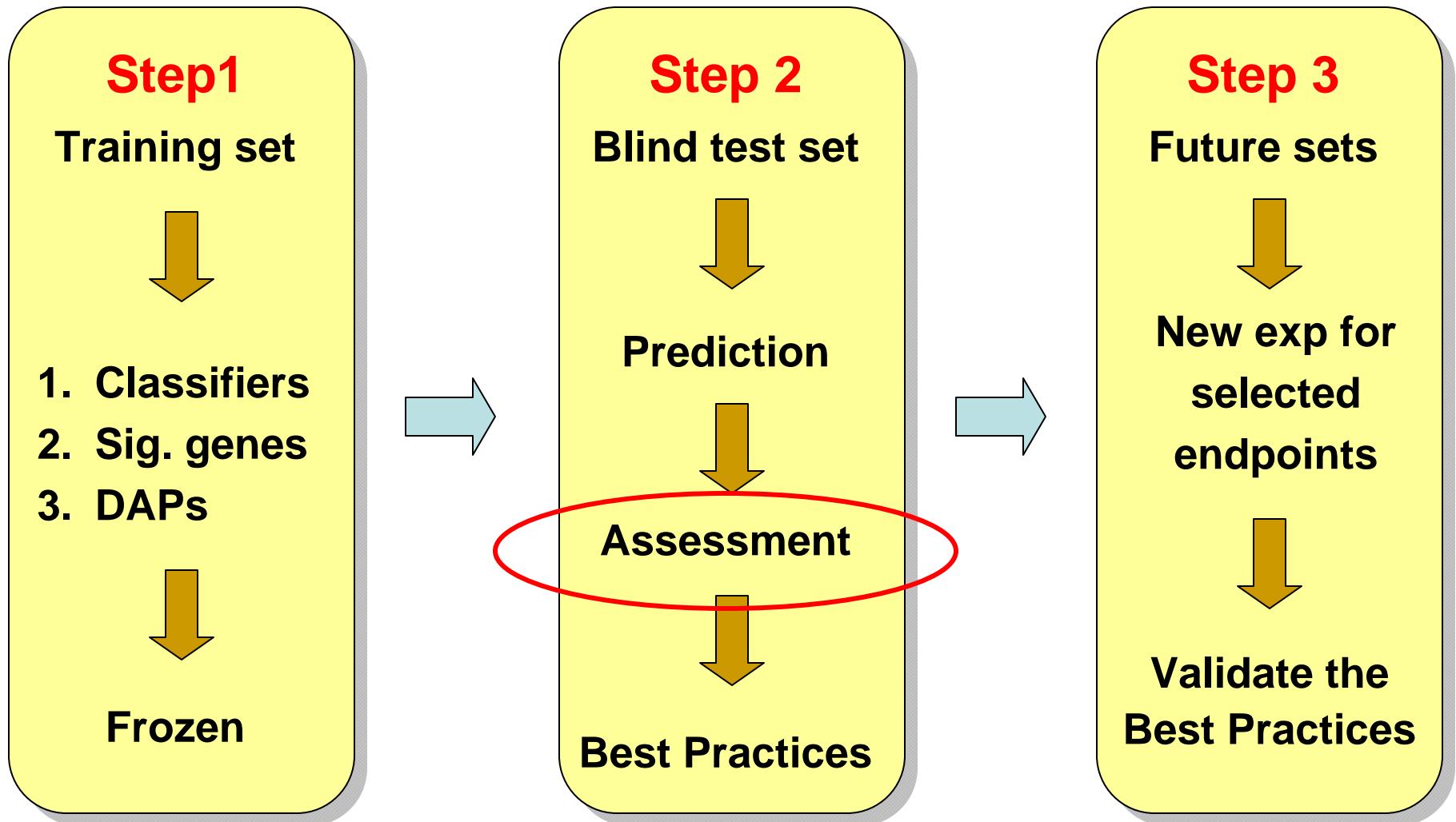
New Patient

$$Y = f(X)$$

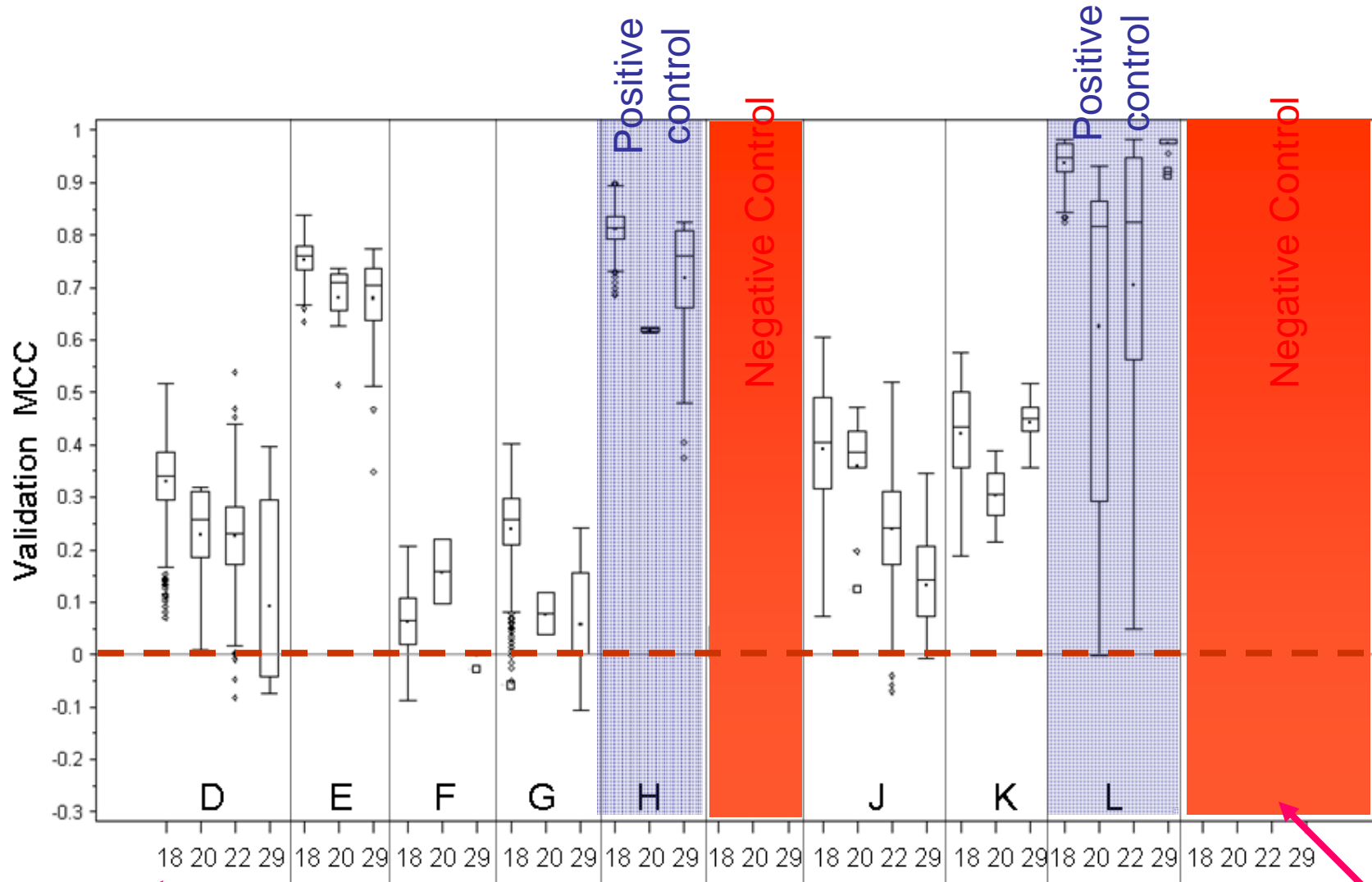


Emphasis on validation

# Three-Step Approach



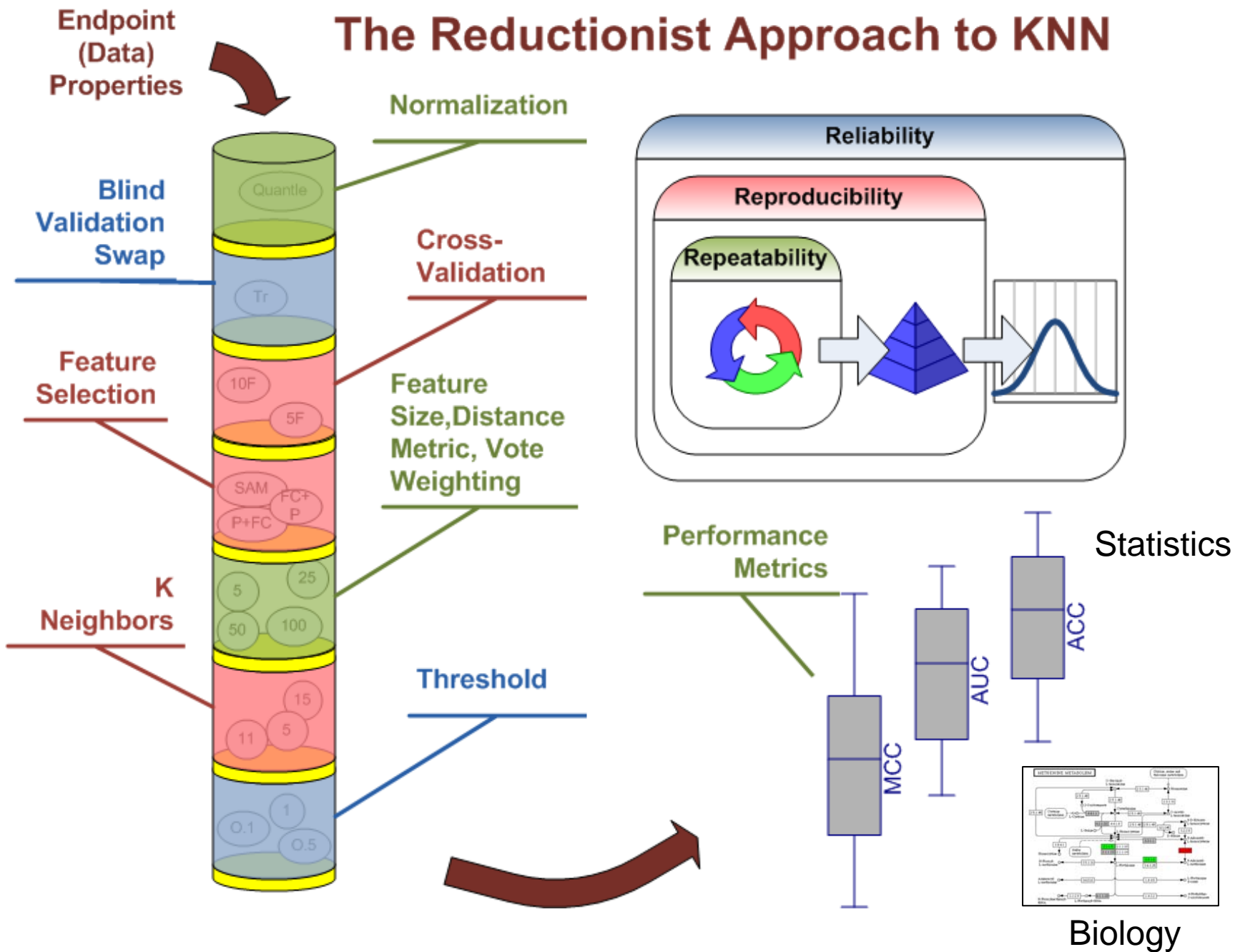
# MAQC-II KNN Results



Analysis Teams

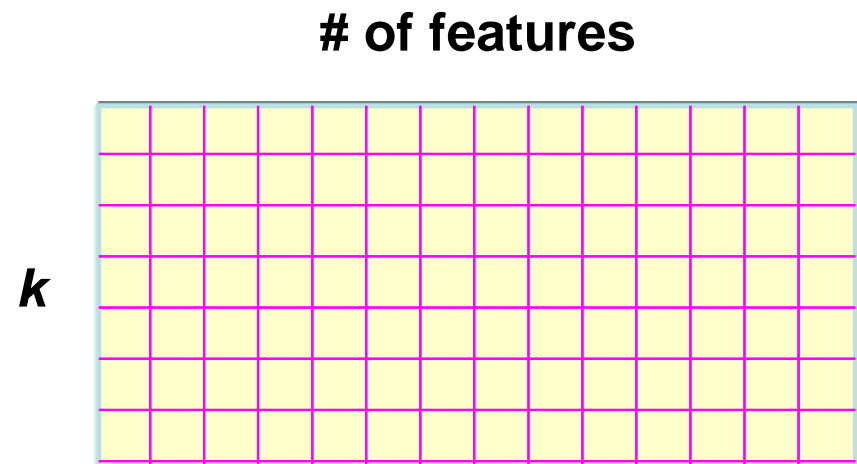
Endpoints

# The Reductionist Approach to KNN

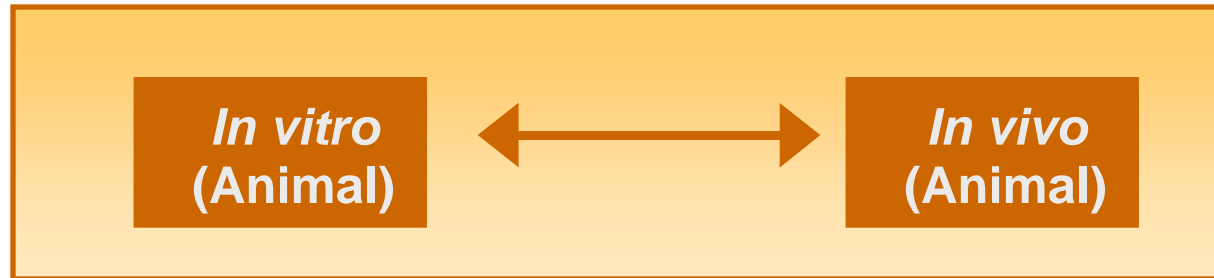


# Lessons Learned From MAQC-II for KNN Through Systematic Evaluation

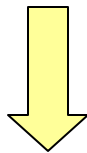
- Why Team18 outperformed other teams:
  - Balance the sample classes
  - Batch correction
  - Systematic exploring the  $k$ -feature space



# Two Key Data Preprocessing



- Two types of data
  - *In vitro* assay data (binary)
  - Calculated descriptors (continuous)



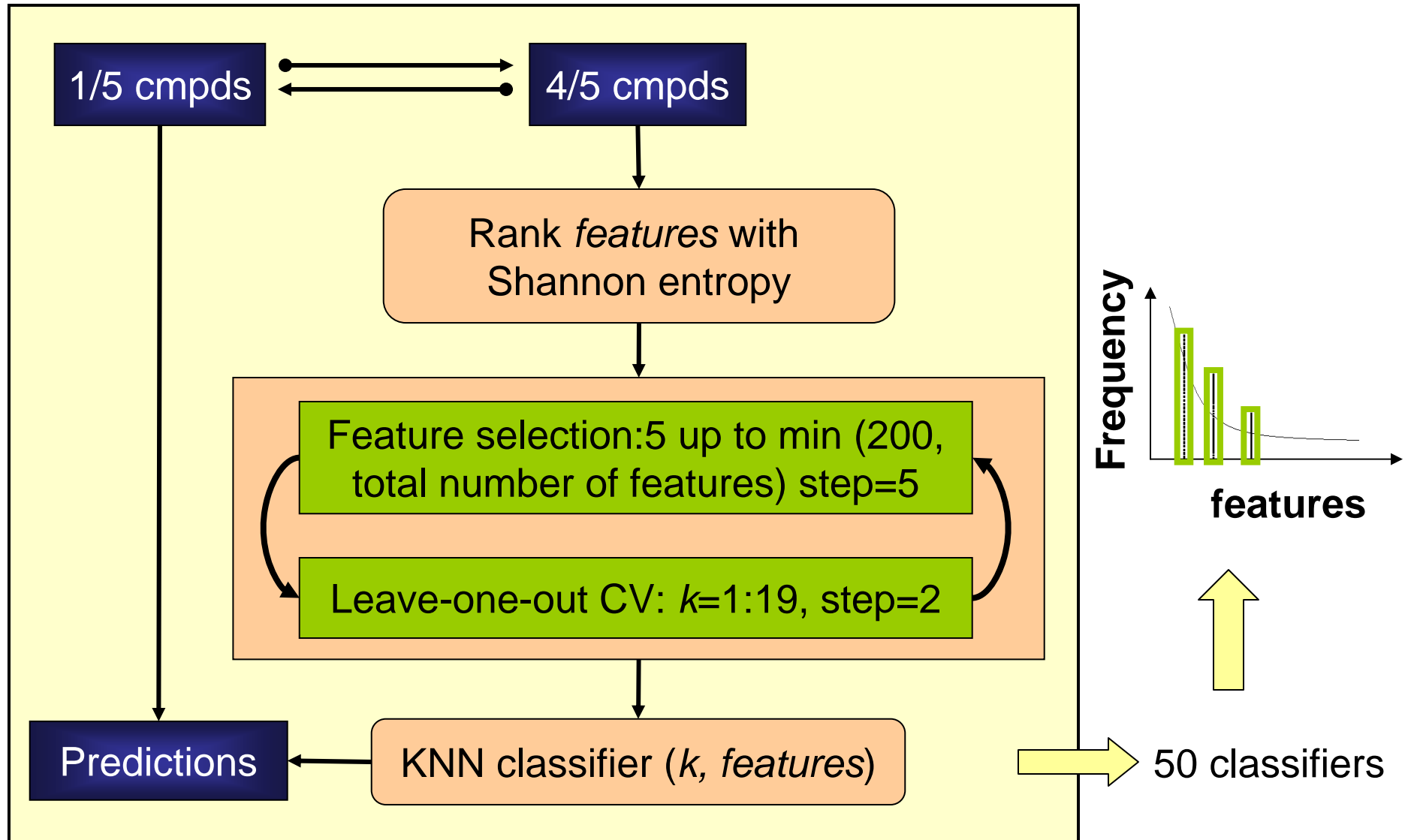
Remove the “batch effect” by only including IVA

- Liver tox endpoints for both rat and mouse
  - Hypertrophy
  - Necrosis
  - Proliferation Lesions
  - Tumors

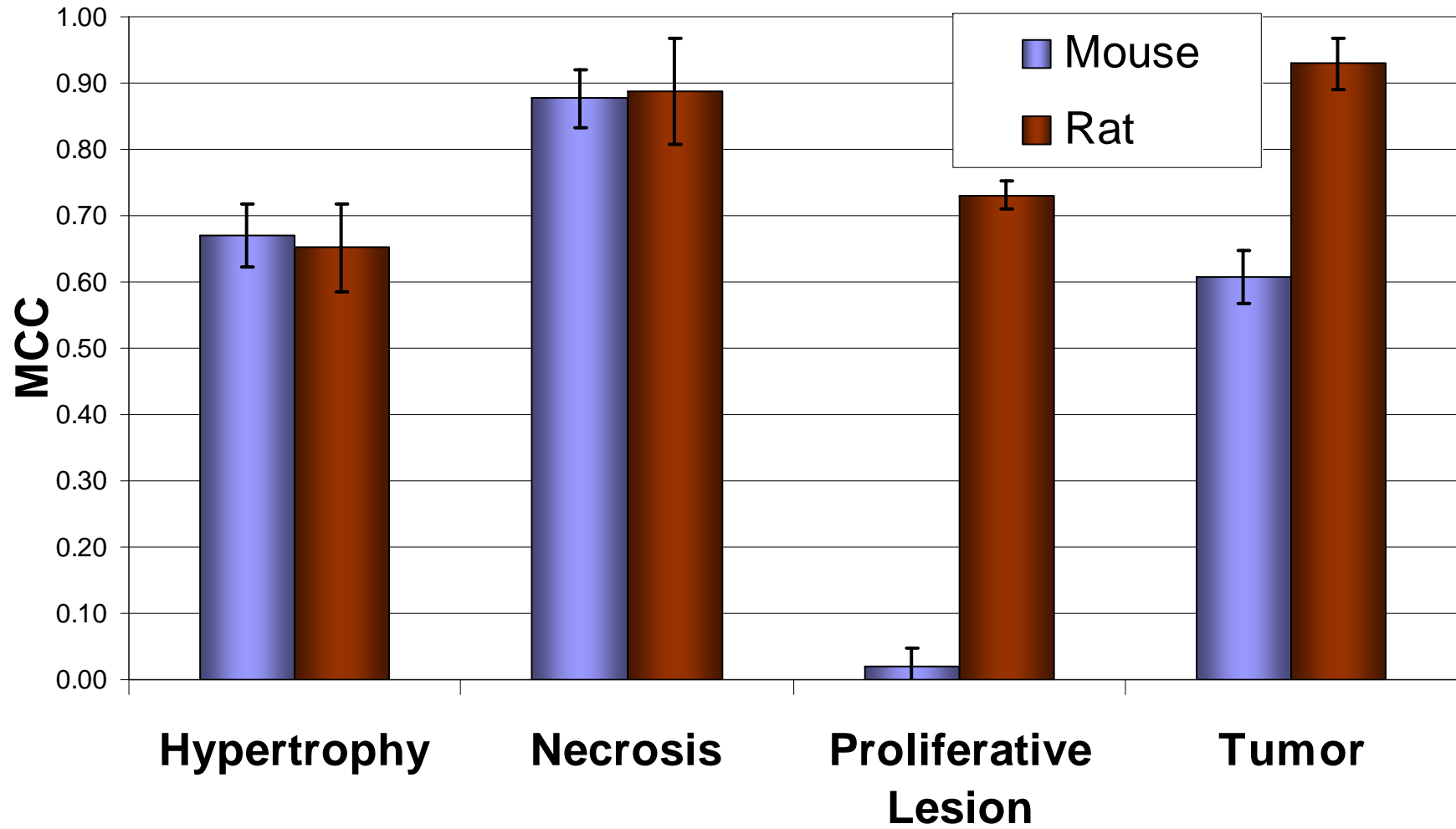


Over-sample the minority class by multiplying its sample size to approximately match the number of samples of the majority class

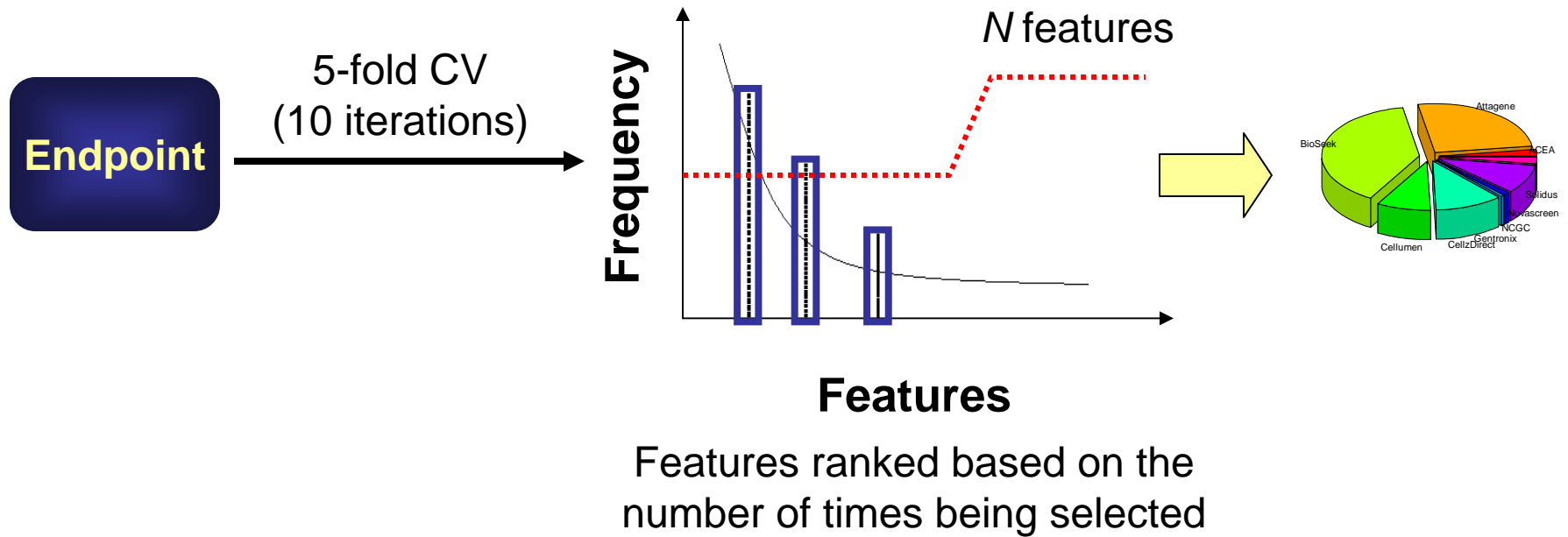
# 5-Fold Cross-Validation (10 iterations)



# 5-Fold Cross-Validation Results (MCC)



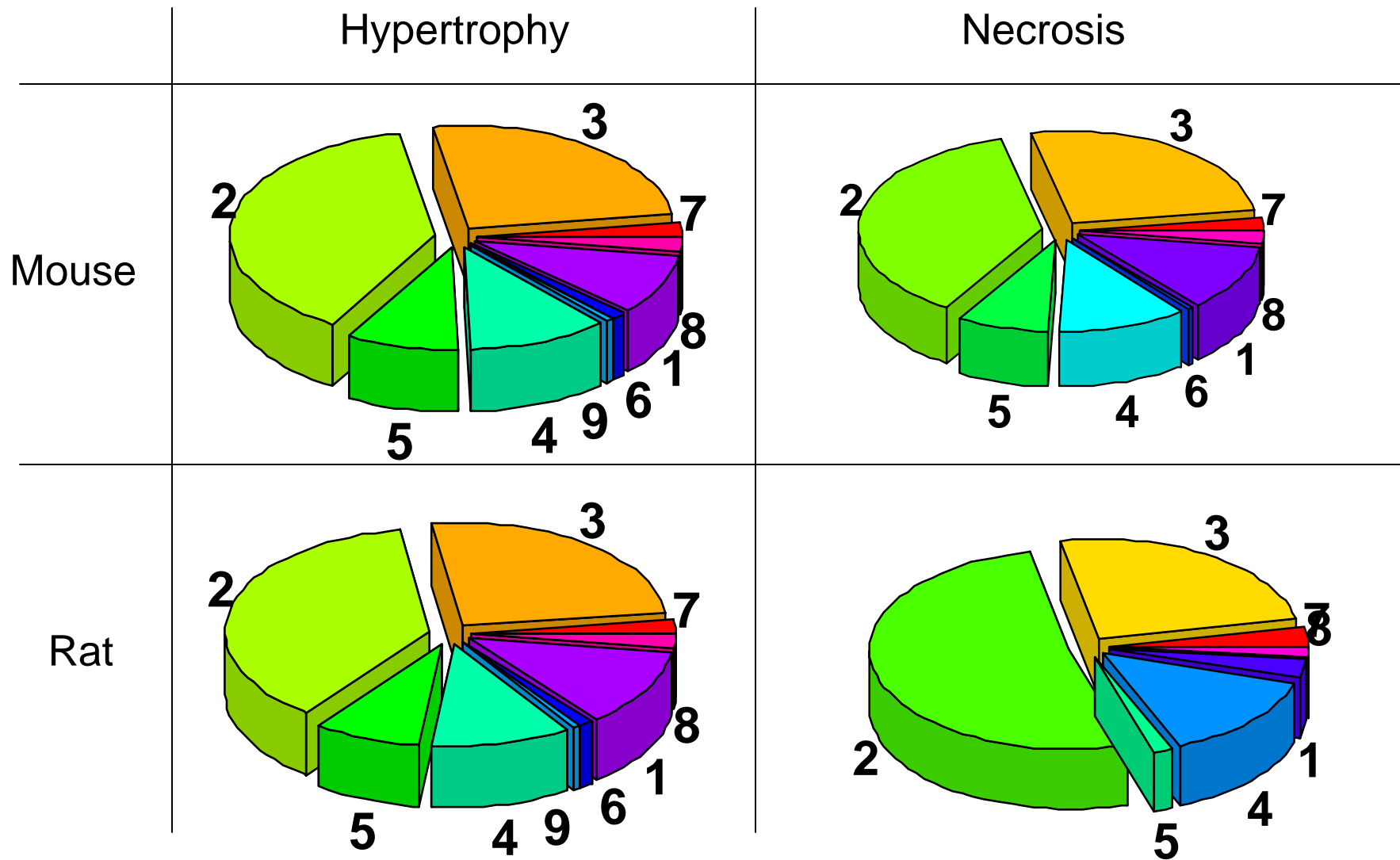
# Which IVAs Contribute Most to The Predictive Models?



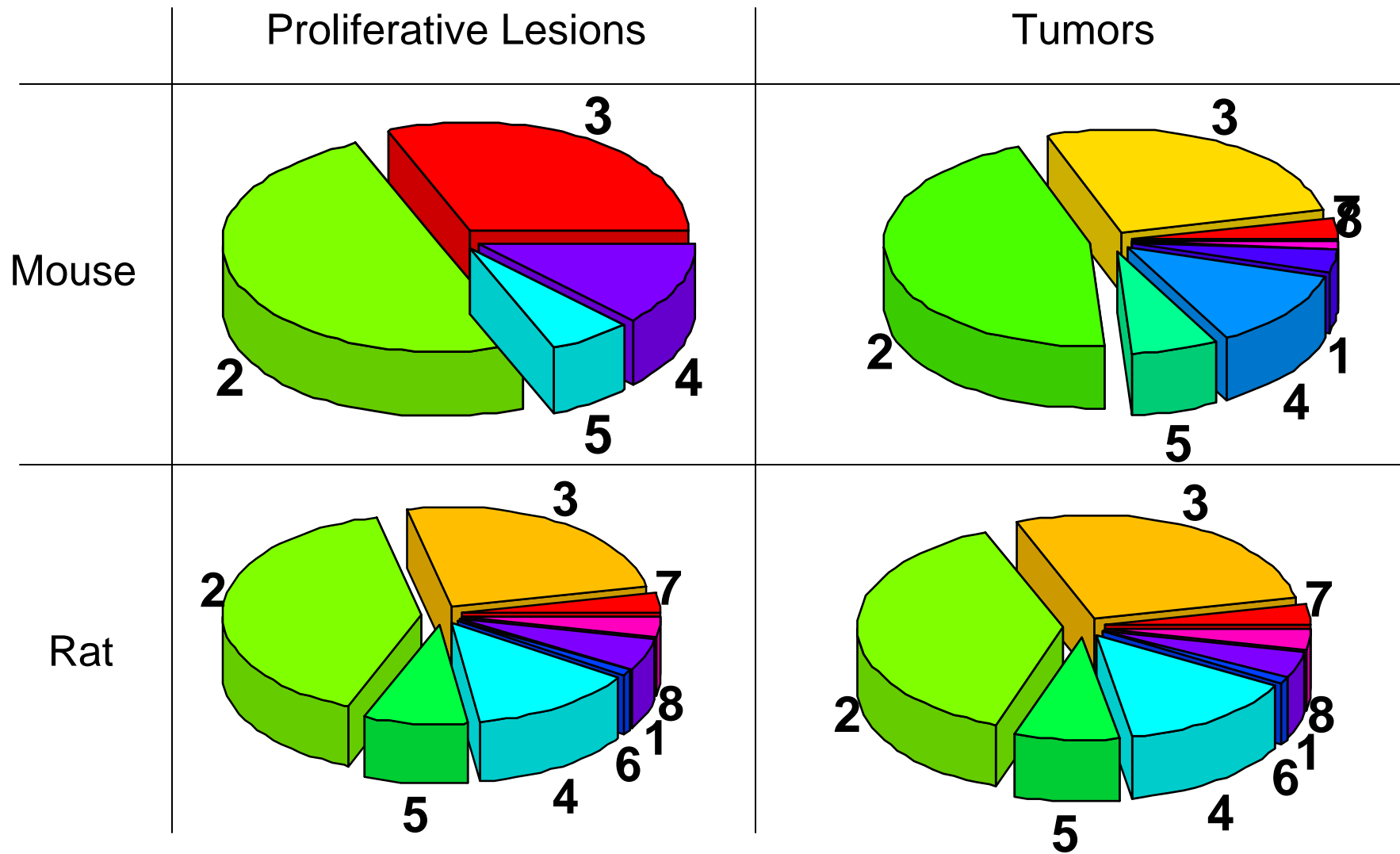
# *In Vitro* Assays (IVAs)

	<b>Assays</b>	<b># of features</b>
1	Novascreen	239
2	BioSeek	87
3	Attagene	81
4	CellzDirect	48
5	Cellumen	33
6	NCGC	24
7	ACEA	7
8	Solidus	4
9	Gentronix	1
	<b>sum</b>	<b>524</b>

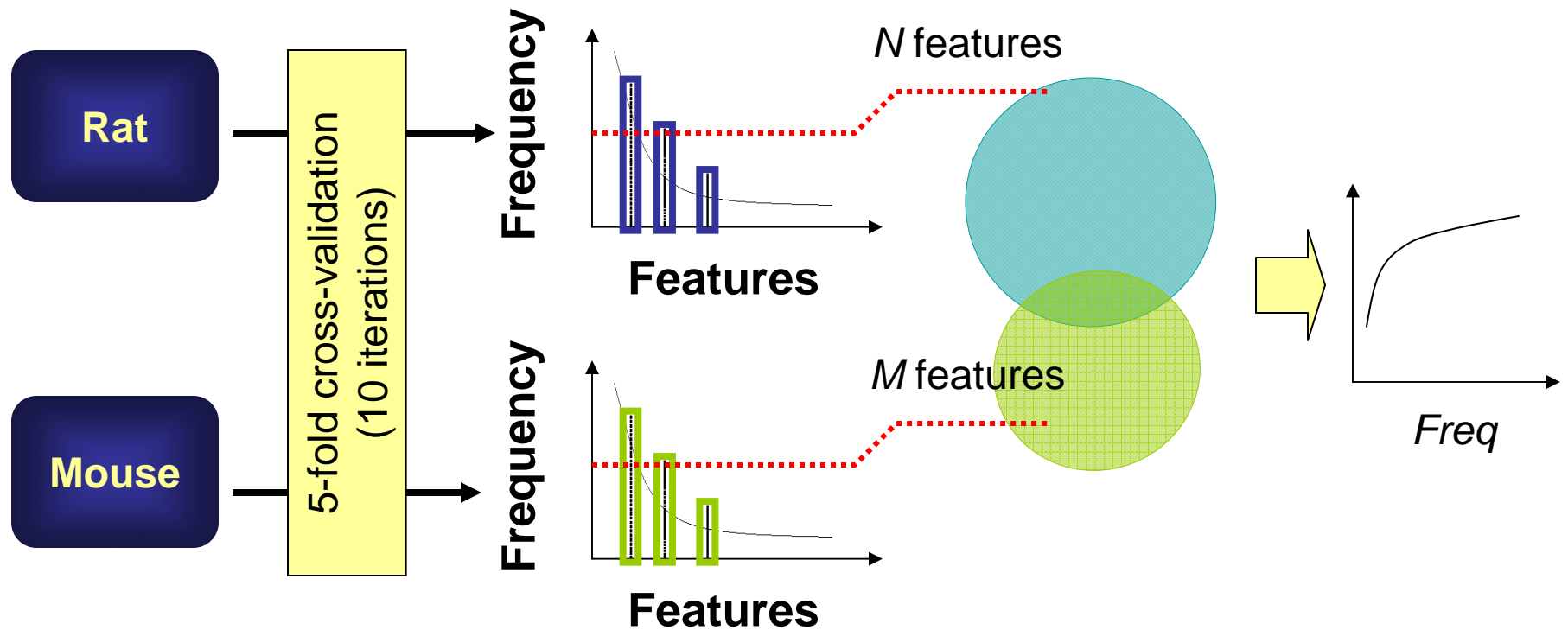
# Distribution of IVA Features (Freq $\geq 40$ ) - Rat vs Mouse



# Distribution of IVA Features (Freq $\geq 40$ ) - Rat vs Mouse

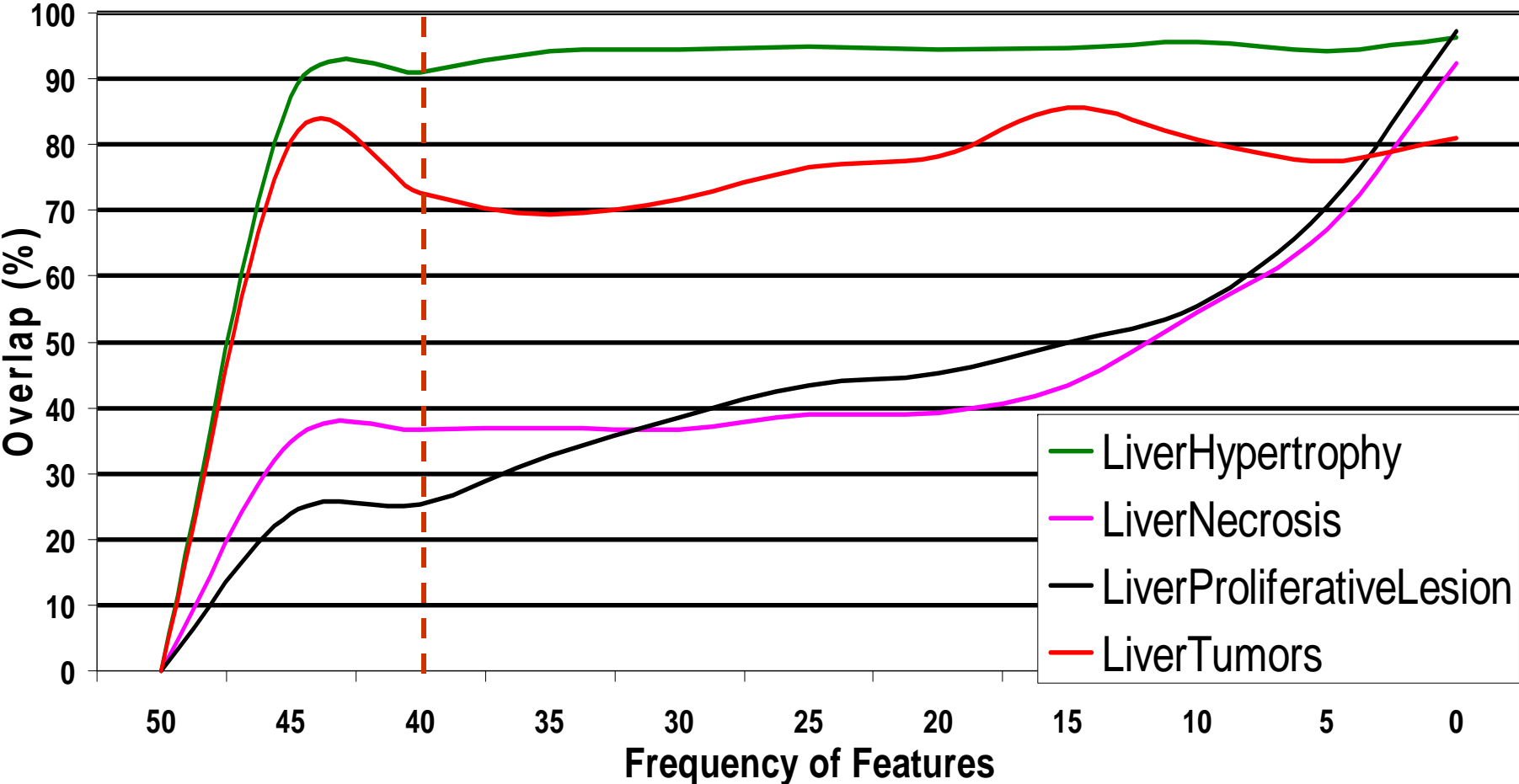


# How much overlap in feature between the rat and mouse models

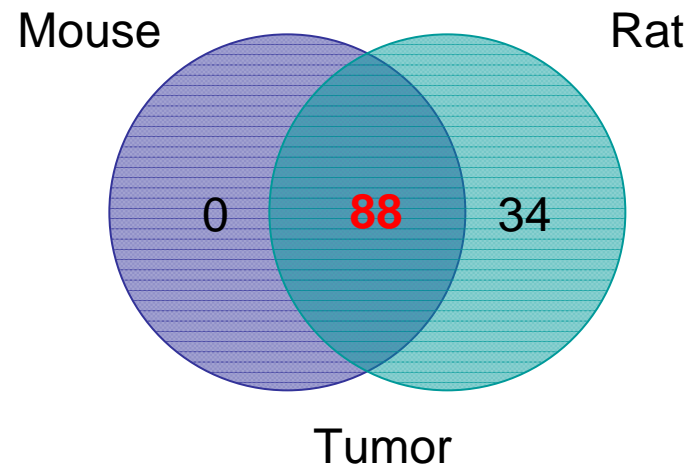
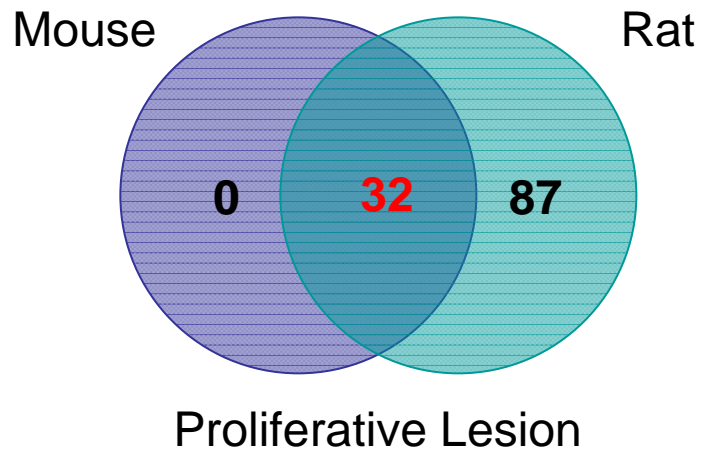
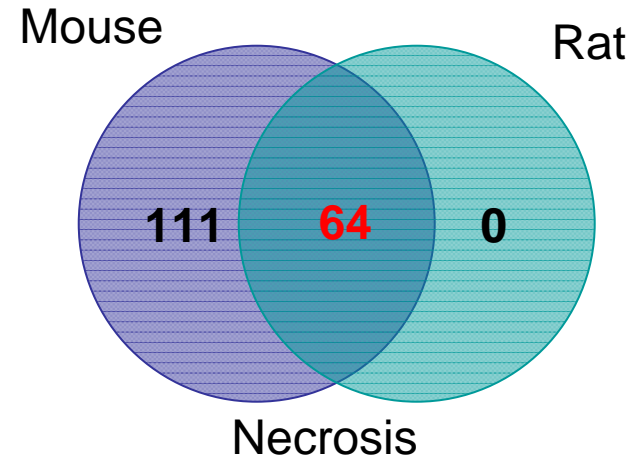
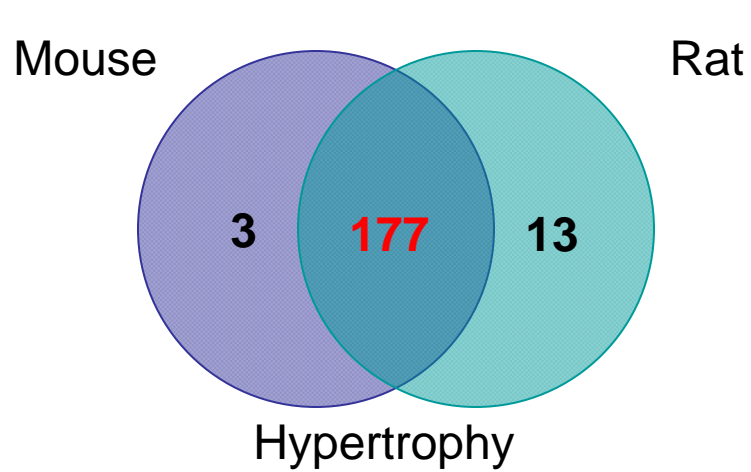


Features ranked based on the number of times being selected

# Overlap of Features



# Overlap of IVA Features



# Common IVA Features (Freq>40)

Hypertrophy	Necrosis	ProliferativeLesion	Tumor
Solidus_AllEnzyme	Solidus_P450	CLZD_CYP2B6_24	Solidus_P450
Solidus_NoEnzyme	NVS_ADME_hCYP2C19	CLZD_CYP2B6_48	NVS_ADME_hCYP2C19
Solidus_P450	NVS_NR_hPXR	CLZD_CYP2B6_6	NVS_MP_rPBR
Solidus_PhaseII	CLZD_CYP1A1_24	CLM_CellLoss_72hr	NVS_NR_hPXR
NVS_ADME_hCYP1A2	CLZD_CYP1A1_6	CLM_MitoticArrest_72hr	CLZD_CYP1A1_24
NVS_ADME_hCYP2B6	CLZD_CYP1A2_24	BSK_3C_Proliferation	CLZD_CYP1A1_48
NVS_ADME_hCYP2C18	CLZD_CYP2B6_24	BSK_BE3C_uPAR	CLZD_CYP1A1_6
NVS_ADME_hCYP2C19	CLZD_CYP2B6_48	BSK_hDFCGF_CollagenIII	CLZD_CYP1A2_24
NVS_ADME_hCYP2C9	CLZD_CYP2B6_6	BSK_hDFCGF_MMP1	CLZD_CYP1A2_48
NVS_ADME_hCYP3A5	CLZD_CYP3A4_24	BSK_hDFCGF_Proliferation	CLZD_CYP2B6_24
NVS_ADME_rCYP2A1	CLZD_CYP3A4_48	BSK_hDFCGF_VCAM1	CLZD_CYP2B6_48
NVS_ADME_rCYP2B1	CLM_MitoticArrest_72hr	BSK_LPS_PGE2	CLZD_CYP2B6_6
NVS_ADME_rCYP2C11	BSK_3C_hLADR	BSK_SAg_CD38	CLZD_CYP3A4_24
NVS_ADME_rCYP2C6	BSK_3C_MCP1	BSK_SAg_CD40	CLZD_CYP3A4_48
NVS_ADME_rCYP2D2	BSK_3C_Proliferation	BSK_SAg_CD69	CLZD_HMGCS2_48
NVS_ADME_rCYP3A1	BSK_3C_Vis	BSK_SAg_Eselectin	CLM_CellLoss_72hr
NVS_ADME_rCYP3A2	BSK_4H_MCP1	BSK_SAg_PBMCCytotoxicity	CLM_MicrotubuleCSK_72hr
NVS_MP_hPBR	BSK_4H_VCAM1	BSK_SAg_Proliferation	CLM_MitoMass_24hr
NVS_MP_rPBR	BSK_BE3C_hLADR	BSK_SM3C_Proliferation	CLM_MitoMembPot_1hr
NVS_NR_hPXR	BSK_BE3C_IP10	ATG_Ahr_CIS	CLM_MitoticArrest_72hr
NCGC_PXR_Agonist_human	BSK_BE3C_uPA	ATG_AP_1_CIS	CLM_OxidativeStress_72hr
NCGC_PXR_Agonist_rat	BSK_BE3C_uPAR	ATG_BRE_CIS	BSK_3C_hLADR
xT.xTID..4.dim.xT..2..	BSK_hDFCGF_CollagenIII	ATG_CMV_CIS	BSK_3C_MCP1
CLZD_ABCB11_48	BSK_hDFCGF_IP10	ATG_ERa_TRANS	BSK_3C_Proliferation

# Summary

- Approaches
  - Focused on 4 liver tox endpoints
  - Only used the *in vitro* data (treated as a binary number 0/1)
  - KNN with balancing the sample classes
  - 5-fold cross-validation with exploring the entire k-feature space
- Results
  - Good models were obtained for all the endpoints for both species except for the mouse proliferative lesions
  - Rat models generally performed better than the mouse models, particularly, for proliferative lesions and tumor
  - Similar features were used in both species for hypertrophy and tumor
  - CYP 450 are among the important features (mechanistically informative IVA endpoints)

# Acknowledgement

- Zhenqiang Su
- Huixiao Hong