

# Integrated analysis of transcriptomics and kinase activity data for better characterization of cancer models

# #LB060

# Introduction

## Background:

Quantitative measurements of transcripts and proteins only provide information about the basal state of a biological system, in contrast, functional proteomics are essential to investigate the active state of regulatory networks. Thus, the application of "multi-omics" strategies and their integration could improve understanding of the mechanisms underlying cancer.

## Aim of the study:

Integration of transcriptomics (RNAseq) and kinomics (Pamgene kinase activity) data for :

- Better characterization of various cancer models.
- Improve identification of new cell signaling targets

## Method:

- Kinase activity was profiled using Pamchip<sup>®</sup> Technology (Fig.1).
- RNAseq was performed on an Illumina NovaSeq platform.
- Analysis was performed on 63 Patient Derived Xenografts (PDX) from six cancer types (Fig.2).
- Kinase signaling-specific genes (n=2932) were selected from the Reactome database Signal Transduction Pathway (n=2560) and additional kinases (n=372) represented on Pamchip.



Fig.1. Kinase activity profiling on PamChip<sup>®</sup> microarrays

		1	I	I	I		1
Non	i small ce	II lung ca	ncer (LEX	F)			
Colc	on cancer	(CXF)					
Trip	le negativ	ve breast	cancer (N	/IAXFTN)			
Ova	rian canc	er (OVXF	)				
Mel	anoma (I	VIEXF)					
Acu	te myeloi	id leukem	ia (LEXFA	M)			
0	2	4	6	8	10	12	14

Fig.2. Sample distribution across six cancer types

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Fig.3. Workflow for integration of RNAseq and kinase activity data. MOFA implements dimensionality reduction to find factors (i.e. correlated RNAs and phosphosites) capturing variability shared across data sources. For features within a factor, pathways were analysed using Metascape.



Fig.4. Principal component analysis (*left*) using only RNAseq data and MOFA analysis (*right*) using integrated data describing variation between different PDX models.

### Integrated data identifies new pathways Integrated data RNAseq data PI3K-Akt signaling pathway PI3K-Akt signaling pathway Ras signaling pathway Chemokine signaling pathwa Chemokine signaling pathway egulation of actin cytoskeleto EGFR tyrosine kinase inhibitor resistance **MAPK** signaling pathway Axon guidance Adherens junction Leukocyte transendothelial migration Axon guidan aposi sarcoma-associated HSV infectior enic Escherichia coli infectio Prostate cancer **Tight junction** Human papillomavirus infection PD-L1 and PD-1 pathway in cancer acterial invasion of epithelial cells Pathogenic Escherichia coli infection **Relaxin signaling pathway** Tight junction Hippo signaling pathway Insulin resistance Arrhythmogenic right ventricular... Alcoholism entral carbon metabolism in cancer Osteoclast differentiation JAK-STAT signaling pathway -log<sub>10</sub>(pValue Salmonella infection Fluid shear stress and atherosclerosis Lipid and atherosclerosis 10 15 20 -log<sub>10</sub>(pValue)

Fig.5. Predicted pathways on 100 genes from Dimension 1 of RNAseq data (*left*) or 50 genes and 50 phosphosites from Dimension 1 of integrated data (*right*). New pathways in integrated data are highlighted.





Fig.6. (*Left*) RNAs (*black*) and phosphosites (*green*) for individual CXF or LEXFAM PDX model. CXF is shown as representative of 5 tumor clustering separately from LEXFAM in Fig.4. (Right) Protein-protein interaction for PI3K-AKT (*top*) and RAS (*bottom*) signaling pathway.





# Integrated data helps to select PDX models

Insulin signaling pathway





Fig.7. (A) Percentage correlation of 29 RNAs and phosphosites from insulin signaling pathway in 12 CXF (Colon cancer) PDX models. Samples are ranked based on correlation score. (B) Heat map depicting signals of correlated (box) phosphosites (P) and RNAs (R) in two CXF PDX model.

# Conclusions

- Kinase related signaling genes capture variation in different PDX tumor models.
- Compared to RNAseq alone, integrated RNAseq-phosphosite data:
  - Explain more variance between PDX tumor models.
  - Enhances statistical score of biological pathways.
  - Enrich for additional relevant pathways
- High correlation between two datasets improves target characterization within a tumor group and enables selection of relevant animal models addressing specific research questions.

Integration of the active kinome and **RNAseq data strengthens biological** interpretation and identifies hidden targets and pathways.