Your Applications add colour to our technology

Kinase activity profiling providing vibrant applications

Sensitive, multiplex measurement of kinase activity in real-time for discovery, biomarker and clinical research

Introduction

With PamGene's kinase activity profiling platform, the activity of kinases in a wide range of cells and tissues can be measured in real-time. This ability can be applied in research areas encompassing the spectrum: From pre-clinical and discovery research to biomarkers and clinical research.

PamGene's comprehensive kinase activity profiling platform can open new opportunities for a wide range of applications that provide insights into molecular mechanisms of diseases and can lead to discovery of biomarkers. Many of PamGene's applications are in the oncology area, with novel applications emerging in several other disease areas such as neurobiology, cardiovascular biology and immunology.

We use our proprietary 3D peptide microarray technology (PamChip® and PamStation®) which offers a multiplex method for global kinase activity profiling. The assay is very sensitive, requiring only a small amount of lysate to measure the activity of kinases in various samples including cell lines, xenografts and human tissues. Lysates obtained from a few thousand cells can suffice to obtain a kinomic profile of the multiple kinases present in these samples.

This is accomplished by incubating the sample lysates across 144 tyrosine or 144 serine/threonine kinase peptide substrates immobilized on the 3D surface of the PamChip® microarray. Kinases present in the lysates will phosphorylate the peptide substrates which is detected using fluorescently labelled antibodies.

Kinase activity profiling services

PamGene provides access to this unique technology through services and sales of products (PamStation®12, and PamChip® microarrays). Services are provided in several research areas (Table 1), and include a full range of assays, technologies and bioinformatics analysis adapted to your specific needs.

Fundamental and discovery research	Biomarker and clinical research
• Pathway elucidation • Compound mode of action • Target discovery • Target interaction • Biochemical characterization • Substrate identification	• Classification biomarkers • Prognostic biomarkers • Therapy-predictive biomarkers • Pharmacodynamic biomarkers

Table 1: Key application areas

PamChip® Technology

- Based on 2nd generation microarrays: The 3D, flow-through PamChip®.
- Up to 144 peptides with kinase phosphorylation motifs are immobilized per microarray

Technology outline

PamGene's microarray assay for kinase activity profiling is based on measuring peptide phosphorylation by protein kinases. The PamChip®4 or PamChip®96 disposable consists of 4 or 96 identical arrays, each array containing 144 (STK) or 196 (PTK) peptides immobilized on a porous ceramic membrane (Fig. 1). The peptide sequences (13 amino acids long) harbor phosphorylation sites derived from literature or computational predictions and are correlated with one or multiple upstream kinases (Protein tyrosine kinases for the PTK PamChip® and Serine-threonine kinases for the STK PamChip®). Fluorescently labelled anti-phospho antibodies are used to detect phosphorylation activity of kinases present in the sample (Fig. 2).

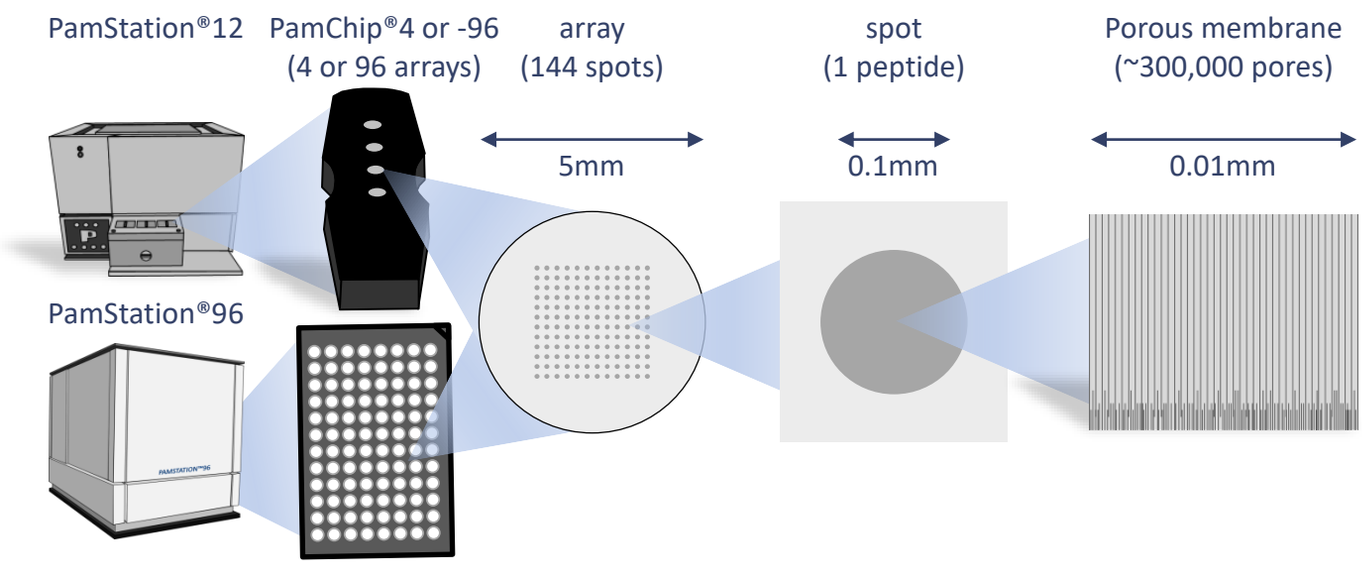


Figure 1: Outline of PamStation® instruments and PamChip® microarray disposables

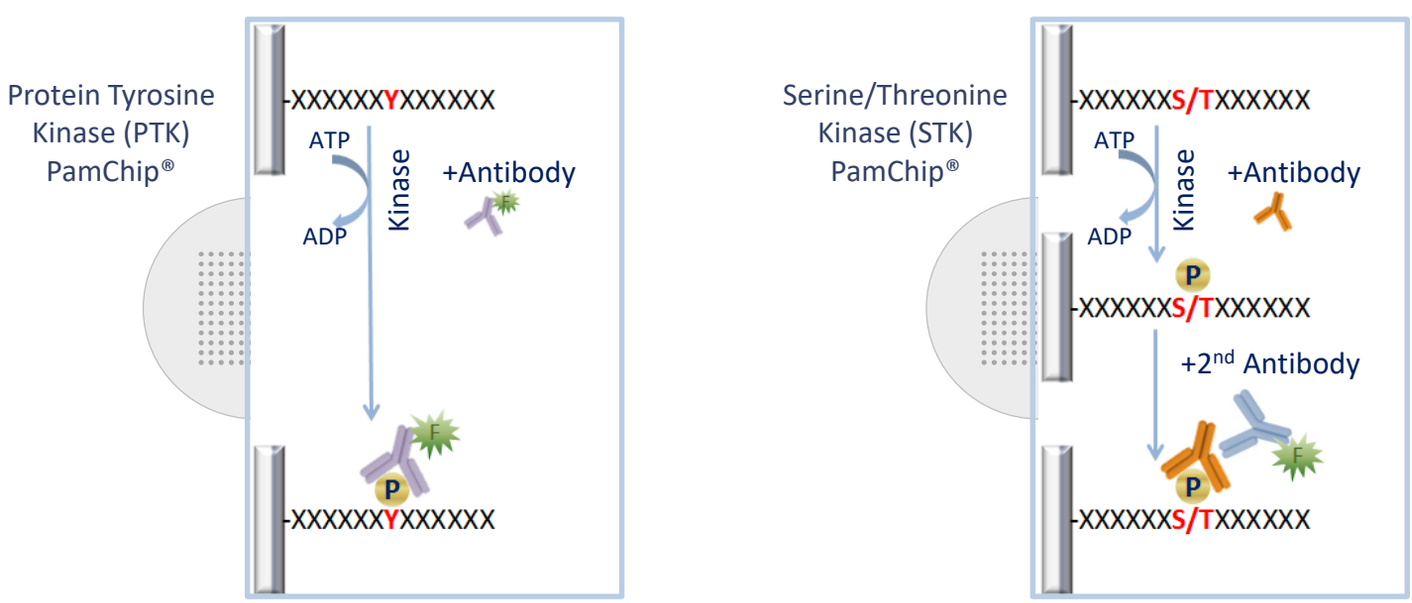


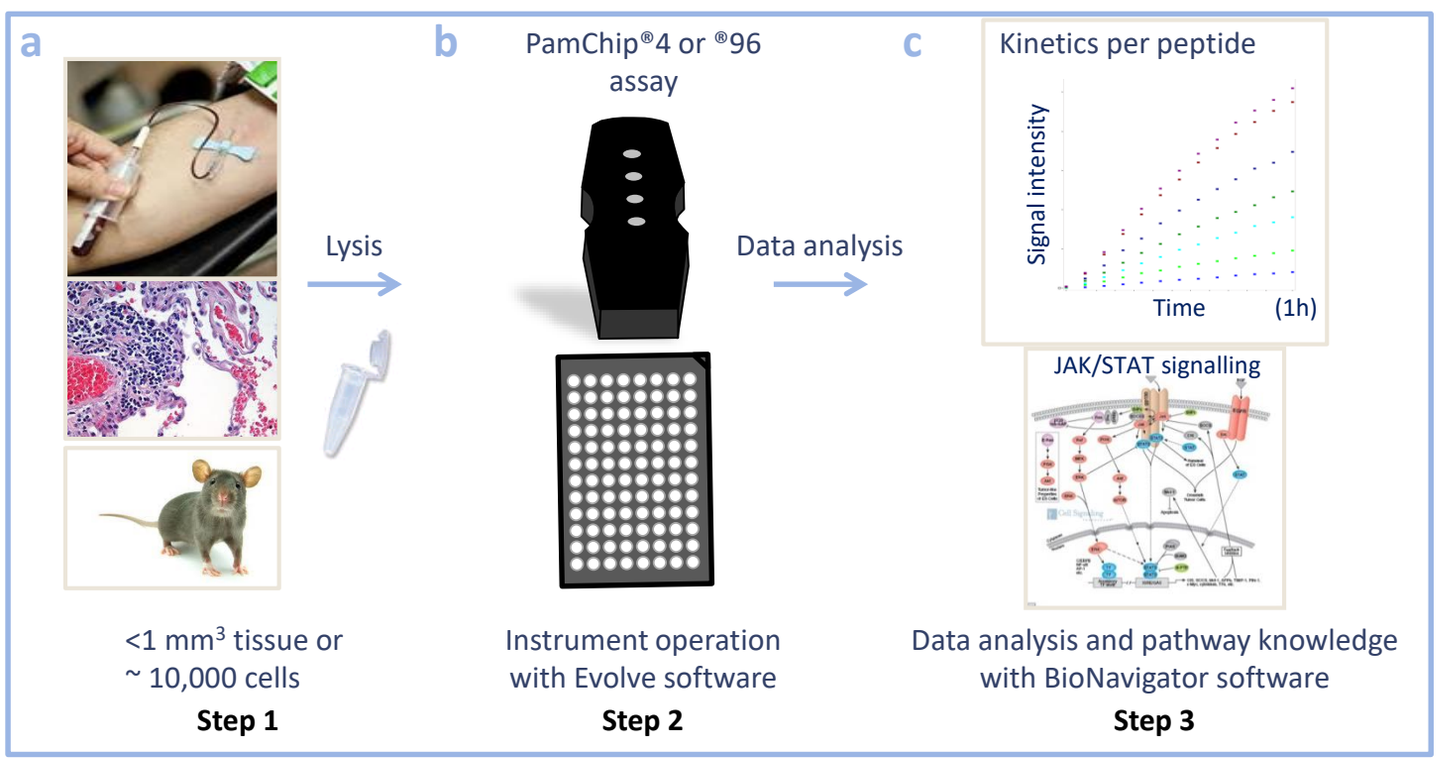
Figure 2: Outline of the detection of kinase activity on the PamChip® array

PamChip® assay workflow

- Providing options to measure kinase activity, including compound selectivity and inhibition
- Both 12- and 96- well array platforms generate high-quality and robust kinase activity data.

Kinase assay workflow

The PamChip® kinase assay workflow (Fig. 3a-c) is comprised of 3 steps: (1) Sample preparation (cell or tissue lysis); (2) PamChip® assay, using our proprietary Evolve software; (3) Data analysis and knowledge integration, using our proprietary BioNavigator software. During the assay, the sample solution is pumped through the porous membrane, allowing for faster kinetics and real-time measurements. When the solution is underneath the array, images of each array are taken at several exposure times by the CCD camera in the workstation (Fig. 3d). Images are later used by the BioNavigator® software to generate kinetic data curves of each peptide (Fig. 3c). The data workflow consisting of image quantification, quality control, statistical analysis, visualization and interpretation is performed using the BioNavigator® software.



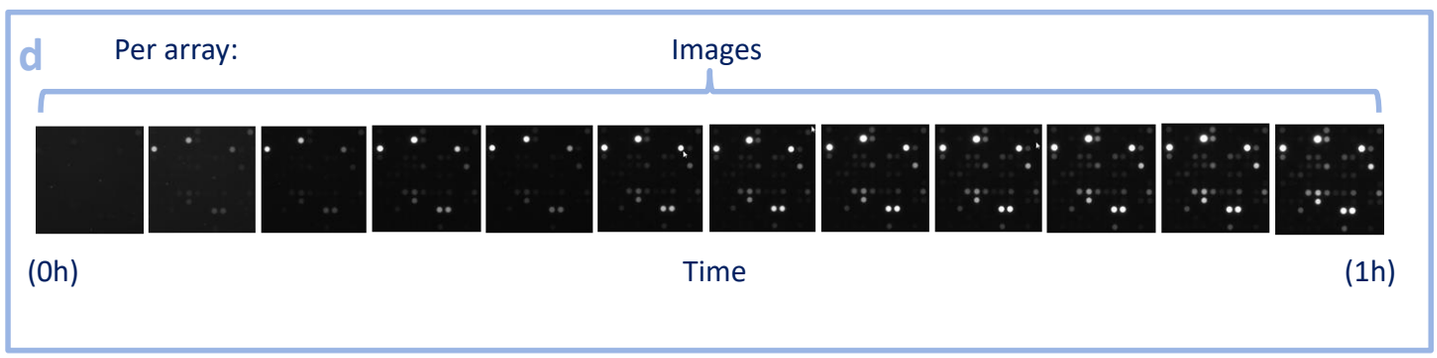


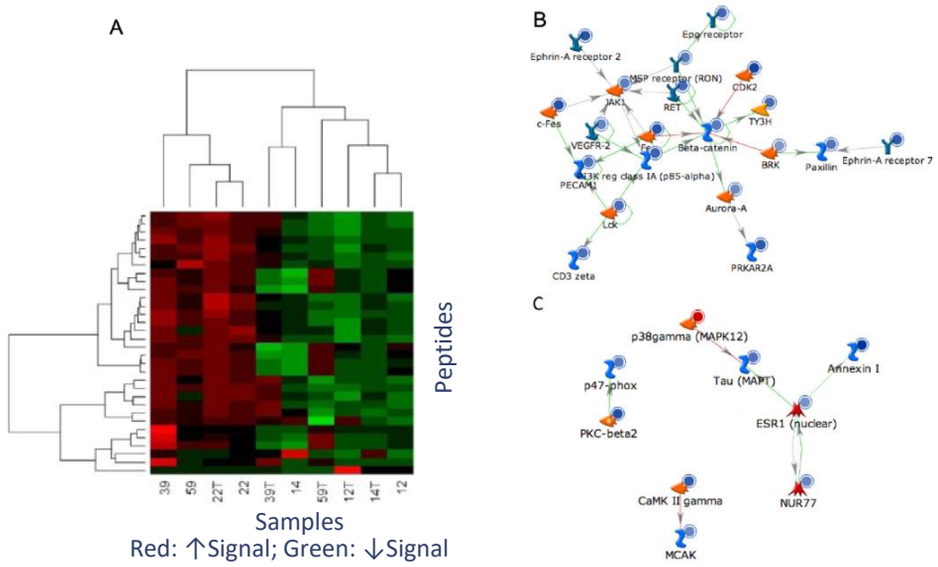
Figure 3: Kinase assay workflow and measurement of kinase phosphorylation activity in real-time

Pathway and Network analysis

PamChip® data leads to new discoveries and pathway knowledge.

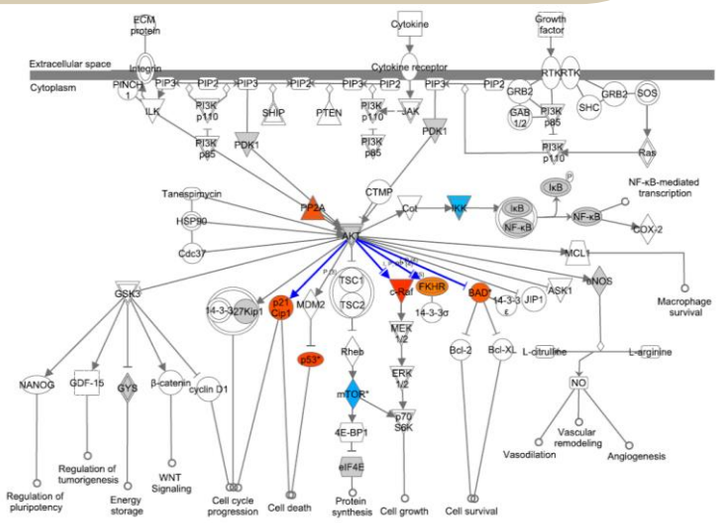
Figure 4: Examples of Pathway and Network analysis of PamChip® data resulting in important findings are illustrated.

Kinase networks involved in radiation resistance in glioblastoma xenolines



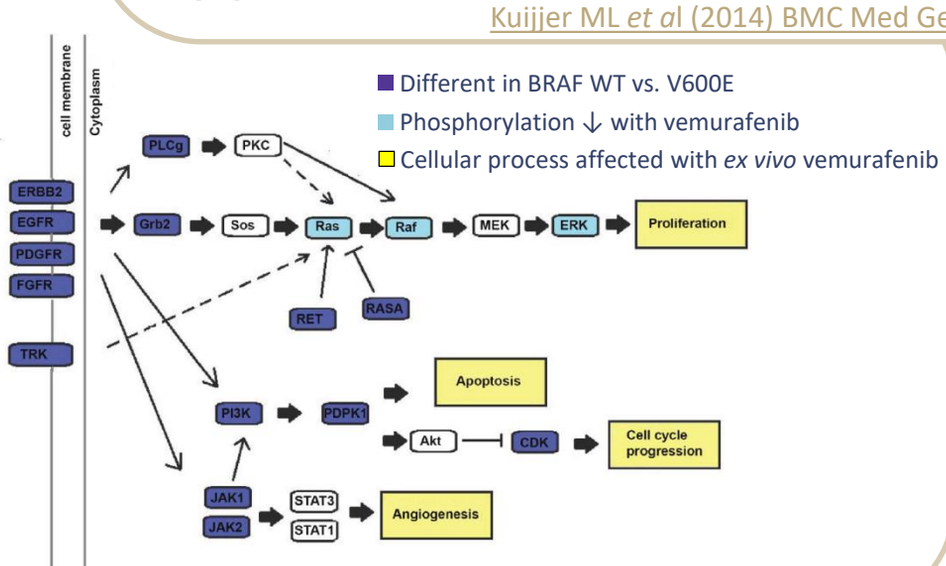
Anderson JC *et al* (2014) Radiother Oncol. 111(3):468-74

PamChip® phosphorylation profiles correlated to AKT signalling networks in osteosarcoma cell lines



Kuijjer ML *et al* (2014) BMC Med Genomics. 21;7:4

Kinase networks affected by *ex vivo* vemurafenib in BRAF(V600E) melanoma patient tissues

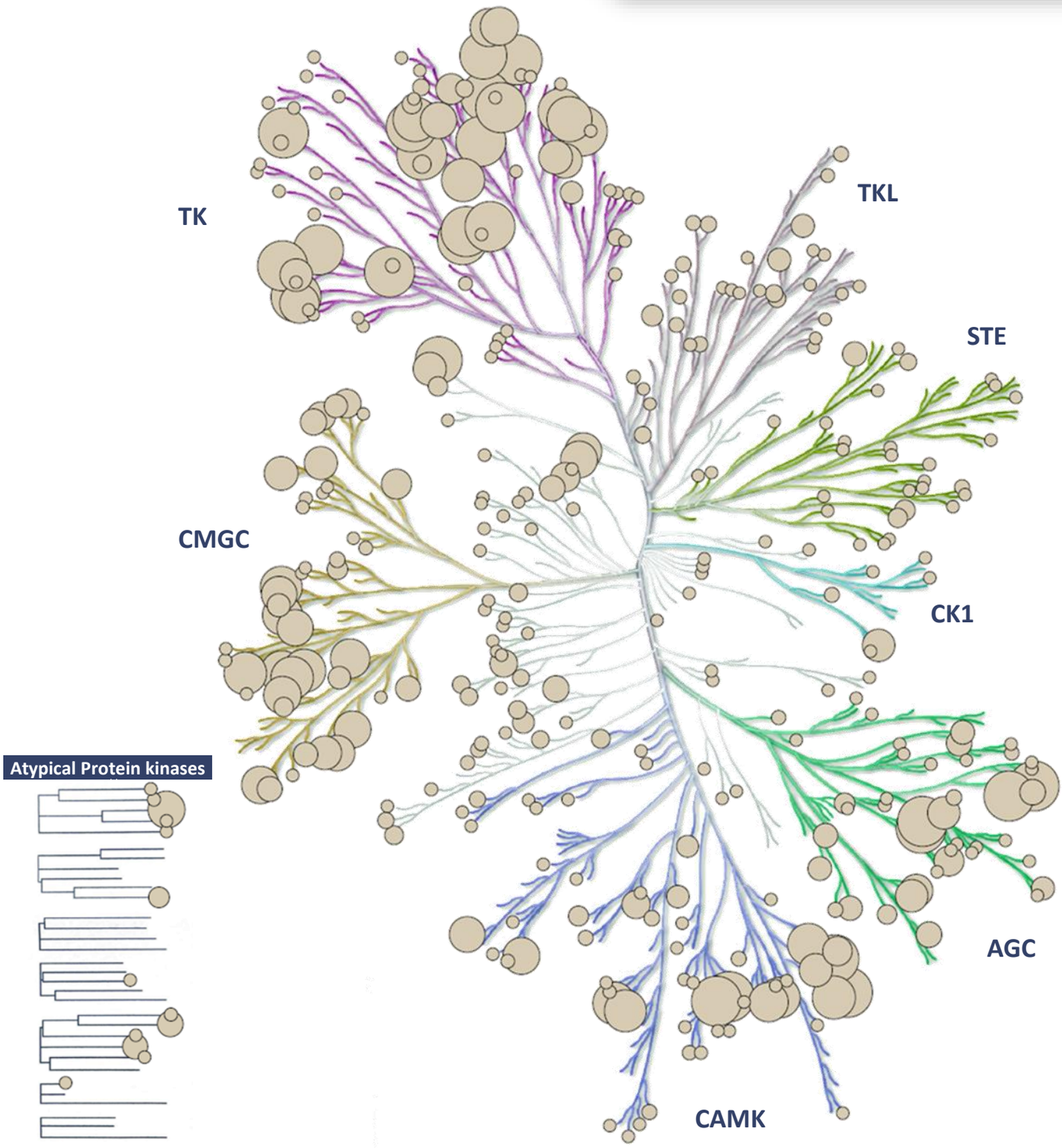


Tahiri A *et al* (2013) PLoS ONE. 8(8): e72692

Upstream kinases

- Putative upstream kinases can be inferred from the peptide substrates spotted on the PamChip®: Protein Tyrosine Kinases (PTK) and Serine Threonine Kinases (STK)

Figure 5: Kinases that are known or predicted to phosphorylate substrate peptides on the PTK and STK PamChip® are mapped from select databases (HPRD, Kinexus, Phosphosite, Reactome) and projected on the kinome tree.



"Illustration reproduced courtesy of Cell Signaling Technology, Inc. (www.cellsignal.com)", using the Kinome render tool (http://bcb.med.usherbrooke.ca/kinomerender.php). Circles represent kinases and circle size the number of peptides on the PamChip® (scale 10 to 70), as derived from the respective databases. In empirical data from PamGene (not shown) all 80 recombinant kinases assayed actively phosphorylated specific substrates on the PamChip®. The kinases shown are putative and project the information provided in the indicated databases.

Applicable in many disease areas

From substrate identification and target discovery towards disease biology

Table 2: Applications of PamChip® kinase activity profiling in different disease areas are illustrated.

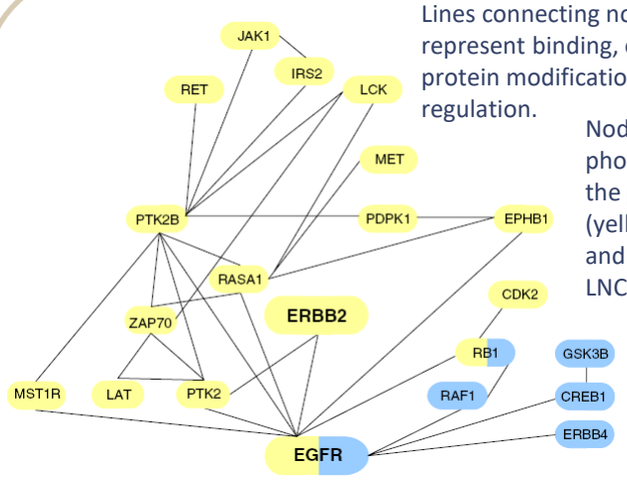
Disease areas	Targets	Pathways	Publications
Alzheimer's Disease (AD)	IRAK-4	IRAK-4 and cytokine signaling	Hoozemans JJM *et al* (2014) J Clin Cell Immunol 5: 243; Hoozemans JJM *et al* (2012) Neurodegener. Dis. 10 (1-4): 46-8
Brain tumors	SRC	JAK/STAT/PI3K/STAT	Sikkema AH *et al* (2009) Cancer Res. 69 (14): 5987-95
Breast Cancer	EGFR, VEGFR2 and PLCγ	Angiogenesis	Lindholm EM *et al* (2012) Mol Oncol. 6 (4): 418-27
Glioblastoma	FAK, FGFR1	VEGFR1/2 and c-Raf; PKC, JAK1, PI3K, CDK2, and VEGFR	Anderson JC *et al* (2014) Radiother Oncol. 111 (3): 468-74
Glioma	PDGFRb, PI3K, and VEGF	Fes signaling	Stoltz K *et al* (2014) Neuro Oncol. Epub. pii: nou320
Infantile hemangioma	VEGFR2	VEGFR2 pathway	Jinnin M *et al* (2008) Nat Med. 14 (11): 1236-46
Ischaemic stroke, myocardial infarction	EPHA4	P2Y1-12/EphA4	Tournoij E *et al* (2012) Platelets. 23 (8): 617-25
Leukemia	EGFR, PDGFR, NTRK1/2, MST1R, MAP2K2	RAS/RAF/MEK/ERK	Ter Elst A *et al* (2011) Leuk Lymphoma. 52 (1): 122-30
Leukemia (AML)	FLT3	c-Myc	Eriksson A *et al* (2014) Biochem Pharmacol. 87 (2): 284-91
Leukemia (CLL)	BCR-ABL1	BCR-ABL1, NUP214-ABL1	De Keersmaecker K *et al* (2008) Leukemia. 22 (12): 2208-16
Lung cancer (NSCLC) and other solid tumors	EGFR	EGFR; EGFR/ PTEN/ PI3K; EGFR/ cMET/ SRC/ RO; MET/ ALK/ RON/ ROS; CBL, PI3K, and STAT3	Anderson JC *et al* (2014) PLoS One. 9 (12): e116388; Kawada I *et al* (2014) Cancer Res. 74 (3): 884-95; Vivanco I *et al* (2010) Proc Natl Acad Sci USA. 107 (14): 6459-64; Versele M *et al* (2009) Mol Cancer Ther. 8 (7): 1846-55
Melanoma	BRAF	RAS/RAF/MEK/ERK	Tahiri A *et al* (2013) PLoS ONE 8 (8): e72692
Myeloproliferative neoplasms	JAK2 JAK2-V617F/JH2	JAK2/STAT5 JAK2/JH2	Bar-Natan M *et al* (2012) Leukemia. 26 (6): 1407-10 Sanz A *et al* (2011) PLoS One. 6 (4): e18522
Oropharyngeal squamous cell carcinomas (OPSCC)	p16INK4a	EGFR, MMP1-MMP3, STAT3, ZAP70/Syk	Isayeva T *et al* (2014) Mod Pathol. 28 (5): 631-53
Osteosarcoma	Akt	PI3K/Akt/mTORC1 and AMPK	Kuijjer ML *et al* (2014) BMC Med Genomics. 7:4
Prostate cancer (Androgen-sensitive) (Castration-Resistant)	EGFR/ERBB2 STAT5A	EGFR/ERBB2 JAK/STAT	Bratland A *et al* (2009) Clin Exp Metastasis. 26 (5): 485-96 Røe K *et al* (2013) PLoS ONE 8(5): e63723
Rectal cancer (Locally advanced rectal cancer)	PI3K	EGFR/PI3K and KRAS/BRAF	Ree AH *et al* (2015) Crit Rev Oncol Hematol. pii: S1040-8428(15)00004-9; Ree AH *et al* (2012) PLoS One. 7 (11): e50806; Saelen MG *et al* (2011) Angiogenesis. 14(4):481-9; Folkvord S *et al* (2010) Int J Radiat Oncol Biol Phys. 78 (2): 555-62
Schizophrenia		Homeostasis	McGuire JL *et al* (2014). Brain Res. 1568: 42-54
Uveal melanoma	SRC	Src/ERK1-2	Maat W *et al* (2009) Br J Cancer. 101 (2): 312-9

Key application areas: Fundamental and discovery research

Figure 6: Representative applications of PamGene's kinase activity profiling assay in fundamental and discovery research

A. Pathway elucidation

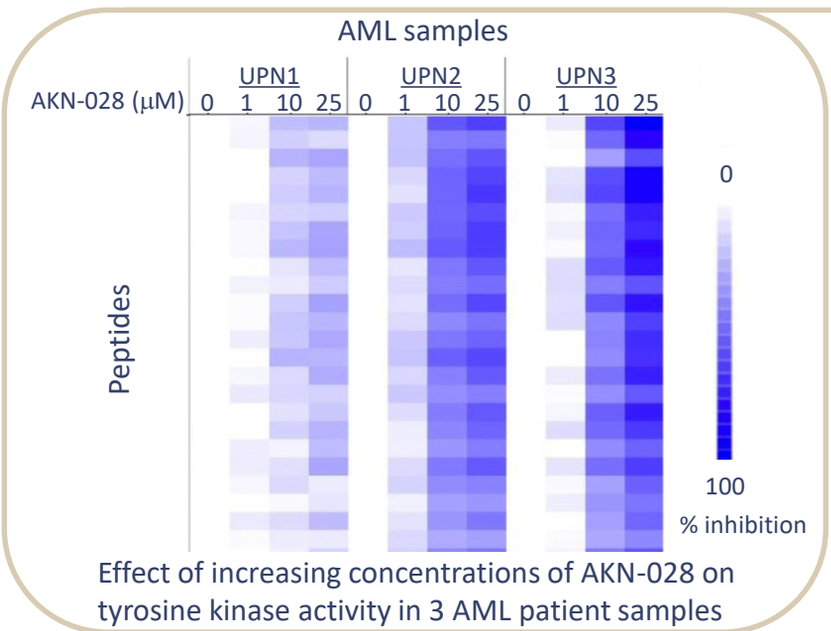
Elucidate various pathways in parallel in diverse cell lines or tissues. Prior knowledge of involved targets and pathways is not required, and interpretation is not limited to existing knowledge.



Interconnected signalling pathways activated in LNCaP cells by influence of osteoblastic cells or androgen treatment.

- Plaza-Menacho I *et al* (2011) J Biol Chem. 286(19):17292-302.
- Bratland A *et al* (2009) Clin Exp Metastasis. 26(5):485-96 (*Fig. 6A*)

B. Compound mode of action



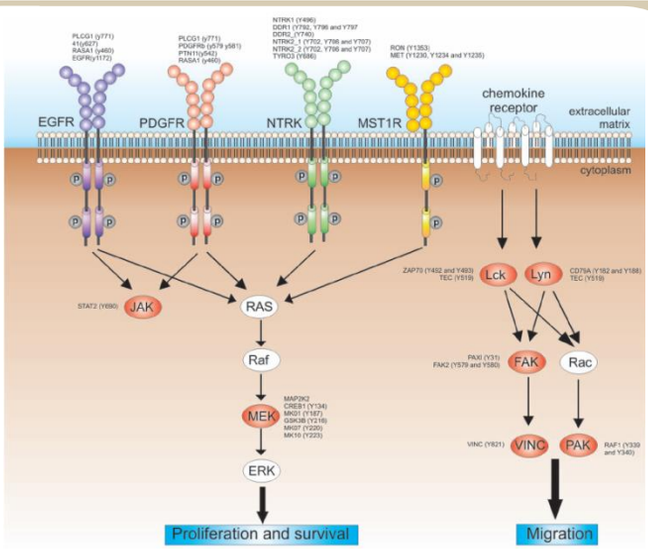
Effect of increasing concentrations of AKN-028 on tyrosine kinase activity in 3 AML patient samples

Study the mode of action of compounds (kinase inhibitors) and/ or structural analogs directly by *ex vivo* spike-in with cell lysates in the assay.

- Eriksson A *et al* (2014) Biochem Pharmacol. 87(2):284-91 (*Fig. 6B*)
- Versele M *et al* (2009) Mol Cancer Ther. 8(7):1846-55

Key application areas: Fundamental and discovery research

C. Target discovery



Red: proteins corresponding to peptides phosphorylated on the PamChip® array

Signaling pathways common in 3 types of leukemia

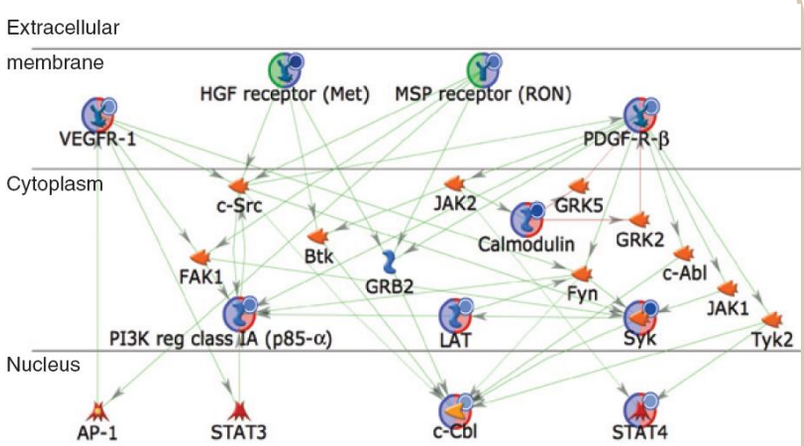
Discover novel targets using cell lines, xenografts and also clinical tissues. Prior knowledge of involved targets and pathways is not required.

- Kuijjer ML *et al* (2014). BMC Med Genomics. 21;7:4
- Ree AH *et al* (2012). PLoS One. 7(11): e50806.
- Ter Elst A *et al* (2011) Anal Biochem. Leuk Lymphoma. 52(1):122-30 (*Fig. 6C*)

D. Target interactions

Perform target interaction/ engagement studies in cell lines, xenografts and also clinical tissues. The unique possibility of spiking-in kinase inhibitors *ex* vivo ("On-Chip pharmacology") is an advantage.

- Kawada I *et al* (2014) Cancer Res. 74(3):884-95. (*Fig. 6D*)
- Bar-Natan M *et al* (2012) Leukemia. 26(6):1407-10
- Jarboe JS *et al* (2012) Radiother Oncol. 103(3):380-7

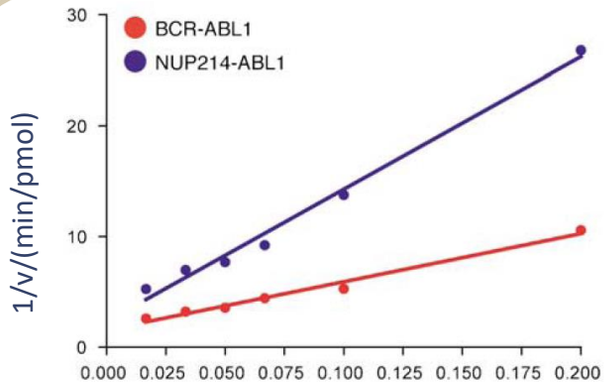


Network between the significant proteins associated with treatment of H1993 cells with LY2801653 (3 nmol/L)

Key application areas: Fundamental and discovery research

E. Biochemical characterization

Enzymatically characterize novel kinases or mutated kinases



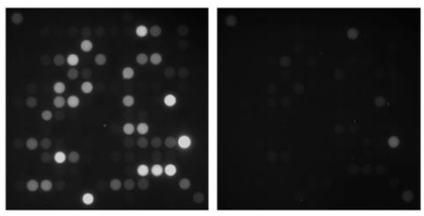
	BCR-ABL1	NUP214-ABL1
Vmax (pmol/min)	0.50 +/– 0.02	0.27 +/– 0.02
K_M (μM)	17.7 +/– 1.9	24.5 +/– 3.7

Catalytic properties of oncogenic fusion kinases: NUP214-ABL1 is a weaker kinase than BCR-ABL1

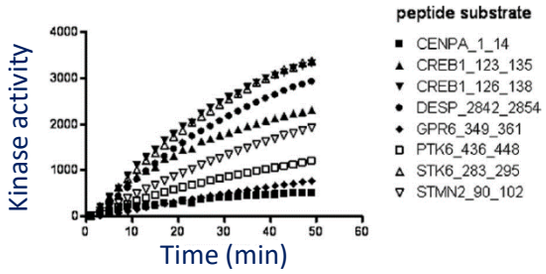
- Sanz Sanz A *et al* (2014) Biochim Biophys Acta. 1844(10):1835-41
- De Keersmaecker K *et al* (2008) Leukemia. 22(12):2208 (*Fig. 6E*)

F. Substrate identification

PamChip®



+ PKA - PKA



Time course of PKA-catalyzed phosphorylation on the array

Identify peptide kinase substrates for a kinase of interest, novel kinases, mutated kinases or post-translationally modified kinases

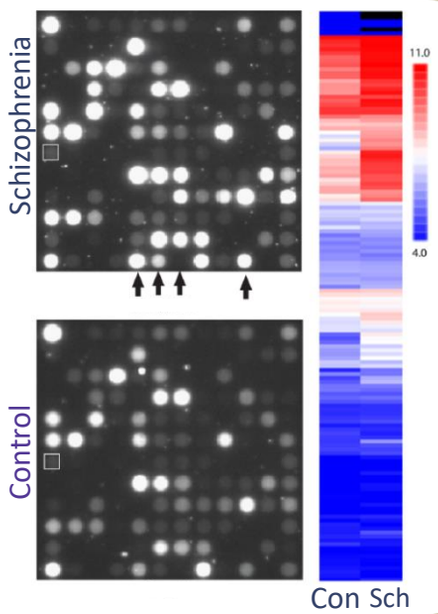
- Sanz A *et al* (2011) PLoS One. 18;6(4): e18522.
- Hilhorst R *et al* (2009) Anal Biochem. 2009 Apr 15;387(2):150-61 (*Fig. 6F*)

Key application areas: Biomarker and clinical research

Figure 7: Representative applications of PamGene's kinase activity profiling assay in biomarker and clinical research

A. Classification Biomarkers

Differential substrate phosphorylation comparing schizophrenia and control samples, showing representative serine-threonine kinase activity profiles and corresponding heatmaps.

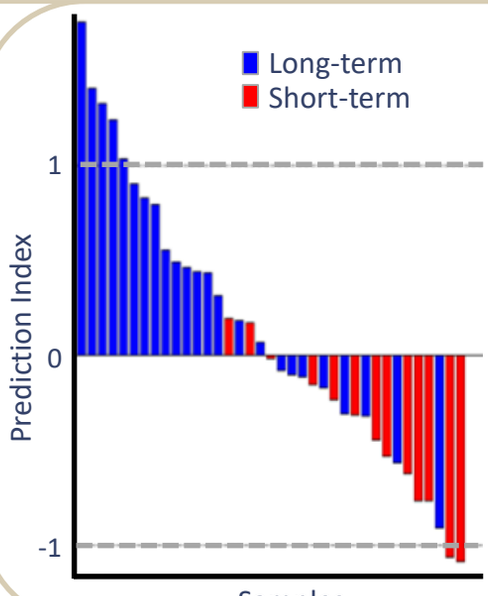


Determine kinomic signatures that classify clinical samples based on phenotypes, disease states, mutation status and more.

- McGuire JL *et al* (2014) Brain Res. 1568:42-54 (*Fig. 7A*)
- Tahiri A *et al* (2013) PLoS ONE. 8(8): e72692

B. Prognostics biomarkers

Develop prognostic biomarkers to assess disease progression or risk of recurrence using patient samples by correlation of PamChip® data to clinical outcomes.



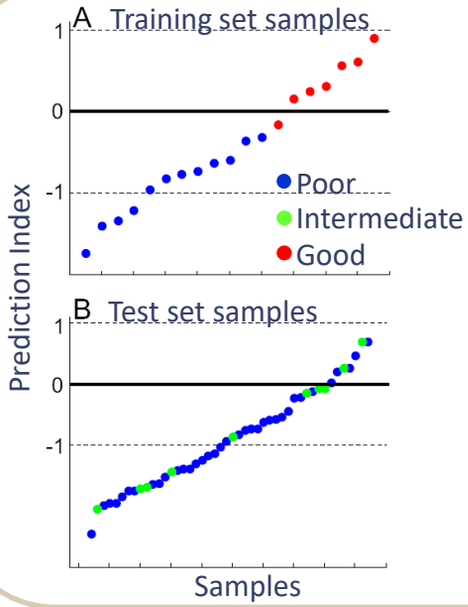
Prediction of short-term survivors in early stage lung adenocarcinoma (37 samples; 73% correct prediction). Patients with a prediction index > 0 are predicted to be long-term survivors and < 0 to be short-term survivors.

- Arni S *et al* (2013) AACR; Cancer Res. 73(8 Suppl): Abstract nr 2366 (*Fig. 7B*)
- Ruijtenbeek R *et al* (2011) AACR; Cancer Res. 71(8 Suppl):Abstract nr 4113.

Key application areas: Biomarker and clinical research

C. Therapy-predictive Biomarkers

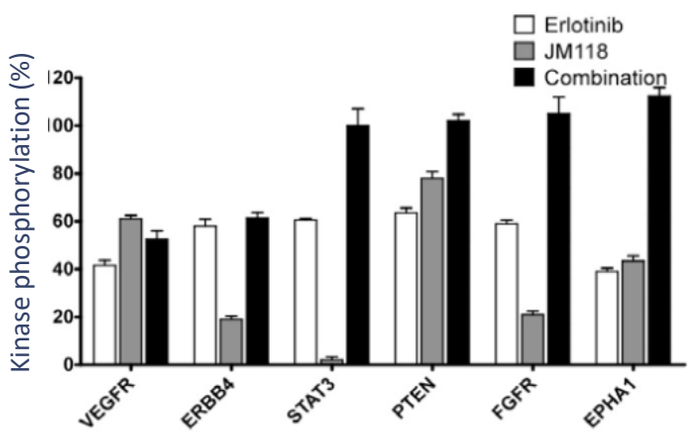
Use kinomic profiles, and/or the effect of your drug on these profiles, to identify subpopulations of patients which are most likely to respond to a given treatment.



Prediction of tumour response to chemo-radiotherapy in locally advanced rectal cancer (LARC) patients. Patients with a prediction index > 0 are predicted to have a good response and < 0 to have a poor response. Test set samples (B) were predicted using the prediction model generated from the training dataset (A).

- Hilhorst R, *et al* 2011) J Clin Oncol 29 (Suppl; Abstract nr 10521)
- Folkvord S *et al* (2010) Int J Radiat Oncol Biol Phys. 78(2):555-62. (*Fig. 7C*)
- Versele M *et al* (2009) Mol Cancer Ther. 8(7):1846-55.

D. Pharmacodynamic biomarkers



Relative kinase phosphorylation levels in A549 lung cancer cells exposed for 2h to IC50 concentrations of erlotinib and/or JM118 a satraplatin metabolite

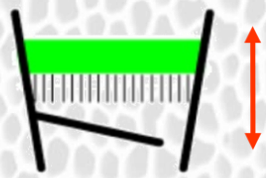
Identify which drug dose or combination therapy to use for an individual and determine molecular indicators of drug effect on the target directly in patient biopsies.

- Avan A *et al* (2014) Curr Drug Targets. 15(14):1312-21. (*Fig. 7D*)

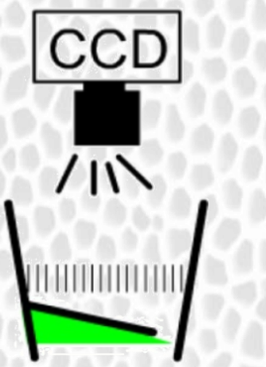
Key Advantages

- The novel 3D, 2nd generation flow-through PamChip® microarrays.
- Unique capabilities

Unique 3D surface



Diffusion-independent reaction rates



Fluorescence-based detection

The aluminum oxide surface of the PamChip® microarrays has a 3D porous structure with long, branched interconnected capillaries, allowing peptide substrates to be deposited at higher concentrations than conventional arrays.

Faster reactions

During the assay, the sample solution is pumped back and forth through the porous membrane resulting in faster kinetics and enabling real-time measurements of kinase phosphorylation activities.

High sensitivity

Dynamic enzymatic reactions and the 3D surface reduces sample input requirements significantly, to 0.1 to 5 µg/array compared to conventional assays (0.1 to 5 mg/assay). Testing precious clinical samples from individual patients for the field of personalized medicine is feasible.

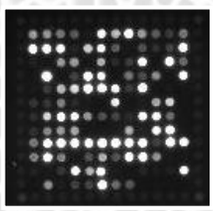
Full-length protein activity

Enzymatic characterization of not only recombinant kinases but also of kinase activity present in cell lysates from a wide variety of samples (cell lines, xenografts and human tissues), with physiological relevance.

On-Chip pharmacology

Patient's (tumor) tissue sample

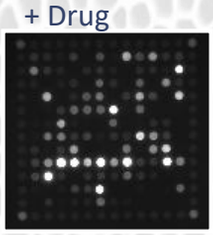
No Drug



peptide phosphorylation

Kinase activity

+ Drug



inhibition of peptide phosphorylation

Kinase activity inhibition

The direct effect of drugs and other compounds on the sample can be determined *ex-vivo* by adding them to the lysate, providing an additional new dimension of "On-Chip Pharmacology".

Ex-vivo inhibition profiles

Inhibition profiles can be used to elucidate drug mode of action, resistance mechanisms, class prediction analyses, compound screening and a multitude of basic and clinical interrogations.

Broad interrogation

The PamChip® assay does not require prior knowledge of the involved kinases, positioning it as a target discovery platform.

PamGene's Services & Products

- Distinct services
- Continued sales support

Services

PamGene's kinase activity profiling services are ideally suited to support the individual needs, thereby providing comprehensive solutions.

- Every project can be customized, with defined goals, milestones and interactive discussion.
- The PamStation®96 provides a high throughput platform where many samples can be simultaneously processed in a robust manner .
- Extensive, high quality reporting is provided, with complex data from large data sets that can be relevantly interpreted with our proprietary, powerful BioNavigator software.

Products and Sales support

- PamGene's Products are the PamStation®12 instrument, PamChip®4 disposables and Reagents, as well as the BioNavigator® data analysis software combined with training from our PamAcademy.
- PamGene offers various options for the purchase of the PamStation®12 instrument, PamChip®4 disposables and Reagents. To know more you can directly contact us: info@pamgene.com
- PamGene provides Customer support both before and after the sales process

We make the difference for you

- We have an active online research and applied sciences community to support our clients.

PamGene's kinase assay profiling services and products give you the data you need to advance your research faster.

PamGene's kinase activity assays bring together the power of a 2nd generation PamChip® microarray platform, a multiplex set of peptides representing a wide range of substrates for upstream kinases and biological pathways, and our BioNavigator bioinformatics suite to provide you with the data you need.

At PamGene, we have more than 10 years experience in functional proteomics research including profiling kinases and nuclear receptors. Our researchers and collaborators have published in more than 70 peer-reviewed papers.

Our PamCloud Community supports you

PamWiki

An up-to-date documentation maintained by the community of PamChip® users.

PamForum

Community posts topics on applications, bioinformatics, screening wet lab procedures for groups who have access to our platform in their laboratories, and personalized medicine. A place to ask questions and get answers.

PamAcademy

Educate yourself or follow a training to increase your skills starting from basic knowledge, up to advanced level in various aspects of our functional proteomics platform.

Your notes

How to order

Our Services

The *kinase activity profiling* services are fully tailored to your needs. Please contact us for more information.

We use the following stepwise process:

- Request information about kinase services at info@pamgene.com.
- Define project scientific goals, milestones, and planning.
- Receive a tailored quotation.
- Supply your purchase order.
- If applicable ship your samples and compounds.
- Receive and retrieve you data from our secure online portal.

Our Products

The PamStation®12 and PamChip®4 products can be purchased from PamGene.

We offer various options to get access to our technology
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