The PamGene technology

1 Description of the device

The PamGene's comprehensive kinase activity profiling platform (Figure 1A) allows a sensitive, multiplexed measurement of kinase activity in real-time for discovery, biomarker and clinical research. Kinase's activity profiling PamGene's microarray assay is based on measuring peptide phosphorylation by protein kinases. The PamChip consists of 4 identical arrays, each array containing 144 peptides immobilized on a porous ceramic membrane (Figure 1B). 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. Chips for tyrosine kinases and Serine-threonine kinases are available. Fluorescently labelled anti-phospho antibodies are used to detect phosphorylation activity of kinases present in the sample (Figure 1C).

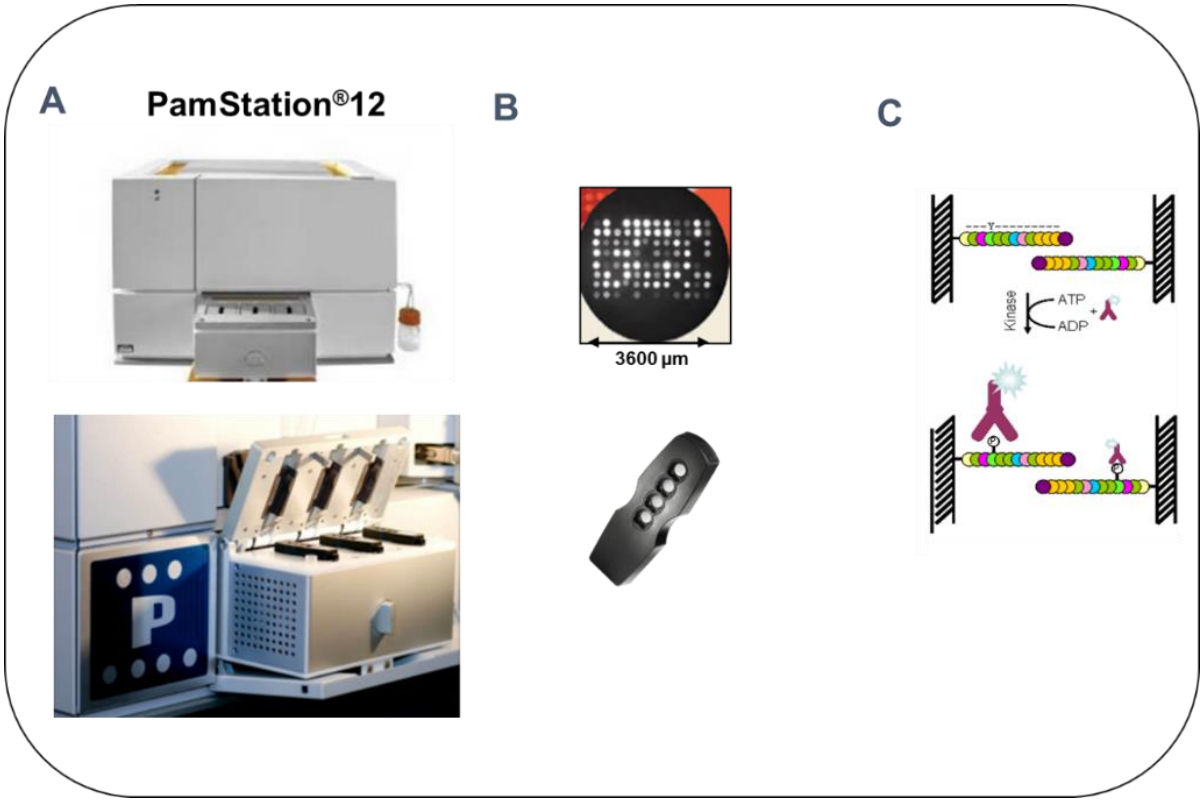

Figure 1: PamGene technology.

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.

2.2 Kinase assay workflow

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 over 200 ms by the CCD camera in the workstation. Images are later used by the BioNavigator® software to generate kinetic data curves of each peptide. Estimated value for luminescence signal at 100 ms was used for analysis after normalization by Z score calculation. The data workflow consisting of image quantification, quality control, statistical analysis, visualization and interpretation is performed using the BioNavigator® software.

2.3 Key applications

Key application areas		
Fundamental and discovery research	Pathway elucidation	Elucidate various pathways in parallel in diverse cell lines or tissues.
	Compound mode of action	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.
	Target discovery	Discover novel targets using cell lines, xenografts and also clinical tissues.
	Target interactions	Perform target interaction/ engagement studies in cell lines, xenografts and also clinical tissues.
	Biochemical characterization	Enzymatically characterize novel kinases or mutated kinases
	Substrate identification	Identify peptide kinase substrates for a kinase of interest, novel kinases, mutated kinases or post-translationally modified kinases
Biomarker and clinical research	Classification Biomarkers	Determine kinomic signatures that classify clinical samples based on phenotypes, disease states, mutation status and more.
	Prognostics biomarkers	Develop prognostic biomarkers to assess disease progression or risk of recurrence using patient samples by correlation of PamChip® data to clinical outcomes.
	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.
	Pharmacodynamic biomarkers	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.
	On-Chip pharmacology	The direct effect of drugs and other compounds on the sample can be determined ex-vivo by adding them to the lysate

Table 1: Key applications that could be performed by using the PamGene technology.

2.4 Prices

The price for a box of chips, plus corresponding reagents is:

- Phospho Tyrosine Kinase (PTK): chips (2900 €) + reagents (290 €) = 3190 €
- Serine /Threonine Kinase (STK): chips (5000 €) + reagents (500 €) = 5500 €

543 € per triplicate or 181 € per point (for PTK + STK)