CDRL Kinome Analysis Omnibus

Cognitive Disorders Research Lab

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Preface

Kinases are an important part of research work. For this reason, we talk about how to screen kinases using the kinome array here.

1 Introduction

Kinases are essential regulatory proteins that play a pivotal role in numerous biological processes, including signal transduction, cell cycle control, and gene expression. Dysregulation of kinase activity has been implicated in various human diseases, including cancer, diabetes, and neurodegenerative disorders (Manning et al. 2002).

While measuring kinase expression levels can provide some insight into their biological function, it is crucial to assess kinase activity levels for a more accurate understanding of their role in biological processes (Zhang, Yang, and Gray 2009). Research has shown that protein expression is poorly correlated with protein abundance and subsequent activity (Smail, Reigle, and McCullumsmith 2021). Kinase activity profiling allows for the detection of post-translational modifications, such as phosphorylation, that regulate kinase activity and, therefore, better represents the actual functional state of the kinase in the cell (Mann and Jensen 2003).

It is also essential to consider the redundancy and fault-tolerant nature of kinase signaling networks. In many cases, multiple kinases can activate the same downstream target, making it difficult to pinpoint the exact kinase responsible for a particular phenotype (Zhang, Yang, and Gray 2009). Therefore, screening multiple kinases simultaneously can provide a more comprehensive understanding of the complex signaling networks involved in a particular biological process.

To enable simultaneous profiling of kinase activity for hundreds of protein kinases in complex biological samples, the PamStation12 microarray platform has been developed. This platform uses peptide array technology, such as the serine/threonine (STK) and phospho-tyrosine (PTK) chips from PamGene, to provide high-throughput and multiplexed analysis of kinase activity (Tacken et al. 2005; McLaggan et al. 2006; Vepachedu et al. 2005).

2 The PamStation® Platform

The PamGene PamStation 12 (PamStation® 12) Platform is a high-throughput peptide array platform that enables multiplexed kinase activity profiling (Breitkreutz et al. 2012; Mertins et al. 2014; McBrayer et al. 2012; Stebbing et al. 2014; Herling et al. 2016; Lee et al. 2009). The technology is based on the PamChip microarray chips, which contain hundreds of unique peptide sequences that are phosphorylated by kinases. The Pamstation® 12 can be used to profile the activity of serine/threonine (STK) kinases or protein tyrosine (PTK) kinases . The platform has two separate components: The Microarray Chip (PamChip® 4), and the Platform (Pamstation® 12) to process the samples. Figure 2.1 shows an overview of the platform.

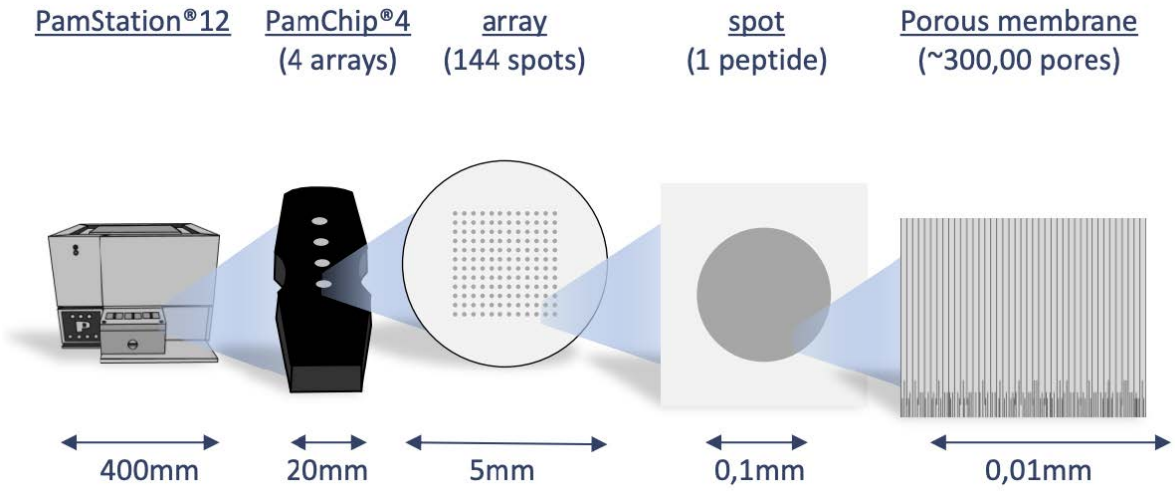

Figure 2.1: An overview of the PamStation® 12 Platform

2.1 PamChip® 4

The PamChip® is a microarray chip that is used for profiling the kinase activity. Each chip contains four wells, with each well being capable of processing a single sample. Figure 2.2 shows a typical PamChip® 4

The basic design of the chips is the same but they differ in the individual reporter peptides that are printed on the chip. The basic design of the chip can be seen in Figure 2.2. The

chip is approximately as long as a credit card and has 4 wells. Each well can be used for one sample.

Figure 2.1 explains the logical construct of the chip. In each well of a chip, there is a grid of either 144 (for STK) or 196 (for PTK) peptides known to be substrates for kinases. Each spot has 300, 000 copies of the same peptide printed on it. The PamStation® 12 instrument accepts 3 chips of one kinds, either PTK or STK. The instrument then goes through several cycles of sample injection and washing. Once sufficient time has been given to the process, a flourescent antibody is added to the chip to allow for visualization. This allows for real-time capture of the activity based on the fluorescence levels. The STK chip requires two different antibodies to achieve fluorescence. The different mechanisms are summarized in Figure 2.3.

2.2 Kinase Coverage in PamChip® 4

The PamGene platform provides coverage for a substantial proportion of the human kinome, enabling a comprehensive analysis of kinase activity. Specifically, the STK and PTK chips have been designed to cover a large number of Ser/Thr and Tyr kinases, respectively. It has been reported that the STK and PTK chips can map approximately 65% and 96% of the known human Ser/Thr and Tyr kinases, respectively (Manning et al. 2002). Furthermore, the platform is able to detect about 18 out of 21 (86%) of the dual specificity kinases (Manning et al. 2002). This broad coverage enables researchers to simultaneously screen the activity of multiple kinases, providing a more complete understanding of the biological system being studied.

Additionally, the STK chip has been shown to have sensitivity for detection into the picogram range for many kinases (Manning et al. 2002). The coverage of kinases in the STK chip is also representative of the abundance of protein kinases in neurons. A study based on the Brainseq neuron database (https://www.brainrnaseq.org/) showed that the STK chip covers similar amounts of low (52%), medium (65%), and high (65%) abundance protein kinases in neurons (Sousa et al. 2017).

Taken together, these findings demonstrate that the PamGene platform provides a comprehensive and sensitive tool for studying kinase activity in a wide range of biological systems.

2.3 PamStation® 12

The Pamstation® 12 system consists of a robotic liquid handling system, a hybridization chamber, and a laser scanner. The robotic liquid handling system is used to apply samples, buffers, and fluorescent antibodies to the PamChips. The hybridization chamber is designed to hold up to three PamChips and provide the necessary conditions for kinase reactions to occur. The laser scanner is used to detect the fluorescent signals from the PamChips.

Figure 2.2: A close-up view of a PamChip® 4

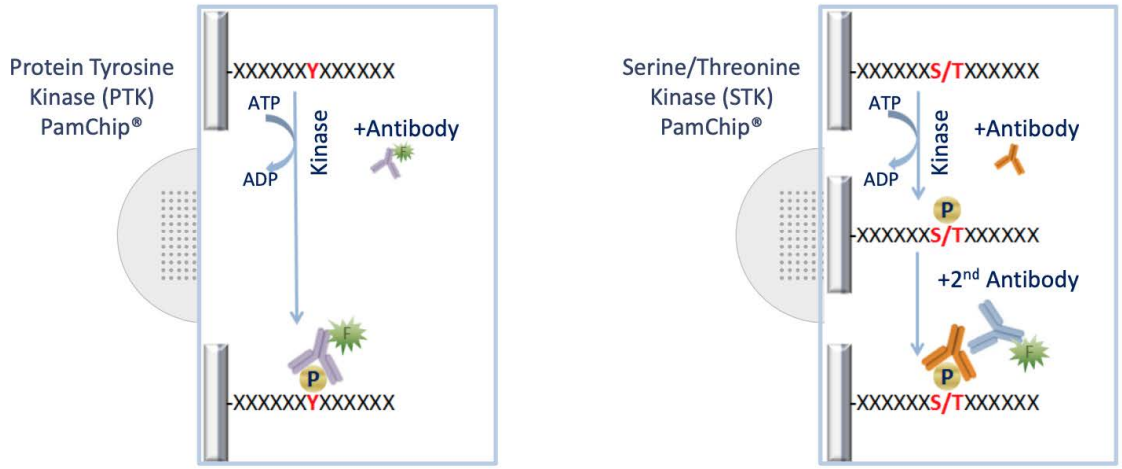

Figure 2.3: Detection Principle for the Protein Tyrosine Kinase (PTK) and Serine/Threonine Kinase (STK Chip)

2.4 Key Advantages

The Pamstation® 12 has several key advantages over other kinase activity profiling methods. First, the platform allows for unbiased detection of kinase activity, as it does not rely on prior knowledge of kinase-substrate interactions. Second, the real-time detection of kinase activity allows for the precise measurement of kinetic parameters, such as reaction rates and enzyme efficiency. Third, the platform is highly sensitive and can detect changes in kinase activity even in complex biological samples, such as cell lysates and tissue homogenates.

The Pamstation® 12 has been widely used in a variety of research fields, including cancer biology, neuroscience, and plant biology. For example, the platform has been used to study the activity of kinases in breast cancer cells and to identify potential therapeutic targets for cancer treatment. Additionally, the Pamstation® 12 has been used to profile the kinome of Arabidopsis thaliana, a model plant organism, and to gain insights into the regulation of plant development and stress responses.

In summary, the PamGene PamStation twelve is a powerful tool for the high-throughput profiling of kinase activity. The platform offers several advantages over other methods, including unbiased detection of kinase activity, real-time measurement of kinetic parameters, and high sensitivity for complex biological samples. The Pamstation® 12 is a registered trademark of PamGene International B.V. and has been widely used in diverse research fields to gain insights into the molecular mechanisms underlying complex biological processes.

3 Data Generation

Data generation for the PamGene® PamStation® 12 takes place in three steps.

1. *The Bench step* deals with acquiring the samples and preparing them for analysis on the PamChip4.
2. *The Imaging step* is the automated step wherein the sample is processed in the Pamstation12 and the image data is acquired
3. *The Preprocessing step* transforms the image data into flourescence values that can be interpreted by other software.

3.1 The Bench Step

The sample preparation protocol for the PamGene PamStation12 platform is provided by PamGene Corporation and follows standardized procedures to ensure reproducibility and accuracy of the results. Prior to analysis, samples are treated with protease and phosphatase inhibitors to control the catalytic activity and stability of kinases, which can be affected by environmental factors.

For sample preparation, PamGene corporation has provided standard protocols for Preparing Tissue Sections (Protocol 1140) (PamGene Corporation 2020), Preparing Lysates from Tissue Sections(Protocol 1150) (PamGene Corporation 2021) and Preparation of Lysates from Cell Lines and Purified Cells (Protocol 1160) (PamGene Corporation 2022). These protocols can be modified to account for specific experimental conditions.

3.2 The Imaging Step

During the imaging step, the sample and the reagent mixtures are added to the chip that is placed in the machine. The machine is then operated with the Evolve® 3 software. Evolve® 3 accepts a series of steps to perform as an input protocol and then executes that protocol using the machine.

The standardized protocol provided by PamGene Corporation, along with internal control tests and normalization strategies, help minimize technical variation and ensure the reliability of the data generated.

To ensure the sensitivity and reliability of the assay, PamGene International has performed various internal control tests. The technical variation between chips and runs is less than 9% and 15%, respectively, as determined by the coefficient of variability (CV). To account for any technical variation between runs, an internal control sample can be included in the analysis to normalize the data.

While the principle of the imaging step remains the same, the details differ between the PTK and STK machines. The general flow of these steps is as follows:

1. The chips are loaded in the PamStation® and blocking buffer is added.
2. Once the blocking step is completed, we add the sample mixtures.
3. After a reasonable amount of time has passed, the machine takes photographs of the flourescence activity.

3.2.1 PTK Imaging Protocol

For PTK PamChip® 4, the process is as follows:

1. Load the chips, blocking buffer and allow for blocking to complete
2. Add the sample mixture along with the PamGene® reagent mix that includes the fluorescent antibody
3. Two minutes after the addition of the sample mixture, the machine takes pictures every 5 minutes until the end of the process. The pictures are taken at the exposure levels of 5 msec, 25 msec and 100 msec.
4. Once all cycles are complete, another picture is taken at the exposure levels of 5 msec, 10 msec, 25 msec, 50 msec, and 100 msec.

3.2.2 STK Imaging Protocol

For STK PamChip® 4, the process is as follows:

1. Load the chips, blocking buffer and allow for blocking to complete
2. Add the sample mixture along with the PamGene® reagent mix that includes the fluorescent antibody
3. Allow the reaction mixture to circulate through the chip for 60 minutes
4. Add the detection mix with the second fluorescent antibody
5. Two minutes after the addition of the detection mix, the machine takes pictures every 5 minutes until the end of the process. The pictures are taken at the exposure levels of 5 msec, 25 msec and 100 msec.
6. Once all cycles are complete, another picture is taken at the exposure levels of 5 msec, 10 msec, 25 msec, 50 msec, and 100 msec.

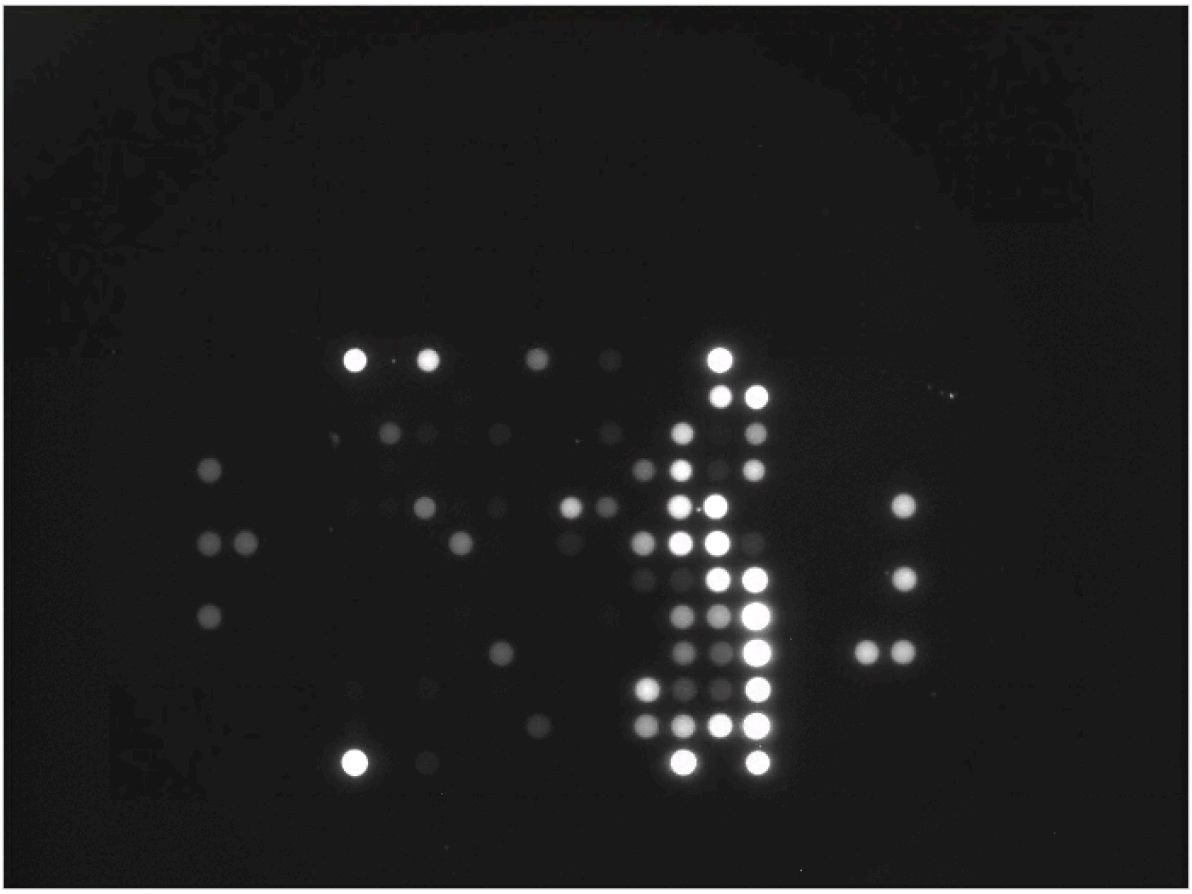

Figure 3.1: A single image taken by the PamStation® 12 during the imaging step

3.3 The Preprocessing step

The final step of data generation is preprocessing. During the preprocessing step the following tasks happen:

1. The image data is analyzed by the BioNavigator® Software to quantify the fluorescence values from the images.
2. Annotation data is added to the extracted value, allowing for identification of data at the resolution level of each image, each exposure level and each sample.
3. Background signal intensity is measured and a final $Signal - Background$ metric of intensity is calculated.
4. The level of *Signal Saturation* is measured for each spot on the array.
5. The data is output in the form of a table that can be processed by downstream software packages.

BioNavigator® is a software provided by PamGene® for performing image analysis and advanced kinase activity anlyses on the datasets.

An example of the outout from this step can be seen on Zenodo (Imami 2021).

4 Analytical Approaches

The PamStation® 12 Platform generates a large amount of data in a single experiment. The raw data obtained from the platform includes fluorescence intensity values for each well, at each cycle and exposure level. This data is rich in information, but in its raw form, it cannot be easily interpreted without further processing. The preprocessing step transforms the raw image data into fluorescence intensity values that can be analyzed using various computational methods. In this chapter, we outline different analytical approaches that can be taken to extract meaningful insights from the processed data. These approaches include upstream kinase analysis, unsupervised and supervised clustering methods, statistical approaches, pathway analysis, and visualization techniques. We also discuss the advantages and limitations of each approach. Through the use of these analytical approaches, we can uncover hidden patterns and relationships in the data and gain a deeper understanding of kinase signaling pathways.

Chapter 5 will walk us through all of the approaches outlined here on an example dataset.

4.1 Approaches

The approaches to analyze the data are driven by two related questions:

1. Which kinases are the most important kinases in my sample?
2. What, if any, are the functional consequences of perturbing a particular kinase?

A well-designed experiment can attempt to answer both these question within the same experiment. However, it is also possible to design separate experiments for each of these questions.

To simplify the process, the analytical techniques can be divided into three broad buckets:

1. Upstream Kinase Identification Techniques
2. Kinase Pathway Analysis Techniques
3. Visualization and Summarization Techniques

5 Case Study

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