Data analysis report

Peter Linders for Odile Filhol-Cochet

January 28, 2022

Table of Contents

[1. Introduction 2](#_Toc94269403)

[1.1. Study Information 2](#_Toc94269404)

[2. Results 2](#_Toc94269405)

[2.1. Differential analysis at the phosphosite level 2](#_Toc94269406)

[2.2. Differential analysis at the kinase level 3](#_Toc94269407)

[

# Introduction

The PamGene assays measure kinase activity in cell and tissue lysates by measuring the phosphorylation of peptide representations of kinase targets/ substrates (referred hereafter as phosphosites) that are immobilized on the PamChip® microarrays. The active kinases in the sample lysates will phosphorylate their target on the array. Generic fluorescent labeled antibodies that recognize phosphorylated residues are used to visualize the phosphorylation. There are two types of PamChip® microarrays; one has 196 protein tyrosine kinase (PTK) and the other 144 serine/threonine kinase (STK) phosphosites. The supplement to this report can be found as 211222\_PamGene\_Supplement.docx, hereafter referred to as Supplement.

## Study Information

We investigated the kinases implicated in treatments T1-96 and T2-97 by measuring the kinase activity of the MCF10A parental cells (C-WT), transduced with an shRNA targeting CSNK2B (T1-96) or a control shRNA (T2-97) using PamGene’s PTK and STK assays.

**Specific aim**

**Comparisons**

# Results

As a first step in the analysis and interpretation of the generated data, the quality of the dataset is assessed based on PamGene’s QC criteria for signal strength, number of peptides and the replicate variation. The data quality can either be “good” (i.e., green flag, ) “fair” (i.e., orange flag, ) or “poor” (i.e., red flag, ). The quality scores serve to assess the reliability of the data. Further details about the definition of QC flags and how they are applied to the data are provided in Supplement section 2.2.2.

We performed statistical analysis on log2 transformed signal intensities from the phosposite level data (Section 2.1). Prior to differential analysis at the kinase level (Section 2.2), VSN normalization was applied1. One outlier was identified in the T1-96 condition and was removed prior to subsequent statistical analyses.

## Differential analysis at the phosphosite level

As a next step, the phosphosites are identified that are statistically significantly different between conditions. We compare the differential phosphorylation of each phosphosite between 2 conditions using statistical tools (T-tests, ANOVA). The signal per phosphosite is the result of the net phosphorylation attributed to the activity of one or more kinases. This information is useful to place the upstream kinase analysis in context and helps get confidence in the data by showing the overall trend and direction of the effect of the treatment/ condition, as well as the effect size. See also Supplement section 2.3. The table below shows the statistical analysis for each comparison.

|  |  |  |
| --- | --- | --- |
| **Phosphosite level data1** | | |
| **Assay Type**  **Comparisons** | **PTK** | **STK** |
| **T1-96 v Control-WT** | 11 / 0 | 1 / 25 |
| **T2-97 v Control-WT** | 1 / 1 | 0 / 80 |
| **T1-96 v T2-972** | 29 / 1 | 71 / 1 |

1. Significance was obtained using a one-way ANOVA followed by a post-hoc Dunnett’s test, p<0.05. Blue indicates the number of phosphosites significantly less phosphorylated compared to control, while red indicates the number that are more phosphorylated.

2 Significance was obtained using a two-sided unpaired Student’s T-test, p<0.05.

For this study we sufficient changes in PTK phosphorylation to draw valid conclusions on the kinase level data. We observed a strong increase in STK phosphorylation, which could be a consequence of the addition of too much ATP in the reaction. Caution must here be applied while interpreting the STK data. Corresponding volcano plots can be found in Supplement section 2.3.

## Differential analysis at the kinase level

Each kinase phosphorylates multiple phosphosites on the PamChip. To address which kinases are responsible for the phosphorylation differences between conditions, we perform an Upstream Kinase Analysis (UKA), explained in Supplement section 2.4. UKA uses sets of phosphosites, rather than singular ones, which are linked to each kinase to predict differential kinase activity. The main results from UKA are shown in the plot below. The kinome tree overview, as well as numerical data and score plots can be found in Supplement section 2.4.

|  |  |  |
| --- | --- | --- |
| **Kinase level data3** | | |
| **Assay Type**  **Comparisons** | **PTK** | **STK** |
| **T1-96 v Control-WT** | Lyn, InSR, LTK, Arg, IGF1R / FmS/CSFR, CSK, FLT4, FLT1, TRKB | ADCK3, CDK9, Pim1, CDK11, CDKL2 / PKG2, PKAα, PKG1, p70S6Kβ, PRKX |
| **T2-97 v Control-WT** | Ron, ITK, Ret, MEK1, Tyro3/Sky / Fgr, Lck, Yes, CTK | ADCK3, RSLK2, PAK1, DAPK3, PKCθ / PKG1, CDK10, p70S6Kβ, PFTAIRE2, IKKα |
| **T1-96 v T2-97** | Lyn, Fgr, Lck, CHK1, InSR / FLT4, JAK1b, TRKB, FLT1, TRKC | p38β, AurA, IKKε, CDK5, CDK11 / PAK1, CDK4, ERK1, RSK3, DCAMKL1 |