

Kinome Profiling;

An unbiased approach for tracking
changes in cellular signaling



Fundamental and Discovery Research

- Pathway elucidation
- Therapy /inhibitor profiling
- Disease model characterization
- Target Discovery
- Immune cell characterization

Biomarker and Clinical Research

- Therapy response /resistance biomarker
- Disease classification biomarker
- Prognostic Biomarker- **IOpener**

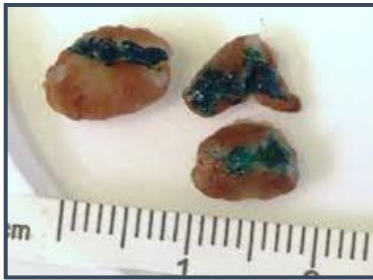
Sample input: fresh (frozen) cell material



Primary and cultured cells

PBMCs, WBC, platelets, Bone marrow

Primary cells, Culture cells (adherent or suspension)

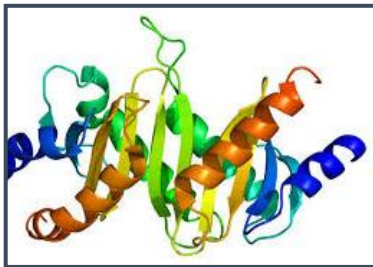


Primary biopsies, slices, clinical samples

Freshly frozen (alternatively Tissue-Tek)

Tumor content advise >70%

Different tissues (FNA) e.g. Colon, lung, liver, breast, brain, prostate, skin, thyroid, CSF

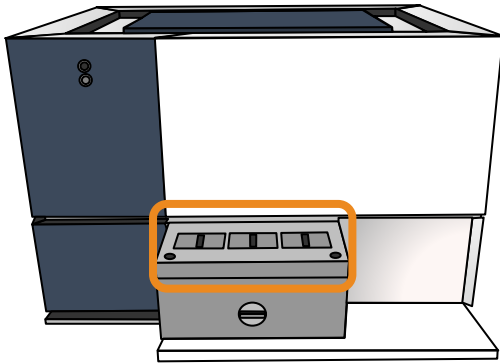


Purified proteins

Recombinant kinases

Collect samples
over time, fresh
freeze, and store
for long periods at
-80°C

PamStation 12[®]



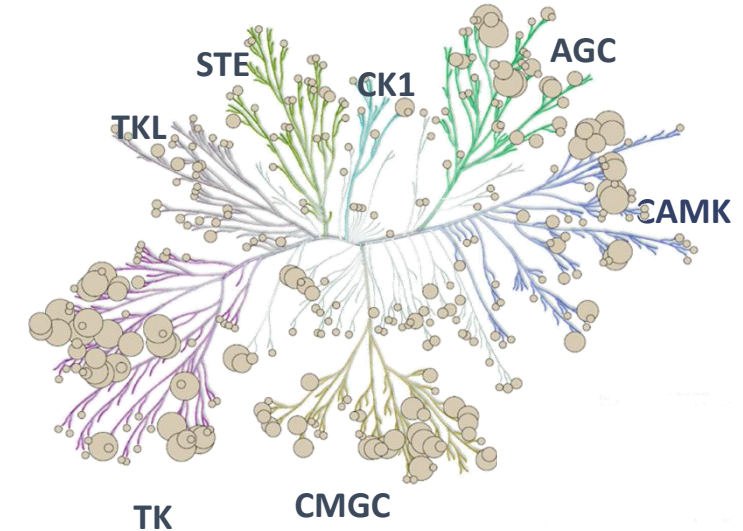
Pamchip 4[®]



Array spots



Bionavigator tool



Reader instrument

- Load 3 Pamchips per experiment (12 samples)
- Combine 4 units together to run 48 samples in parallel.

Array types

- Serine/Threonine (STK) Pamchip with 144 substrates
- Tyrosine Pamchip (PTK) Pamchip with 196 substrates

Sensitive read-out

- 0.5 – 5.0 μ g total protein is required (100.000 – 200.000 cells)
- Generic antibody detecting all phosphorylated substrates

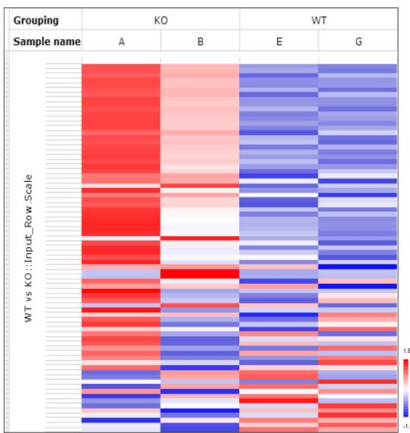
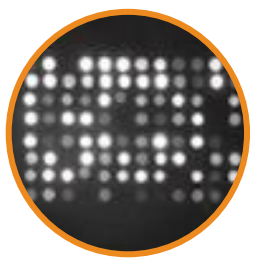
Robust interpretation

- Kinome coverage >380 Kinases
- Mapped from six major databases

Substrate phosphorylation to kinases and cellular signaling



Phosphorylation profile

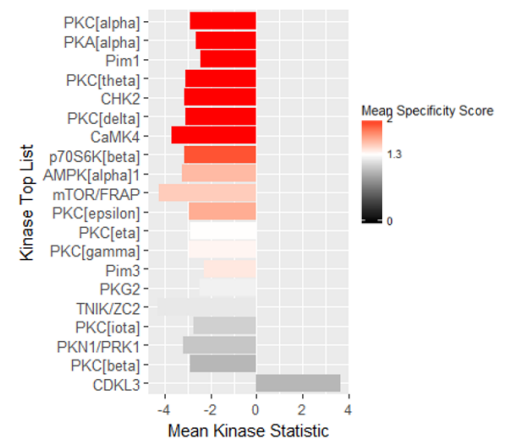


Bioinformatic analysis pipeline

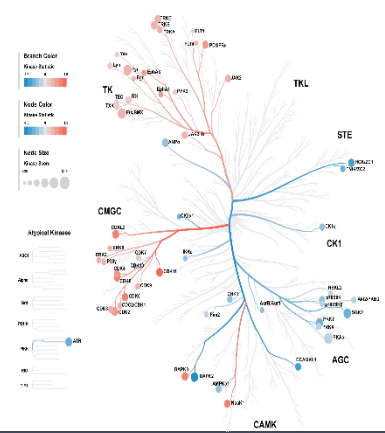


Top predicted kinases: list and plots (covers >380 functional kinases)

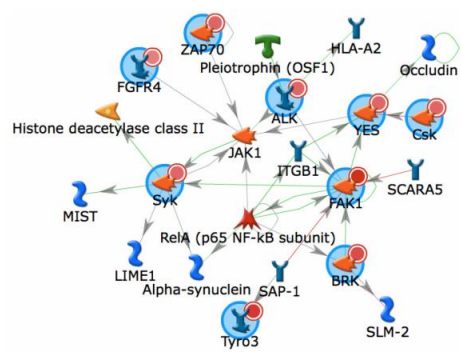
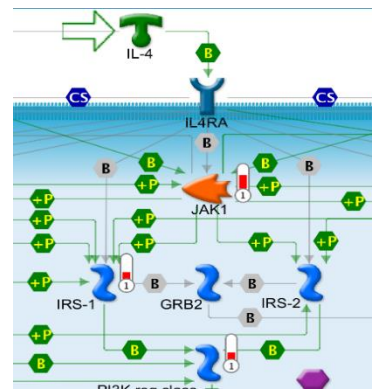
Ranking	Kinase Name	Kinase Score
1	PKC[alpha]	3.06
2	PKA[alpha]	2.86
3	PIM1	2.78
4	PKC[theta]	2.61
5	CHK2	2.55



Kinome tree



Pathways and Network Analysis



- **Full-length kinases** – We measure the kinase activity of full-length proteins from lysates of various cell lines and tissues, a feature not offered by other recombinant-based kinase activity assays.
- **Wide coverage** – Covering 380+ active kinases PTK and STK
- **Reproducibility** – The straightforward protocol ensures consistent results over time.
- **Sensitive** – Only small amounts of protein input (0.5 to 5 μg per array), making it more sensitive than alternative approaches.
- **No specific antibodies** – Data quality is independent of the specificity of phospho-antibodies since specificity is determined by the phosphosites.