Adapting high-content microscopy screening to study dynamic signaling mechanisms

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Abstract

Recently, we have demonstrated the feasibility of performing quantitative FRET/FLIM based genetic screens to assess signaling dynamics in the arrayed format[1]. Such functional microscopy screens provide a wealth of information on cellular signaling in real time. However, due to various reasons, large genetic screens are difficult to combine with multi-well dynamic read-out. Thus, here we aim to perform dynamic screens in the pooled format. We demonstrate a proof-of-concept pooled 'dynamic screen' to identify bottlenecks and optimize the pipeline, taking the cAMP pathway as an example.

Using cells expressing our Epac (FRET) sensor and a photoactivatable mCherry , we generated 2 knockout (KO) cell lines and mixed them with wildtype cells. Then, we imaged, segmented, analysed lifetime traces from single cells and detected hits, i.e., the KO cells that show aberrant cAMP kinetics, using custom written Fiji scripts. Next, coordinates of the 'hit cells' were determined and they were photoactivated, following which they were FACS sorted and grown out. Revalidation tests were performed to calculate false positive rates and overall efficiency of the pipeline.

We could reliably identify the KO populations as "hit populations" with a 10 percent false positive rate, therefore setting the stage to scale up to bigger CRISPR screening libraries. In conclusion, we present a robust FLIM enabled screening platform that provides detailed kinetic analysis of cellular signals in individual cells, paving the way for genome wide screens into the determinants of signal transduction dynamics.

References

1. Harkes, R., Kukk, O., Mukherjee, S., Klarenbeek, J., van den Broek, B. and Jalink, K., 2021. Dynamic FRET-FLIM based screening of signal transduction pathways. *Scientific Reports*, *11*(1), p.20711.