TECHNICAL NOTE

Utilizing CodePlex for Single-Cell Assay Development

Optimization for stimulation conditions, donor selection, and new cell types

In this Technical Note we outline:

- Optimization of stimulation conditions
- Optimization for donor selection
- Optimization for new cell type feasibility

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Prep, Run, Analyze

Before starting your single-cell experiments, CodePlex can be used to optimize stimulation conditions and donor selection, as well as screen for new cell types to analyze on Bruker platforms. By leveraging CodePlex's highly multiplexed fully automated abilities, selecting the best candidates in the most optimal conditions now takes days instead of weeks. Optimization of stimulation conditions on CodePlex prior to proceeding with single-cell experiments will help ensure that you achieve a robust signal within your downstream experiments, and will lead to clarity of results in your analysis.

Optimization of Stimulation Conditions

The optimization of stimulation conditions will be critical to achieve clear biological insights. The concentration of stimulation conditions, or the ratio of effector cells to target cells, can affect the robustness of immune response, and the viability of the cells. The duration of stimulation time of a sample may also affect the immune response. In order to fully capture the immune response of your samples, you can utilize CodePlex to optimize for several different conditions.

Workflow (Figure 1):

1. Each condition is prepared in a well plate and incubated according to experimental design

2. Stimulus is removed, cells are pelleted, supernatant is collected and saved, cells are washed and re-plated in complete RPMI

3. Cells are incubated without stimulus for 13h to mimic IsoLight single cell workflow

4. Supernatant is collected at the end of incubation

5. Supernatant is pipetted into the CodePlex chip and run on Bruker's IsoSpark or IsoLight system

Using CodePlex to Optimize Stimulation Conditions

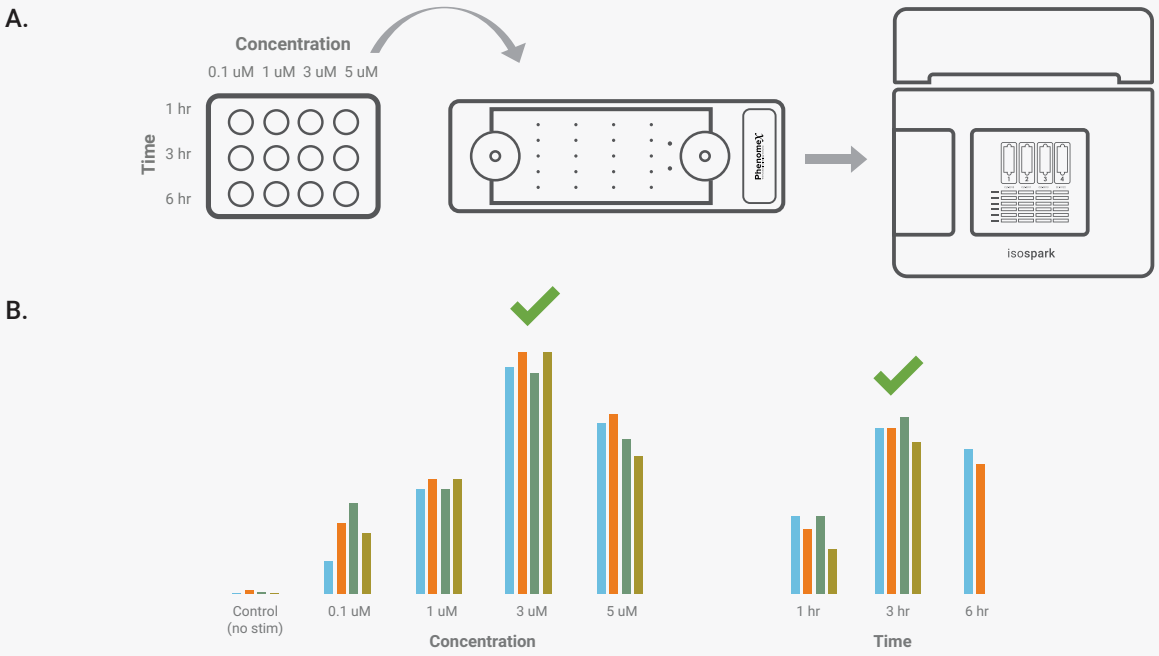

Figure 1 | a) Workflow for stimulation optimization. b) CodePlex can be used to optimize stimulation concentrations and/or time for downstream experiments.

Using CodePlex to Screen for an Ideal Donor for Positive Control

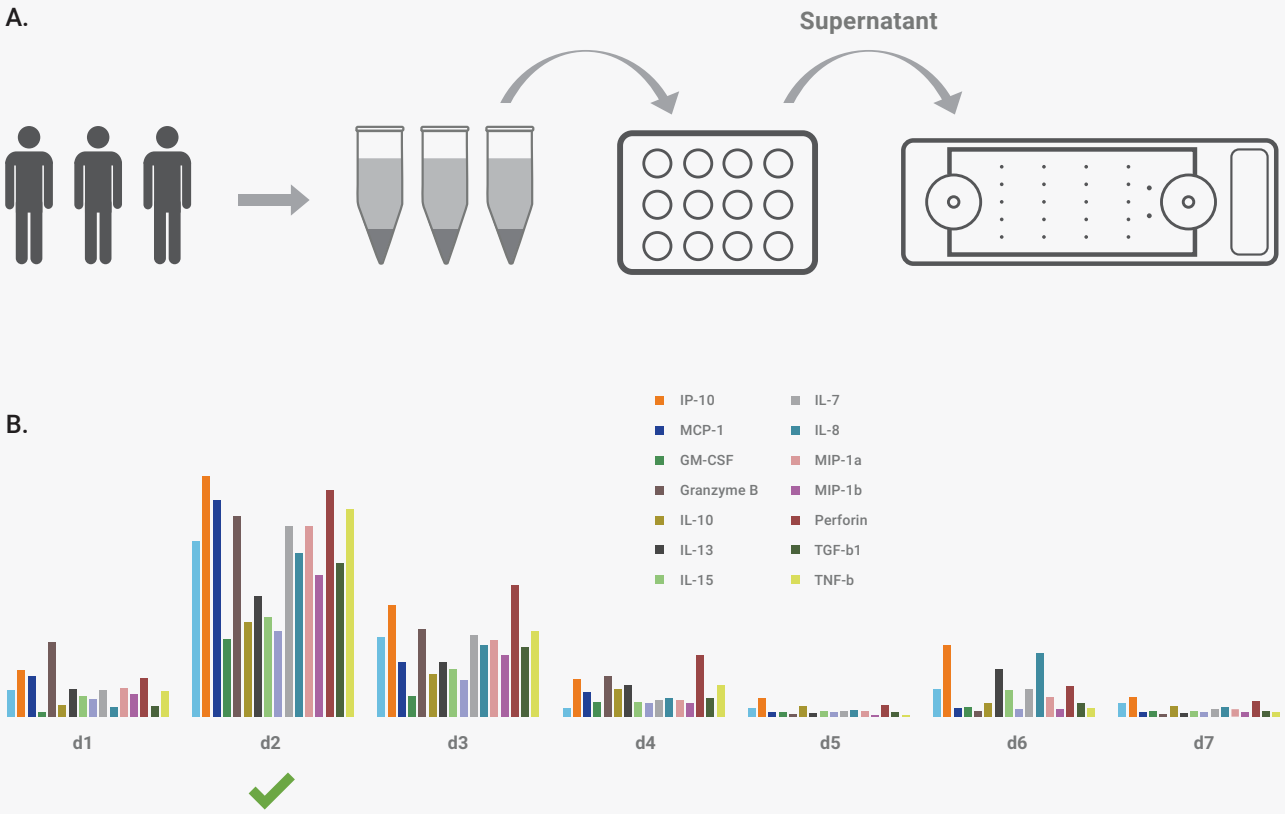

Figure 2 | a) Workflow for donor screening. b) CodePlex can be used to optimize for donor selection. The donor cells that produce the most robust secretions are generally good donor samples for use as controls.

After obtaining the results, identify the concentration and timepoint that led to the most robust immune response. Proceed to Bruker single-cell experiments with these stimulation conditions.

Optimization for Donor Selection

Optimization for robust donors will be critical for identifying and selecting donors to use as positive controls. These donor controls should provide a robust immune readout.

Due to biological heterogeneity, donor response can be variable, and CodePlex optimization can help identify which donor to use as controls in experimental selection.

Workflow (Figure 2):

1. Cells from each donor are collected and stored

2. Cells from each donor are stimulated for that particular cell type, then the supernatant is pipetted into the CodePlex chip and run on Bruker's IsoLight or IsoSpark systems.

3. Run and analyze the donor response

After obtaining the results, identify which donor shows the most robust immune response. Proceed to Bruker single-cell experiments with these donors as a positive control.

Using Codeplex to Identify the Feasibility of New Cell Type Usage

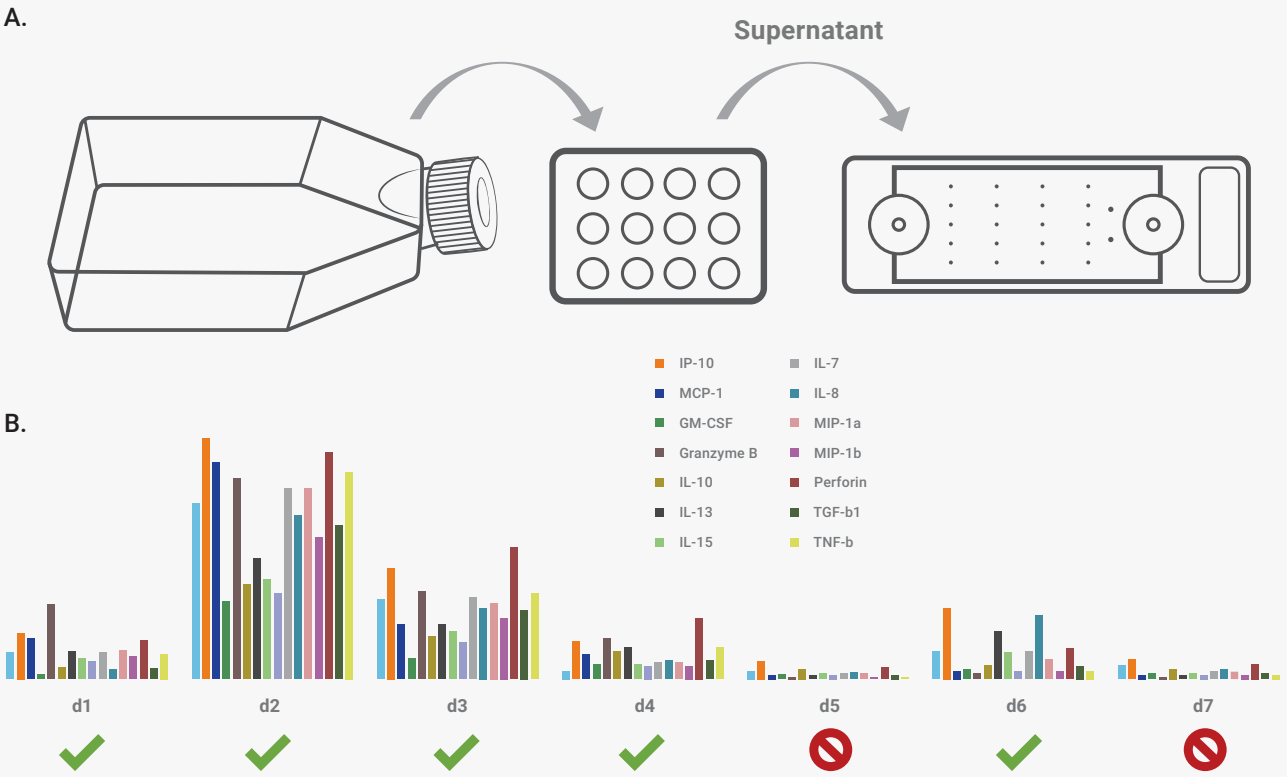

Figure 3 | a) Workflow for new cell type feasibility. b) CodePlex can be used to test the feasibility of new cell types on the CodePlex and IsoCode panels. Combined with stimulation condition optimization, the cell types that show positive secretion of cytokines can be used on the CodePlex and IsoCode panels.

Optimization for New Cell Type Feasibility

When running Bruker panels on cell types that have not yet been validated, optimization for immune response will be critical for obtaining clear biological insights on these new cell lines and cell types. Various cell lines and cell types have varying degrees of cytokine secretion.

Identify the panel you would like to test on new cell types. The optimization will be performed with this panel. The panel should cover a range of cytokines that you expect to be secreted by the cell type of interest. Before proceeding to single-cell experiments, it is critical to verify these cytokine panels for new cell types and the optimized stimulation conditions.

Due to biological heterogeneity, cellular responses can be variable. CodePlex optimization can help validate new cell lines and cell types as well as optimize stimulation conditions, such as concentration and times.

Workflow (Figure 3):

1. Identify the panel

2. Each cell type is prepared within a 96-well plate

3. The cell type is stimulated via the chosen compound of interest for the time of interest

4. The supernatant is collected and pipetted into the CodePlex chip and run on Bruker's IsoSpark or IsoLight Systems*

***NOTE:** It is important to ensure that cells can remain viable in RPMI at 37° 5% CO2 for a minimum of 13-13.5 hrs

Prep, Run, Analyze

After obtaining the results, identify the cell types that show immune response, and then optimize for stimulation conditions via workflow in Figure 1. Proceed to Bruker single-cell experiments with these new cell types.

Conclusion

By using CodePlex's highly multiplexed automated workflow system, you have the ability to optimize your experiment to ideal concentration and time, positive control donor selection, and identify new cell line and cell type feasibility.

Please contact your local FAS for any of your assay development requests.