Duolink® PLA for Detection of Protein Interactions and Modifications in Immune Cells by Flow Cytometry

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Introduction

Duolink Proximity Ligation Assay (PLA) has revolutionized the study of endogenous protein function in biomedical research. This technology can be combined with qualified antibodies to outperform traditional immunoassays through sensitive in-situ detection and visualization of individual endogenous proteins, protein-protein interactions (PPI) and protein-nucleic acid interactions including weak and transient interactions as well as post-translational modifications (PTM). Through rolling circle amplification, Duolink provides a thousand-fold signal amplification, enabling single molecule sensitivity without any overexpression or genetic manipulation. Duolink can be used to easily assess the activation status of specific proteins including low-abundance proteins and signaling pathways in established cell lines as well as patient biopsies or xenografts. Duolink was originally developed for use with bright-field or immunofluorescence microscopy, but we developed techniques to combine Duolink with flow cytometry. Coupling Duolink with imaging flow cytometry enables rapid in-situ visualization of protein events in a high-throughput manner. This poster presentation highlights latest developments and applications of this powerful, investigative tool in the study of immunology from current literature.

References:
- Avin et al. (2017). Quantitative analysis of protein-protein interactions and post-translational modifications in rare immune populations. *Nature Communications,* 8:1524.
- Burns et al. (2017). High-throughput precision measurement of subcellular localization in single cells. *Cytometry Part A,* 91A: 180.
- Lof et al. (2017). Flow Cytometric Measurement of Blood Cells with BCR-ABL1 Fusion Protein in Chronic Myeloid Leukemia. *Scientific Reports,* 7: 623.
- Gordon et al. (2020). A SARS-CoV-2-Human Protein-Protein Interaction Map Reveals Drug Targets and Potential Drug-Repurposing. bioRxiv preprint doi: https://doi.org/10.1101/2020.03.22.002386

Methods

How Duolink flowPLA Works

1) Two primary antibodies from different species bind to protein(s) of interest (within 40nm proximity)
2) Secondary antibodies with conjugated oligos (PLUS and MINUS) bind to primary antibodies
3) Connector oligos and ligase form closed circles
4) Rolling circle amplification (1000x) by DNA polymerase
5) Hybridization of oligos with fluorescent or HRP detection tags
6) Imaging and data analysis using a high-content screening imager, flow cytometer, or imaging flow cytometer

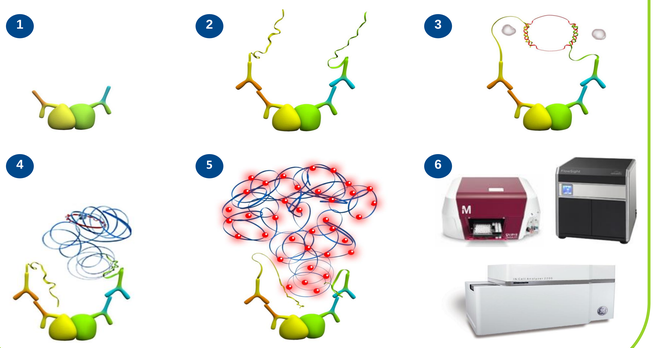

Fig. 1: Detecting PPI by Imaging Flow Cytometry

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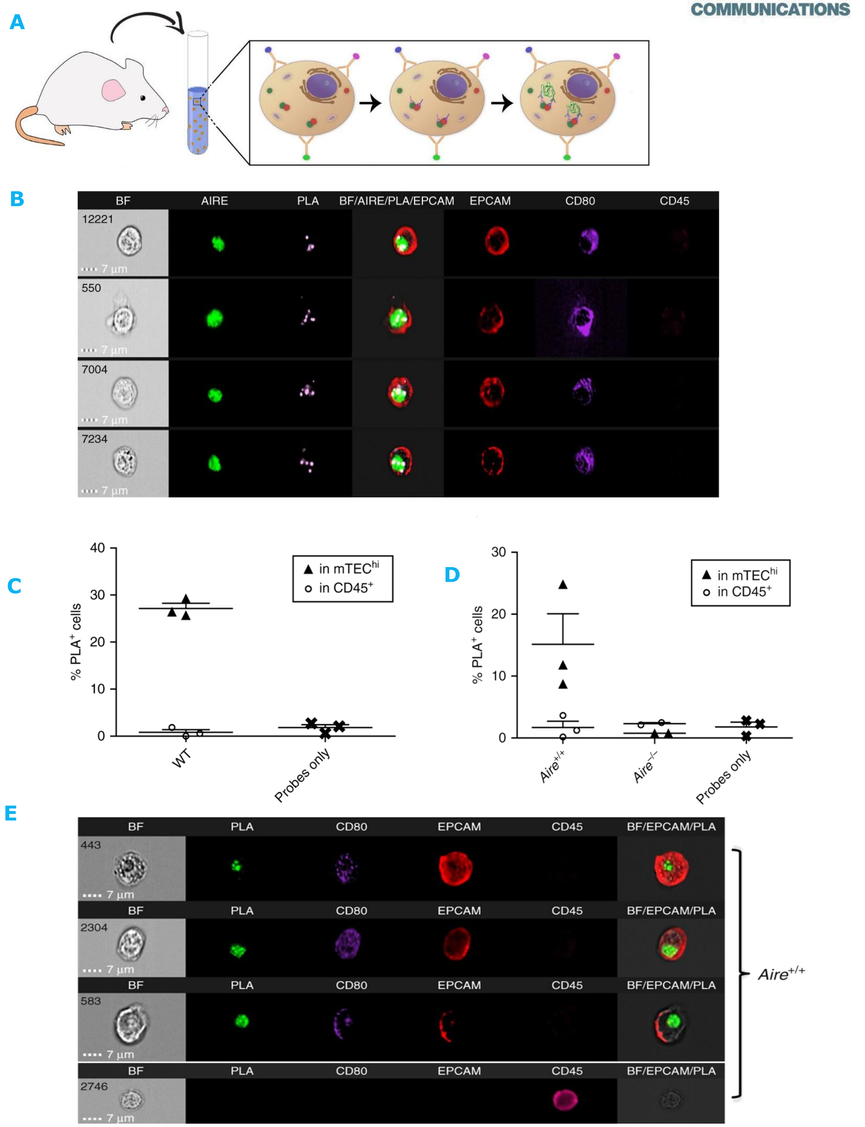

Fig. 1. Detection and quantification of Aire-Sirt1 interaction and Aire oligomerization. **A.** Schematic illustration of experimental protocol. **B.** Representative images of **Aire-Sirt1 interaction** in CD80^{hi} mTECs (mTEC^{hi}): bright field (BF), Aire-AF488, PLA of Aire-Sirt1 interaction (Far-red), BF/Aire/PLA/EpCAM overlay, EpCAM-APC/Cy7, CD80-Pacific Blue (PB) & CD45-PE/Cy7. **C.** Quantitative population analysis of **Aire-Sirt1** interaction in mTEC^{hi} or CD45+ cells isolated from WT mice, compared to probes only background. Each point on the graph represents an averaged value of 2-4 mice from an independent experiment +s.e.m. **D.** Quantitative population analysis of **Aire oligomerization** in mTEC^{hi} or CD45+ cells isolated from Aire$^{+/+}$ and Aire$^{-/-}$ mice, compared to probes only background. Each point on the graph represents an averaged value of 2-4 mice from an independent experiment +s.e.m. **E.** Representative images of **Aire oligomerization** in mTEC^{hi} and CD45+ populations: BF, PLA (green), CD80-PB, EpCAM-APC), CD45-APC/Cy7) & BF/PLA/EpCAM overlay (Avin et al. 2017).

Fig. 2: Identifying PTM by Flow Cytometry

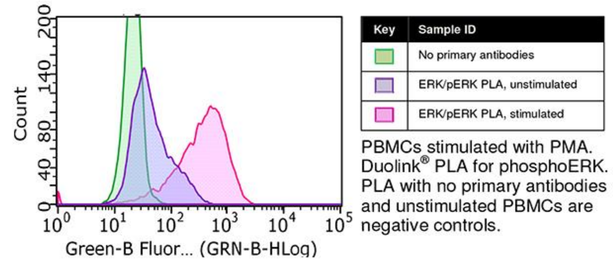

Fig.2. Duolink PLA allowed detection of ERK phosphorylation (pT202/pY204) in response to PMA (phorbol 12-myristate 13-acetate) stimulation of PBMCs by flow cytometry.

Fig. 3: Detecting Fusion Proteins in Clinical Samples

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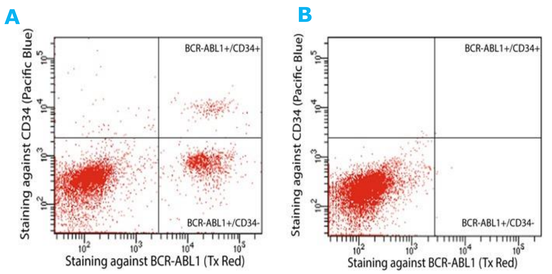

Fig. 3. Simultaneous staining of PBMCs for BCR-ABL1 (PLA) and CD34. **A.** Dot-plot of cells from a newly diagnosed CML patient reveals three distinct populations: major double-negative population, one population (13.8%) expressing BCR-ABL1 but not CD34, and one population (1.8%) positive for both CD34 and BCR-ABL1. **B.** The same analysis was performed on a healthy individual as a negative control (Lof et al. 2017).

Fig. 4: Detecting Protein-DNA Interactions

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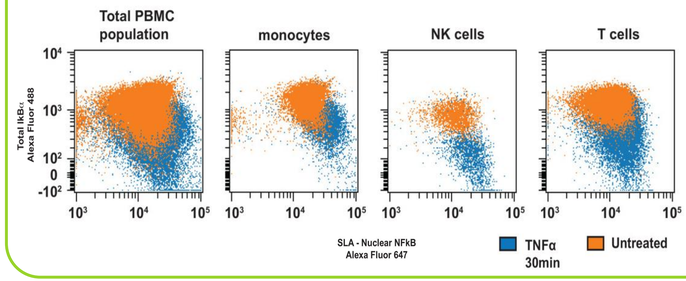

Fig. 4. NF-κB PLA coupled with intracellular staining for negative regulator IκBα in PBMC subsets. PBMCs were treated with TNFα for 30 min. PLA signal from NF-κB interaction with dsDNA indicates nuclear NF-κB (Burns et al. 2017).

Discussion

- Duolink PLA is a versatile technique which can be coupled with brightfield/IF microscopy or flow cytometry to detect PPI, PTM, protein oligomerization, protein fusions, protein-nucleic acid interactions, and low-abundance proteins in cells and tissues without any genetic manipulation or over expression.
- It can be used at all stages of research including pre-clinical models and clinical samples.
- It is often used to validate in-vitro data in an in-vivo model. For example, it can be used to validate the 332 SARS-CoV-2-human PPIs which were recently identified (Gordon et al. 2020).
- Duolink enables us to study PPI by flow cytometry for the first time. Imaging flowPLA provides rapid in-situ visualization of protein events at the single-cell level in a high-throughput manner.
- Duolink flowPLA is, therefore, set to become an essential part of an immunologist's toolbox.