Sensitive and high throughput multiplexed immunoassays for biomarker discovery in biobanked samples using proximity ligation assays and qPCR

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Simple assay procedure

1. Incubate sample with proximity probes
   30-60 min

2. Add, connector, ligase and PCR components
   <5 min

3. Perform quantitative PCR
   60-120 min

The first proximity ligation assay, PDGF-BB

Fredriksson Nature Biotech 2002
Diagnostic challenge in early cancer detection (screening)
- detection specificity (low false positive rate)
- detection sensitivity (low false negative rate)

Solution...
- the use of multiple biomarkers combined in a panel can improve diagnostic accuracy

Research challenge in finding more biomarkers and biomarker panels
- Limited supply of precious biobanked samples
  - Especially with repeat analyses
- Large number of candidate biomarkers
  - Tens or even hundreds

Solution...
- high throughput and multiplexed protein detection tools.
Multiplexed in solution PLA
- a tool for high throughput biomarker research

Features of in solution PLA using antibodies
- high sensitivity
- low sample consumption
- wide dynamic range

Detection of VEGF

Fredriksson et al Nature Methods 2007
Multiplexed in solution PLA

Dual recognition specificity
4 multiplex panels of 7-plex assays

monoclonal or affinity purified polyclonal antibodies

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Pilot biomarker study in pancreatic cancer
A pilot biomarker study in ovarian cancer

Ovarian cancer samples, reproducibility and assay modularity

Cases, RED
Controls, Blue

Luminex data from Scholler et al
PROACTIVE EU-funded technology development and biomarker project

Development of a high throughput plasma biomarker research pipeline
- To profile 180 putative plasma biomarkers
- Pilot project in colorectal cancer detection
- Multiplexed and homogeneus proximity ligation
- High throughput qPCR readout

- Largest ever effort to find new low abundance cancer biomarkers in one and the same biobanked sample collection
- Enabled by low sample consumption of PLA and easy assay development in multiplex (no antibody cross-reactivity)
- €3 million in funding from the European Union for a three year effort
- Will establish a research and data analysis infrastructure for use in other disease areas as well

PROACTIVE partners

Partners

Assay development and reagents : Olink and Innova Biosciences
Statistics : Uppsala University, Integromics
Diagnostics expertise : Fujirebio Diagnostics
Samples and clinical expertise : Copenhagen University
**In situ** proximity ligation

- Localized detection of protein interactions in fixed cells and tissue

- Visualization of signal transduction by localized single molecule detection of protein interactions.

- Objective quantification by counting bright fluorescent spots in each single cell.

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**in situ** proximity ligation assay (PLA) by Duolink™

Her2-Her3 interaction in breast cancer tissue

Single molecule counting

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In situ PLA-Duolink

Incubate with target specific primary antibodies from two different species

In situ PLA-Duolink

Add PLA probes PLUS and MINUS
**In situ PLA-Duolink**

Hybridize connector oligos

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**In situ PLA-Duolink**

Ligation to form a complete DNA circle
In situ PLA-Duolink

Template for rolling circle amplification
**In situ PLA-Duolink**

Hybridization of detection probes

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**SMAD complex formation after TGF-β stimulation**

Images from microscope

Images from BlobFinder software

Untreated MEF cells  |  TGF-β treated MEF cells

In collaboration with Katerina Pandoli, Uppsala University
Relative quantification of interactions using image analysis freeware BlobFinder

Disruption of protein-protein interaction

c-Myc/Max interaction in fibroblasts

Soderberg et al. Nat Methods, 2006
A novel complex between VEGF-R2 and PDGF-Rβ, analyzed using Co-IP and western blotting

Dr. J. Greenberg, Dept of Surgery, Prof. D. Cheresh, et al, Moores Cancer Center, UCSD
*Nature advance online publi. 9 Nov. 2008, doi:10.1038/nature07424*

Confirmation of the VEGF-R2 and PDGF-Rβ complex using Duolink

Dr. J. Greenberg, Dept of Surgery, Prof. D. Cheresh, et al, Moores Cancer Center, UCSD
*Nature advance online publi. 9 Nov. 2008, doi:10.1038/nature07424*
Options for implementing *in situ* PLA

**Duolink research reagents**

Available from our webshop and distributors
[www.olink.com](http://www.olink.com)

Cambridge Bioscience (United Kingdom)

**Contract research projects**

Performed at Olink Bioscience

Assay development and sample analysis service

Developed assays can be transferred to the ordering lab

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Benefits of using *in situ* PLA and Duolink:

- Highly specific target recognition
  - dual recognition assay design

- A prominent fluorescent spot
  - digital counting, as opposed to measuring signal intensity

- No need for overexpression of genetically modified proteins

- Protein interaction quantification in tissue samples and tissue microarrays
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ENLIGHT FP6, PROACTIVE FP7

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Thank you!

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