Technology Development: *in situ* High-Throughput Analysis of Protein-Protein Interactions for Cereals

Naohiro Kato, Louisiana State University

The project is conducted as a two-year pilot project at Louisiana State University.

**Objectives**


2. Conduct proof-of-concept experiments using selected protein-pairs in rice.

**Outcome**

This project will provide molecular tools and protocols for the rice community.
The system we wish to establish in this project

**pENTR-geneA**

**pDuEx-Prey**

**Day 1**

**pENTR-geneB**

**pDuEx-Bait**

*Gateway® in vitro recombination reaction*

*Cre-**loxP** in vitro recombination reaction*

**X**

Day 5

**pDuEx-Bait-Prey**

Bombard the DNA into rice using the biolistic DNA delivery system

Treatments (optional)

Examples: phytohormon, stress

Detect the PPI at tissue levels with a CCD or a scanner

In situ PPI analysis

Detect the PPI at subcellular levels with a fluorescence microscope

The basic structure of plasmids used in this project

**pDuEx-Prey** (8.7 kbp)

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<thead>
<tr>
<th>loxP</th>
<th>Promoter</th>
<th>attB</th>
<th>ccdB/CmR</th>
<th>attR1</th>
<th>Tga</th>
<th>Terminator</th>
<th>CmR</th>
<th>loxP</th>
<th>SacB</th>
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**pDuEx-Bait** (7.3 kbp)

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- For the **Gateway® in vitro recombination**
- For the **Cre-**loxP in vitro recombination

• Plasmids for N-terminal tags were also constructed
• Each functional unit is able to be replaced by commonly used restriction enzymes
pDuEx-Bait-Prey Cloning procedure

Day 1
- LR reaction
- Transformation of E. coli

Day 2
- Picking up colonies from LB plates and culturing in LB media
- Plasmid Mini Prep
- Restriction enzyme digestion and Gel electrophoresis
- Confirmation of pDuEx-Bait and pDuEx-Prey plasmids

Day 3
- Cre-loxP reaction
- Transformation of E. coli

Day 4
- Picking up colonies from LB plates and culturing in LB media
- Plasmid Mini Prep
- Restriction enzyme digestion and Gel electrophoresis

Day 5
- Confirmation of pDuEx-Bait-Prey plasmids

Protein-Protein Interaction assays used in this project

For subcellular levels:
- FRET
  - no-interaction
    - Prey (CFP), Bait (YFP)
    - Blue light, Cyan light, Yellow light
  - interaction
    - Prey (CFP), Bait (YFP)
    - Blue light, Cyan light, Energy Transfer, Yellow light

For tissue levels:
- Luciferase complementation assay
  - Prey (NLuc), Bait (CLuc)
  - Luciferrin
  - Light
A resulted image of the FRET analysis in an leaf epidermal cell of *Arabidopsis*.

The interaction of H2A-H2B was detected in the nucleus.

Tested plasmid

**H2A-H2B: interacting pair**

Or

**H2B-PAN: non-interacting pair**

Full length Firefly luciferase (utilizing a different substrate)
The PPI of H2A-H2B was detected in an onion epidermal cell layer.

The PPI of H2A-H2B was detected in leaves of Arabidopsis growing in soils.