



**Sustainable World,  
Stable GM plant, and  
Ethanol Yeasts  
by NAIST of Japan  
to be presented in  
BioAsia2007**



## Nara Institute of Science and Technology (NAIST) Scientist will be present in the BioAsia 2007

**Date: November 7-9, 2007**

**Place: Queen Sirikit National Convention Center  
Bangkok, Thailand**

Topics:

### **1. Invited Presentation at the conference:**

“Role of Plant Biotechnology for Establishment of Sustainable World”  
by Professor Atsuhiko Shinmyo  
Wednesday 7, 14:30~15:00

### **2. Seminar Presentations**

“Stable plant modification and Yeasts for energy production”  
by Dr. Hiro Kawamoto and Ms. Itsuki Kashin  
Thursday 8, 11:00-12:00

- a. New technologies for gene transfer in plants.
- b. Development of yeast that is applicable for ethanol production..

### **3. NAIST's Booth**

Come visit us to discuss above technologies and others. We can show you NAIST's technologies on Plant Biotechnology.

## Presenters

### Atsuhiko Shinmyo



**Professor**  
**Laboratory of Metabolic Regulation in Plant Cells**  
**Graduate School of Biological Sciences**  
**Nara Institute of Science and Technology**

**Majors in the Biotechnology and the Plant metabolic engineering.**  
**His main interests are as follows;**

- **Development of the expression systems for efficient gene transfer in plants**
- **Molecular breeding of stress-tolerant plants**
- **Utilization of vesicular transport**
- **Bio- and phyto-remediation**

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## Presenters

### HIRO KAWAMOTO



**Professor**  
**Intellectual Property Division**  
**Industry-Government-Academia collaboration Group**  
**Nara Institute of Science and Technology**

**BS from Kyoto University and Ph.D. in EECS from UC Berkeley. Has been promoting NAIST's insect-resistance, photosynthesis-enhancement, and fermentation technologies to the US bio-ethanol industries. Was with Panasonic, UC Berkeley Teaching Staffs, RCA David Sarnoff Research Center, Sony, Sharp, Silicon Image. Fellow of IEEE. Founder and Chairman of Princeton Community Japanese Language School, Princeton, NJ**

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# Summary of Presentation

## 2. Seminar Presentations

“Improved Gene-Modification for Plants and the Yeasts for Ethanol Production”

By Hiro Kawamoto and Itsuki Kashin

### a. Technologies for improving transgene expressions and increasing the productivity by enhancing disease/stress-resistance

The technique of introducing exogenous genes into plant cells has long been established and has enabled us to modify plant functions. However, there are problems such that some genes do not express consistently or the gene product does not accumulate to a high concentration.

We have developed technologies that can solve these problems and enable us to use gene modifications effectively; the technologies that can

1. express the transgene stably in plants, and
2. promote the transgene expression at the translational level.

Also important is to increase the productivity of plants by protecting them from diseases or environmental stresses. We have elucidate the mechanism of some disease-resistance and stress-tolerance system in plants, and thus have developed disease-resistant or stress-tolerant plants.

### NAIST Researchers involved in the development of these technologies



[Prof. A. Shinmyo](#)



[Prof. K. Shimamoto](#)



[Prof. A. Yokota](#)



Prof. H. Sano  
(retired)



## Summary of Presentation

### **b. Improvement of Yeasts and Bio-Ethanol Production**

Yeasts have been improved over years for the purpose of increasing the efficiency of industrial fermentation such as alcohol production and bread making. In those processes, yeasts receive many stresses from alcohol, freezing, and others. We have modified the yeasts for the purpose of improving the stress resistance. We have found that genes, Proline Synthases and Mprs, strengthen the stress resistance against ROS (Reactive Oxygen Species), and thus have generated yeast strains that are tolerant to such stresses.

These yeasts will be helpful in the alcohol production with the method of fermentation such as sake making and bio-ethanol production.

### **NAIST Researcher involved in the development of this technology**



[Prof. H. Takagi](#)



# Summary of Presentation

## 1. Invited Presentation at the conference:

“Role of Plant Biotechnology for Establishment of Sustainable World”

By Atsuhiko Shinmyo

Although we are enjoying convenient and happy life using a large amount of fossil resource, it is believed that petroleum will be exhausted after 40-50 years. Not only the shortage of petroleum, we have to solve many serious problems, such as continuous increasing of world population, shortage of foods, global warming, and destruction of environment of the earth. To correspond to the Kyoto protocol, green chemistry is rapidly progressing, and production of plastic materials and ethanol fuel from corn starch and sugar cane and bio-diesel from plant oil become active in the world. Carbon dioxide produced from bio-products is again fixed by plants, and recycle system of CO<sub>2</sub> is established. However, the increase of industrial use of plant biomass should be compatible with food supply to support world population of 9 billion in 2050.

Solar energy supports all lives on the earth, and plants utilize it efficiently. Annual production of plant biomass energy on the earth is ten times of the energy consumed in the world. If we can increase 10% of productivity of plant biomass, we can establish the replacement of energy source from petroleum to plant biomass without decreasing of food supply. Although it takes long periods in traditional plant breeding for increase of plant productivity, molecular breeding by recombinant DNA technology can be established in short period. Crossing of plants occurs between close species, but any gene can be introduced in molecular breeding, resulting in useful novel plants.

Post-genome project is also active in plant science. Whole genome sequence is established in *Arabidopsis thaliana* and rice, and transcriptome, proteome, and metabolome are rapidly growing in model plant, *A. thaliana* and industrial plants, such as eucalyptus, rubber tree, eucommia, and *Glycyrrhiza*, in METI/NEDO project.

In the 20th century, oil-producing countries became rich, and countries with abundant plant biomass will be rich in the 21st century. Japan was the 2nd economic giant in the world in the 20th century even we were poor in oil, because of high level of science and technology. The most important technology must be plant biotechnology to survive in the 21st century.



## NAIST's Booth at Exhibition

### **Technologies for improvement of transgene expression, and increasing the productivity.**

#### Effective Gene Expression in plants

##### Problems in gene introduction

##### Positional Effects

- Some genes are not expressed.
- There are variation in gene expressions among clones.
- It takes long to obtaining appropriate transgenic plants

##### DNA copy number

- Too many copies of gene cause suppression of the gene expression
- It is difficult to control the copy number to be introduced.

##### Localization

- It is desired to control the expression locus of the recombinant proteins

##### Expression level

- Control of expression level of recombinant proteins at transcriptional or translational level is needed.

We have developed expression systems that eliminate such problems.

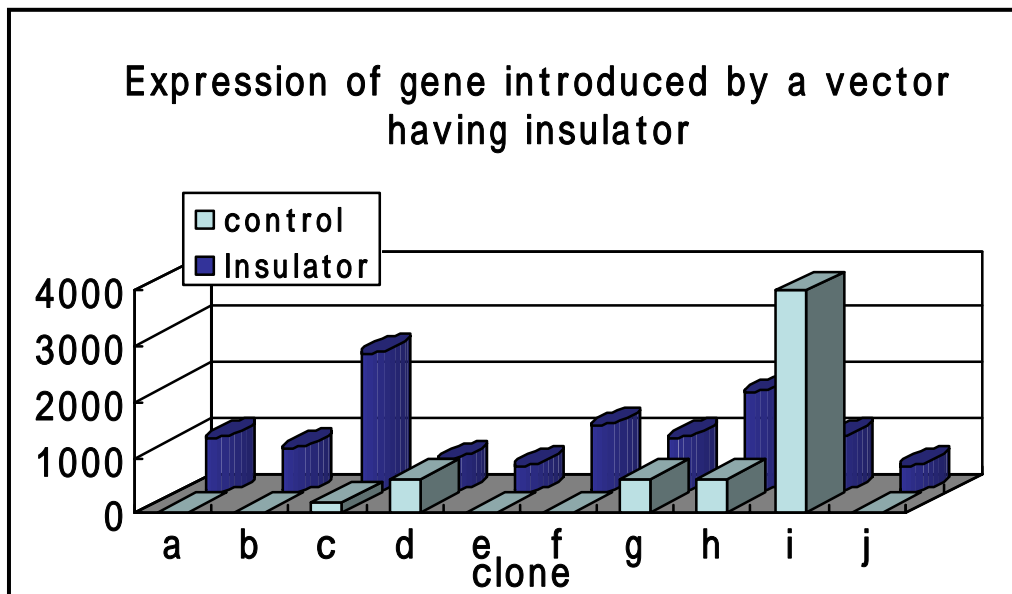
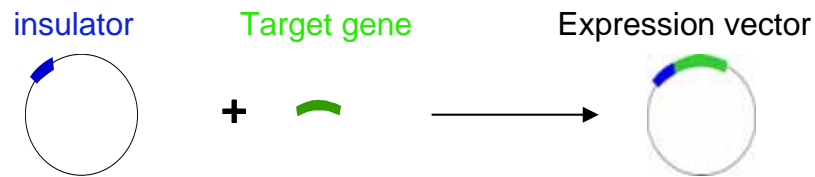
## NAIST's Booth at Exhibition

The problem that happens often is Positional Effect caused by some elements located in the chromosome, which sometimes suppress the transgene expression.

### Suppress the Positional Effects

We have developed a vector that has an insulator that eliminates the positional effect. The target gene is combined with the insulator, and then integrated into chromosome.

All clones transformed by the vector having insulator show the expression of target gene, while clones transformed by the control vector (not having insulator) show the variation in the gene expression.



\*Results with tobacco plants

Ref. JP 6229070 partial revision

## NAIST's Booth at Exhibition

Gene silencing is caused by accumulation of mRNA or aberrant RNA species produced by the interaction between transgene-transgene or transgene-host gene.

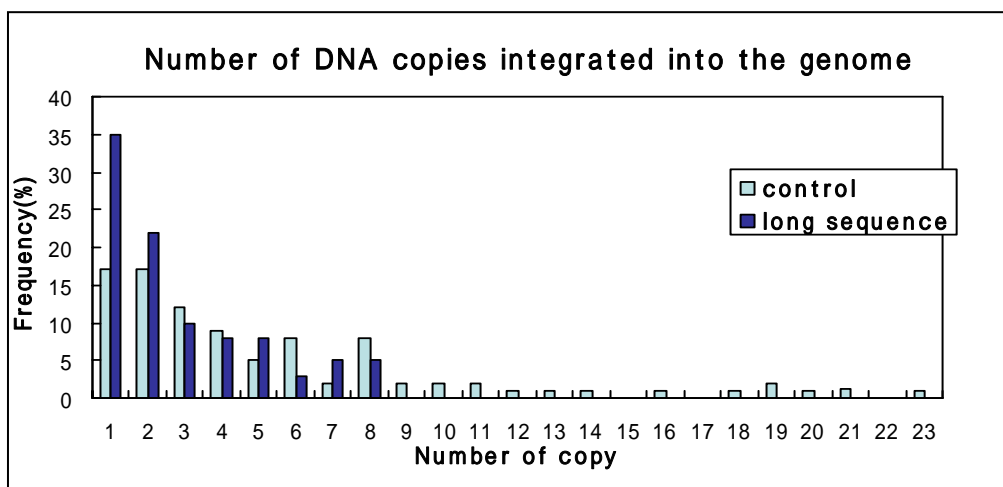
The Gene silencing occurs both at transcriptional level and at post-transcriptional level.

Though MAR elements were thought to cancel this effect when they were combined to the 3'- and 5'-end of transgene, the cancellation was not enough.

### Control the number of copies of gene that is introduced into the genome

We have developed an expression vector that has long DNA sequences on both sides of target gene. The target gene is sandwiched between long DNA sequences, and then is integrated into the chromosome.

As a result of using the expression vector, the number of DNA copy that is introduced into the genome is limited less than 8; most of clone has 1 or 2 copies in its genome.



## NAIST's Booth at Exhibition

Controlling the expression locus of transgene products (recombinant protein) in the transgenic plant has some advantages. For example, it becomes easy to purify the recombinant protein from plant cell when the recombinant protein is designed to be secreted into the culture medium.

### Control the localization of recombinant protein.

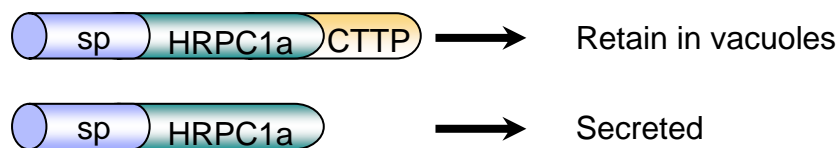
We have found that combination of some signal peptides or 5'UTR sequence with transgenes enable us to control the localization of recombinant protein.

Horseradish peroxidase isozyme C1a (HRP C1a) has signal peptides (sp) at N-terminal and proprotein at C-terminal (CTPP).

We examined the function of sp and CTPP and concluded;

- 1) that sp and CTPP are collectively function to localize the recombinant protein into vacuoles, and
- 2) That, without CTPP, HRP C1a is secreted into the culture medium.

### Different localization of recombinant protein



## NAIST's Booth at Exhibition

Controlling expression level is not only achieved by controlling the number of DNA copies but also by enhancing the translation.

### Control the expression level of recombinant protein.

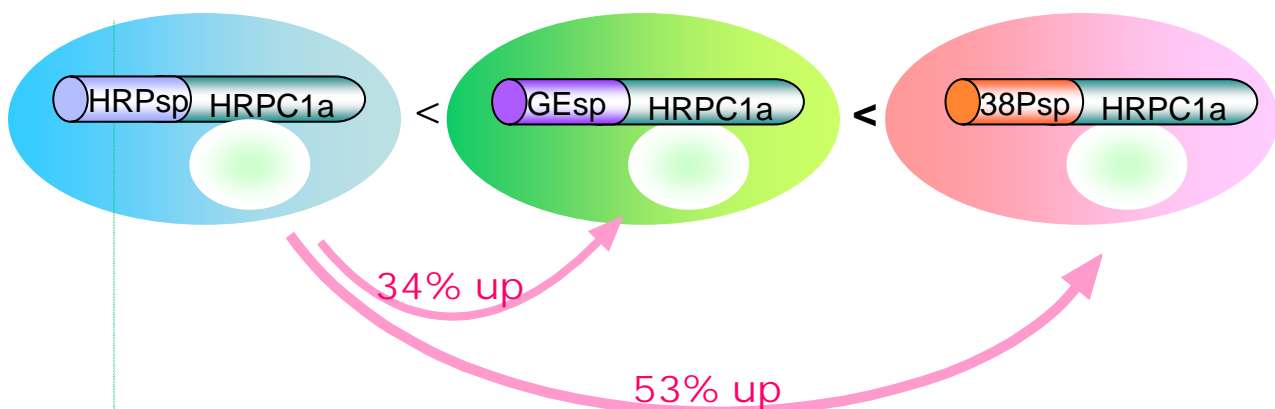
We have found that combination of signal peptides and 5'UTR sequence of some genes with transgenes enables us to increase the expression level without changing the amount of transcript.

Signal peptide (sp) of HRPC1a (lacking CTPP) is replaced by the sp of  $\alpha$ -D-glucan exohydrolase (GE), and the sp of 38kD peroxidase (38P). Each cassette is inserted into the expression vectors and then these vectors separately transform tobacco BY2 cells.

The HRPC1a protein is normally expressed in each recombinant cells.

The accumulation of HRPC1a protein is more pronounced in GEsp-HRPC1a cells and 38Psp-HRPC1a cells than in HRPsp-HRPC1a cells.

### Accumulation level of recombinant protein in recombinant cells



## Growth Enhancement of Plants

Increasing productivity of plants or enhancing the growth of the plants are major public interest as energy crisis becoming more serious.

### Enhancement of the photosynthesis

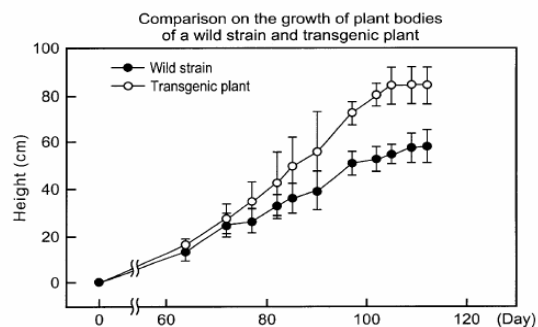
Photosynthesis is the primary metabolism cycle of a higher plant having chloroplasts. RuBisCO, Fructose-1,6-bisphosphatase (FBPase) and Sedoheptulose-1,7-bisphosphatase (SBPase) have been found as being the rate-limiting enzymes in photosynthesis; The substrate specificity of RuBisCO for CO<sub>2</sub> is low, and the amounts of FBPase and SBPase protein in a chloroplast are small.

We have modified the photosynthesis by increasing the amount of FBPase/SBPase amount in plants.

We have produced a tobacco plant that overexpressed the cyanobacterial FBPase/SBPase in nucleus or in chloroplasts.

In the transformed tobacco:

- growth of the plant has been increased,
- starch-, sucrose-, and hexose- content has been increased,
- efficiency of photosynthesis has been increased.



US6528705, JP3357909,  
PCT/JP2005/004037



## NAIST's Booth at Exhibition

### Improvement plants that have resistance to diseases, stresses, or feeding

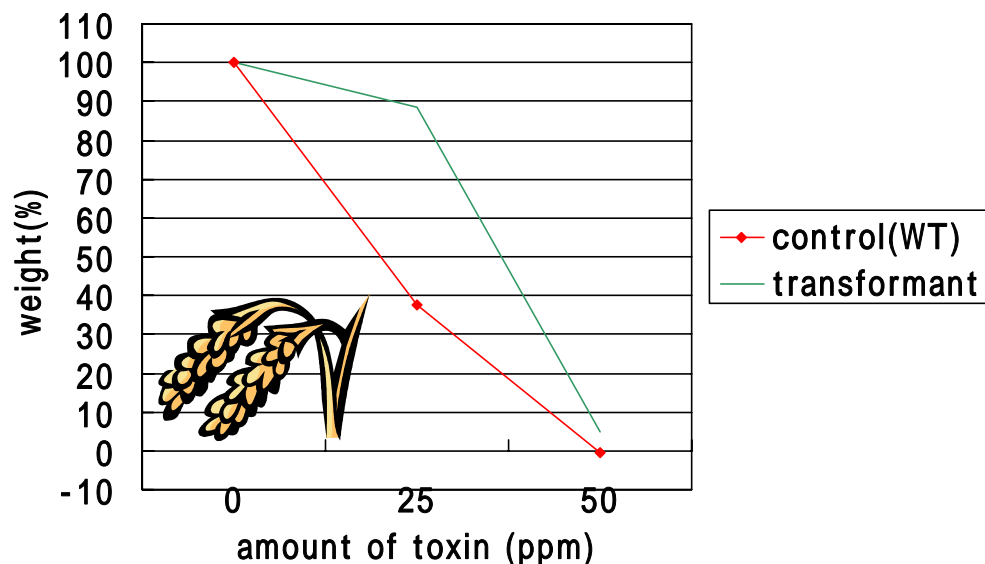
It is important to protect crops from diseases, stresses, and insects' feeding.

We have been engaged in elucidating the mechanism of "resistance" or "tolerance" in plants, and have achieved to provide plants with the property of disease-resistance, insects' repellent, and so on.

#### Disease Resistance plants

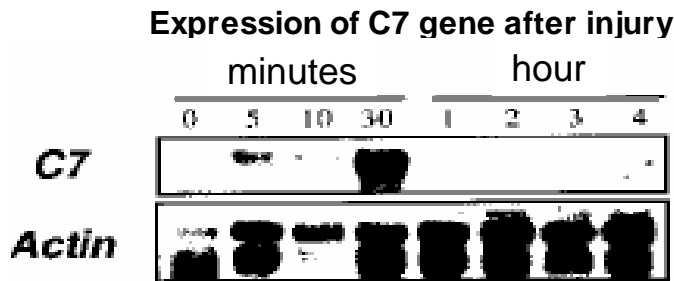
When a plant is infected by a pathogen, sometimes it happens that uninfected parts of the plant show resistance to wide range of pathogen type for a relatively long time; so called acquired resistance. Acquired resistance is significant for plants because it provides plants with resistance against multiple number of bacterial strains, while the resident resistant gene works for only one strain.

We have found that tobacco BlxA2-700 gene (the gene involved in rice-blast occurrence) can provide leaf-blast resistance and bacterial leaf blight resistance to rice when the gene is introduced into rice.



## Stress Tolerance plants

We found a gene C7, a novel gene encoding a receptor-like protein whose expression is induced in response to injury stress, osmotic pressure stress, salt stress or low-temperature stress in tobacco plants. C7 is thought to be a sensor of exogenous stress because its expression is observed about 45min after being stressed.

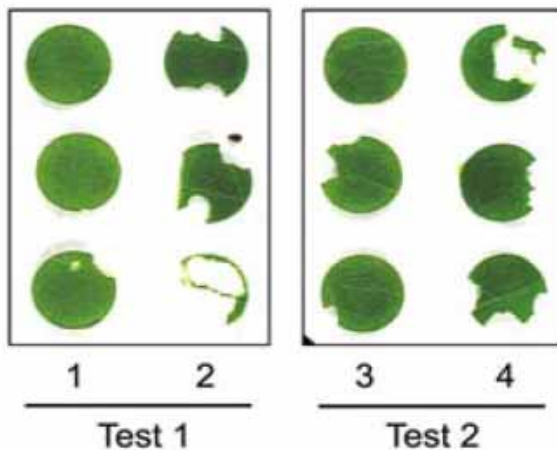


Patent #:JP3448610, US681756

## Insect Repelling plants

We had elucidated the caffeine synthesis pathway and identified those genes involved in the pathway. As a result, we have achieved controlling the caffeine production in plants. When a tobacco plant has been retained these genes of caffeine synthesis, it has produced caffeine. Caffeine can work as an insect repellent chemical in the transformed tobacco. On the other hand, we succeeded to produce caffeine-less coffee plant using RNAi technique.

### Anti-herbivore effects of transgenic tobacco (Feed to Tobacco cutworm larvae)



1&3:Control  
 2: 5  $\mu$ g caffeine producing tobacco  
 4: 0.4  $\mu$ g caffeine producing tobacco



Flower of Caffeine-less coffee plant



## NAIST's Booth at Exhibition

### Enhancing Ethanol Fermentation

Fermentation by yeasts is a common method in alcohol producing, bread making, and biofuel production. In such processes, yeasts are continually put under stresses such as high alcohol content, high temperature, and freezing temperature. Such stresses induce enzyme degeneration and ROS (reactive oxygen species) production in the yeast cell.

We have found some genes that are important for stress tolerance and achieved to produce yeast strains that are tolerant against stresses.

#### **Proline accumulating yeast** JP2006-67806

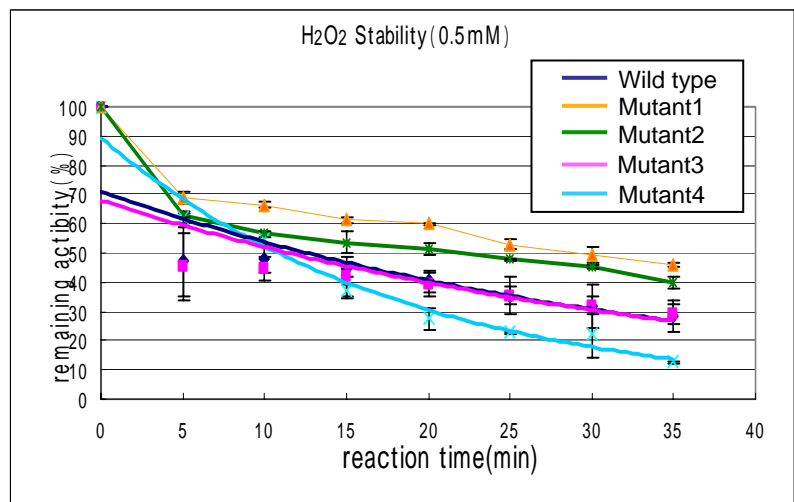
We have found that proline has a function to protect yeasts from freezing stress. We have obtained a mutant yeast that accumulate proline in the cell, and have elucidated the mechanism of proline accumulation in the mutant; proline-degradation gene has been destructed, and the activity of proline-synthesis gene has been enhanced. Based on this finding, we have achieved to produce alcohol-/ freeze-tolerant yeast.

#### **Mpr1 expressing yeast** PCT/JP2007/053667

Mpr1 is an acetyl transferase that reduces the intracellular ROS level.

We have found some Mpr1 mutant genes that can contribute to a high ROS-tolerance in yeasts.

By introducing these mutant genes, we have achieved to produce ROS-tolerance yeasts.





# NAIST's Information

NAIST is a national university composed solely of graduate schools. NAIST conducts advanced research and educates accomplished individuals to support the development of society through science and technology.

We have three graduate schools; Information Science, Biological Sciences, and Materials Science, consisting of about 1000 students and 200 faculty.

NAIST's objectives are

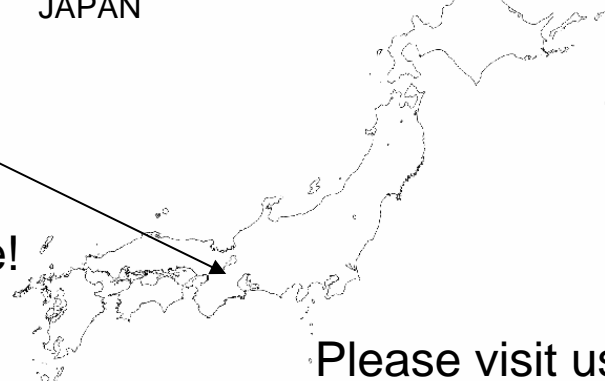
- To promote high-level research in advanced science and technology
- To develop international leaders in research
- To train competent specialists capable of contributing to national society and the economy
- To promote relationships between science and the community and the creation of a scientifically literate population

The department of biological sciences possesses about 20 laboratories and about 100 faculty members, and researches on plant biology and animal biology are mainly performed.



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NAIST is located around here!



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