

APAC DA 2019

Revitalization of drug discovery using
natural products by open innovation in Asia

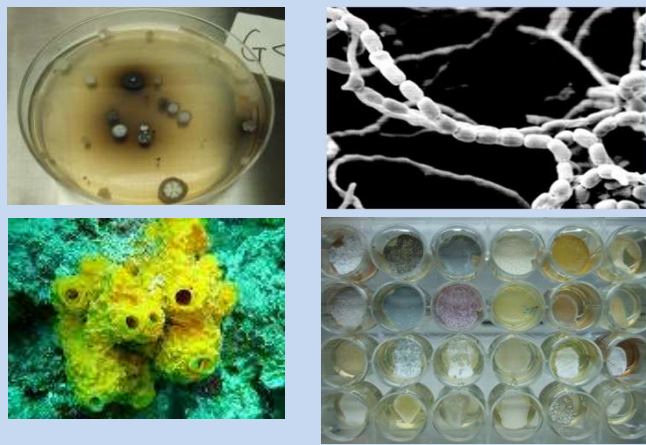
Screening commission system of N²PC
and
new technology for the development of Natural Products

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*National Institute of Advanced Industrial
Science and Technology (AIST)*

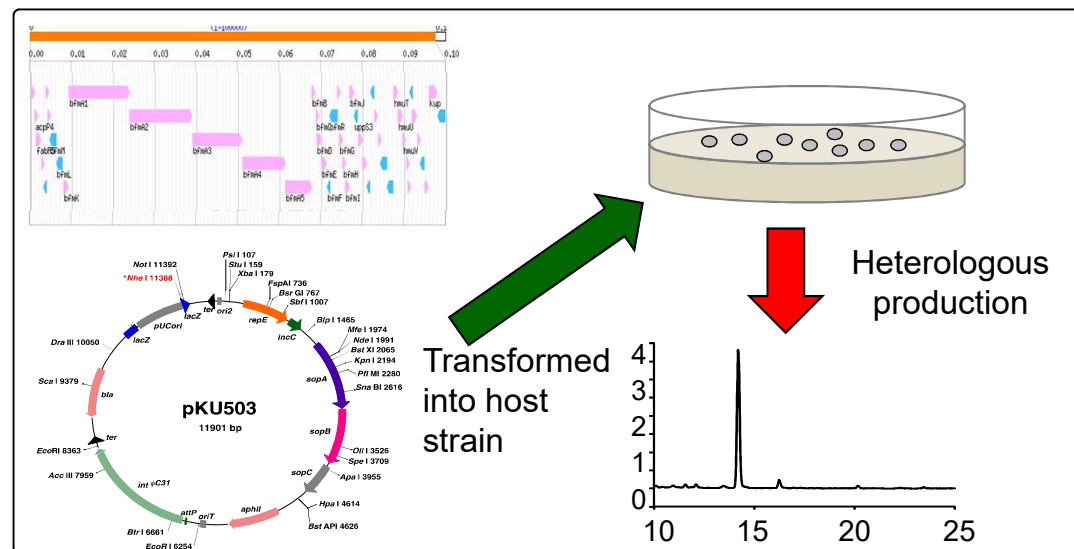
Technology Research Association for Next generation natural products chemistry (N²PC)

- Performing drug screenings with World-largest Natural Library (over 270,000)
- Developing Next-generation technique for heterologous expression system

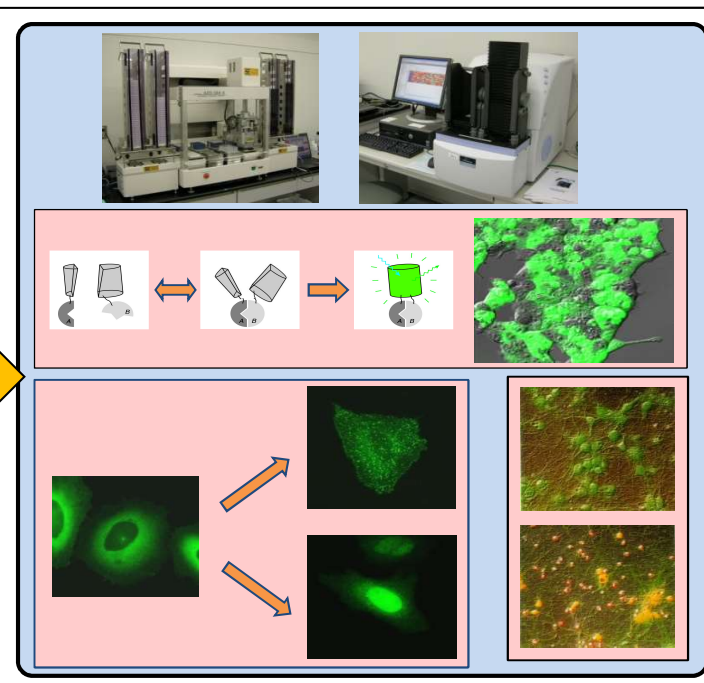
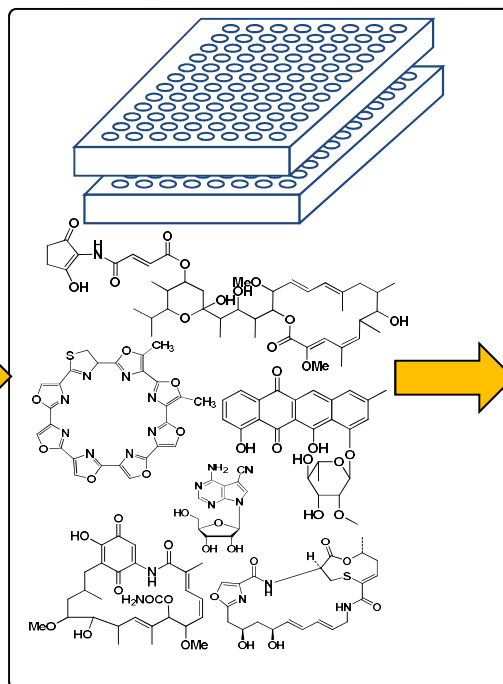
Microorganisms



Obtaining
biosynthetic
gene clusters

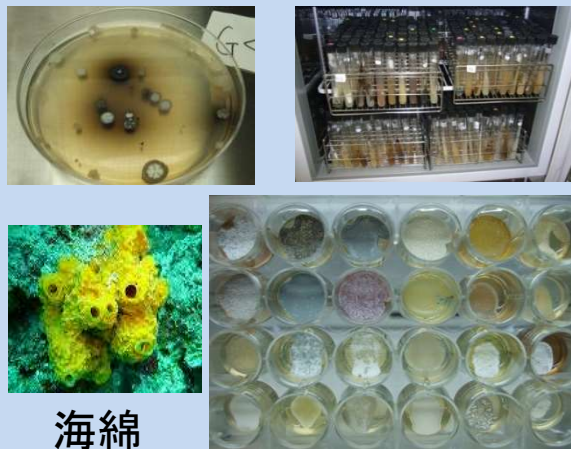


Cultivation, Isolation,
Structure determination



Highly efficient system is required in natural product screening

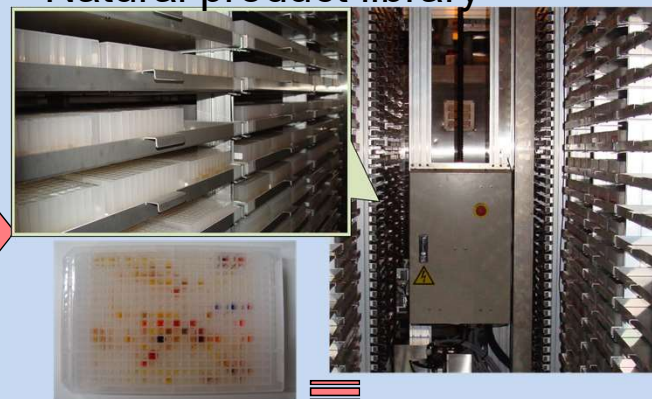
Microorganism collection



Culture, Sample prep



Natural product library



Isolation



Structure determination



Large scale culture

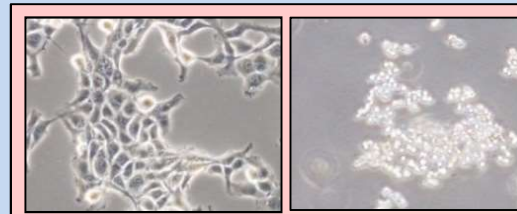


Bioactivity tests
(Pre-clinical)

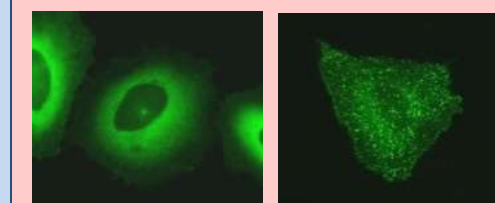
Screenings



Disease models



Antitumor



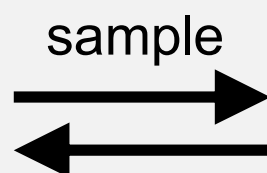
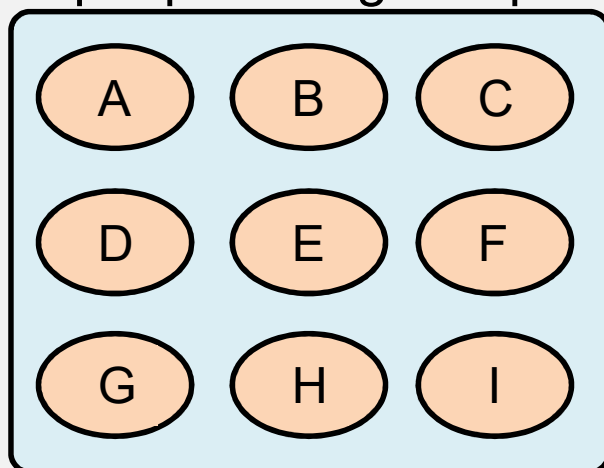
Cell-
signaling
modulators

Technology Research Association for Next generation natural products chemistry (N²PC)

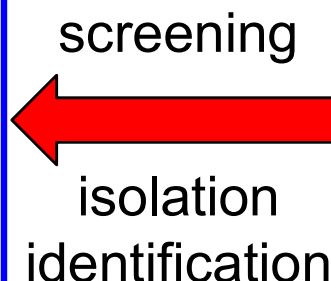
N²PC

Core organization

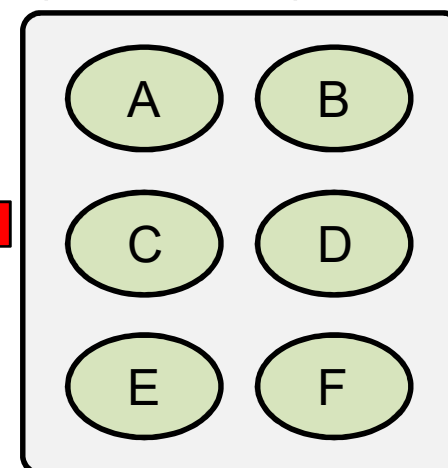
Sample providing companies



N²PC
secretariat
and
laboratory



User companies
(academia)



Total 9 companies (organization) provide natural library

Eisai, OP-BIO factory, Godo Shusei, Shionogi, Meiji Seika Pharma, Microbio Pharma Japan, Daiichi Sankyo RD NOVARE, Kyowa Hakko Kirin, JBIC

N²PC also acts as technology supporting organization in
APAC Natural Products Drug Discovery Consortium

World-largest natural library for drug screenings

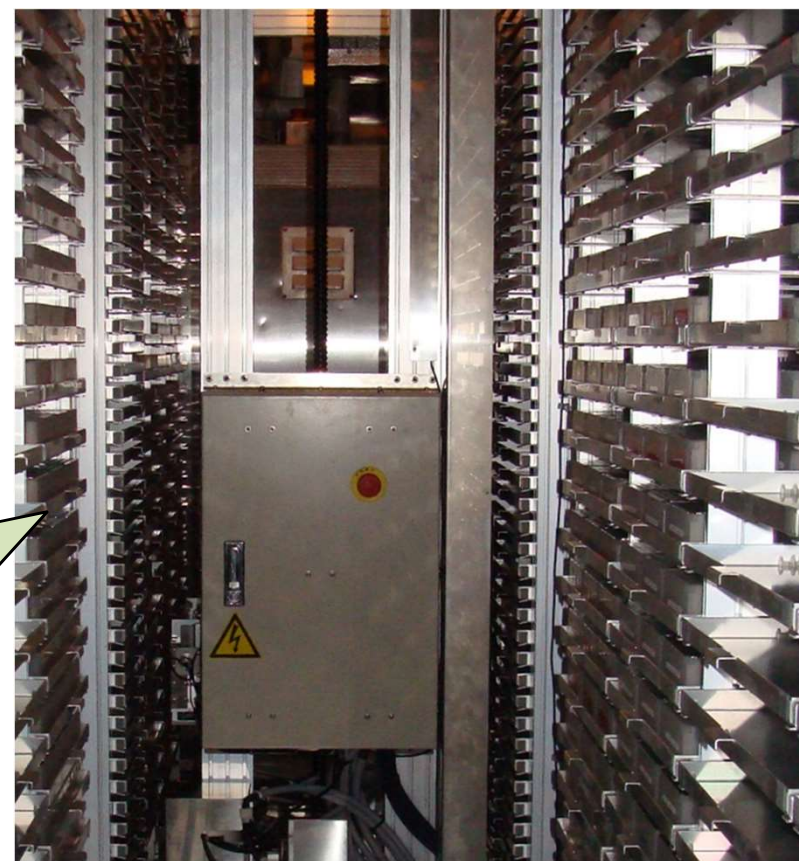
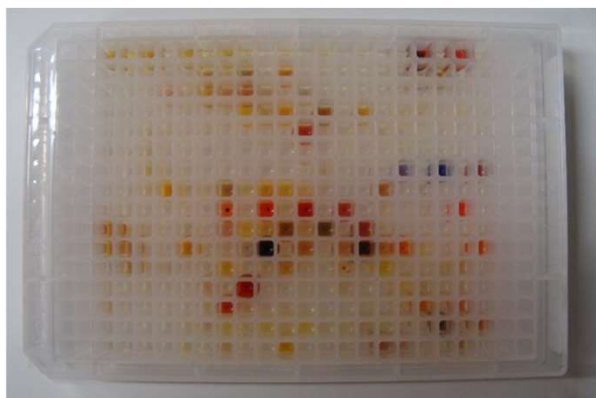
	Crude Extracts	Plant	Marine	Isolated natural compounds	Synthetic compounds
In house	49,920	252		~ 1,600	11,040
Company origin (7 companies)	188,800		640	~ 2,000	

Total: 287,360 sample (2018.10)

Technology Research Association for Next
generation natural products chemistry
(April 15, 2011)

Total 8 companies provide natural library

Eisai, OP-BIO factory, Godo Shusei, Shionogi,
Meiji Seika Pharma, Microbio Pharma Japan,
Daiichi Sankyo RD NOVARE, JBIC



Combination of large natural library and high-throughput random screening system

World-largest natural library

×

Total: > 280,000 sample

0.1 or 0.2 μ L addition

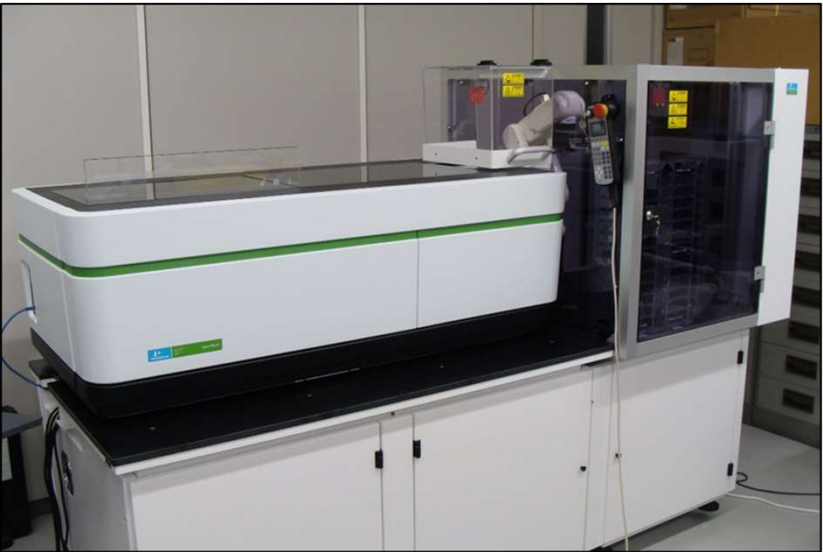
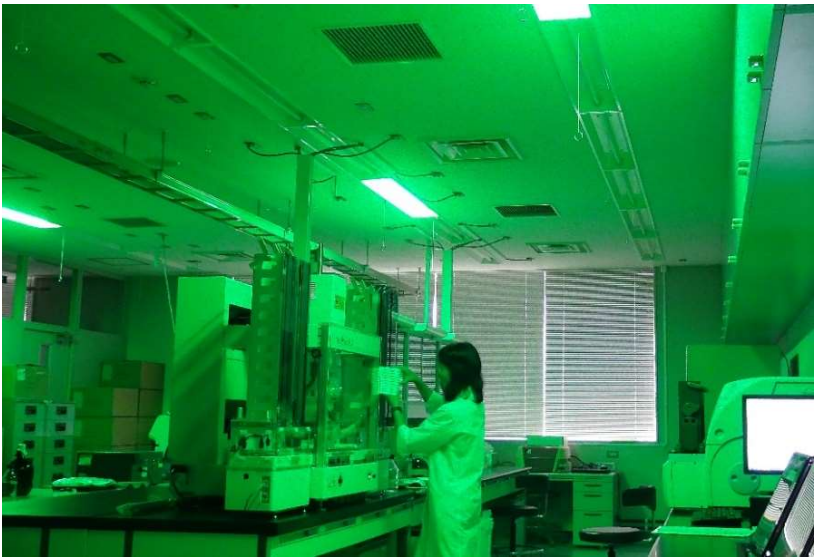
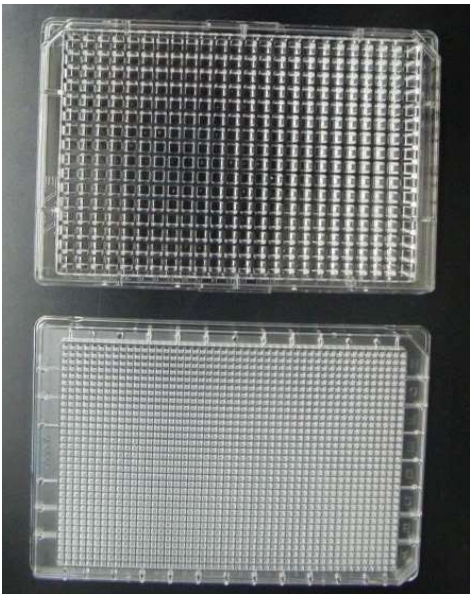
High-throughput and High-content screening system



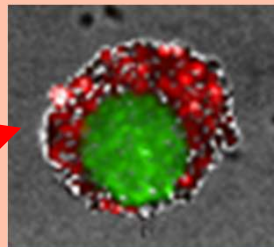
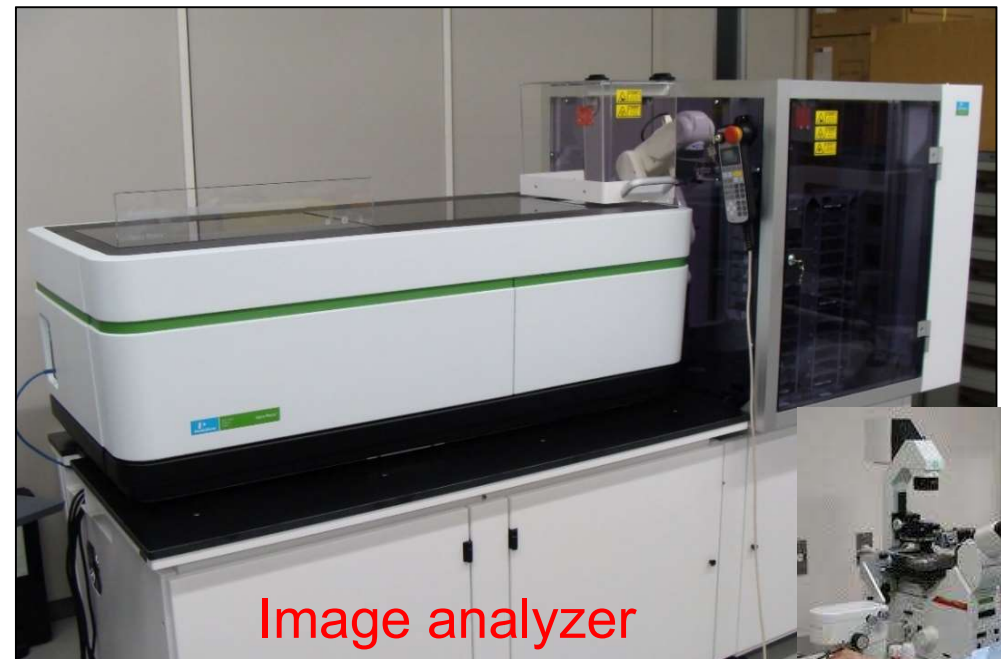
Throughput

In vitro assay > 100,000 assay / week
Cell based assay ~ 100,000 assay / week

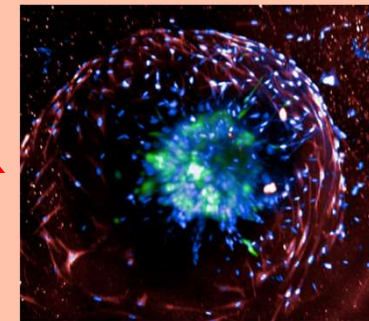
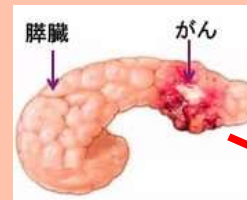
High-performance natural product screening



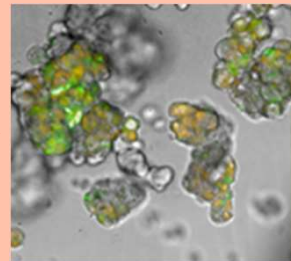
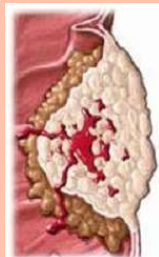
Phenotypic Screening (High Content Screening) by image analyzer



Patient-derived
GBM sphere



Stromal tissue model of
Pancreatic cancer organoid

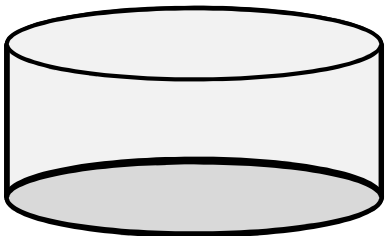


Patient-derived colon cancer organoid

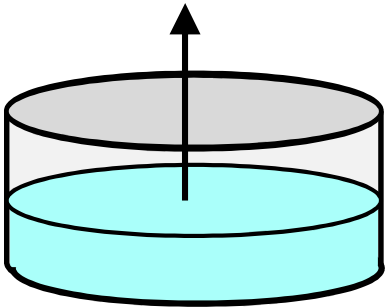
http://www.id.yamagata-u.ac.jp/Gen/Res_stud.html

Preparing new sets of assay plates

Acoustic Transfer System (ATS-100)



1 nL ~ 



Ultra sonic

New assay plates

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL
B	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL
C	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL
D	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL
E	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL
F	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL
G	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL
H	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL
I	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL
J	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL
K	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL
L	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL
M	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL
N	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL
O	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL
P	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL

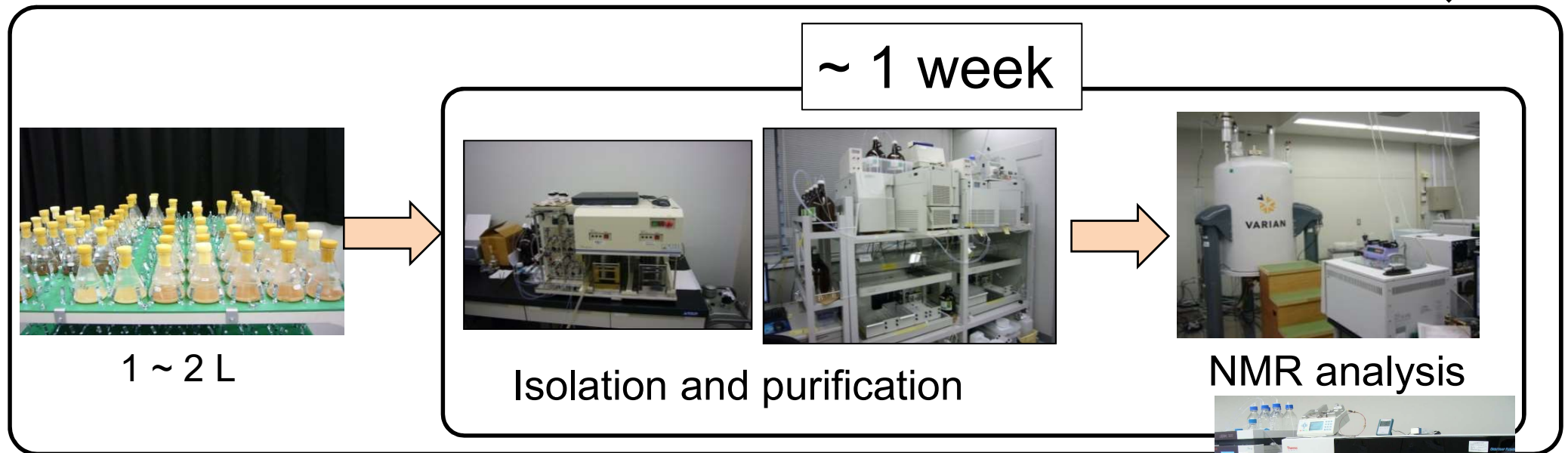
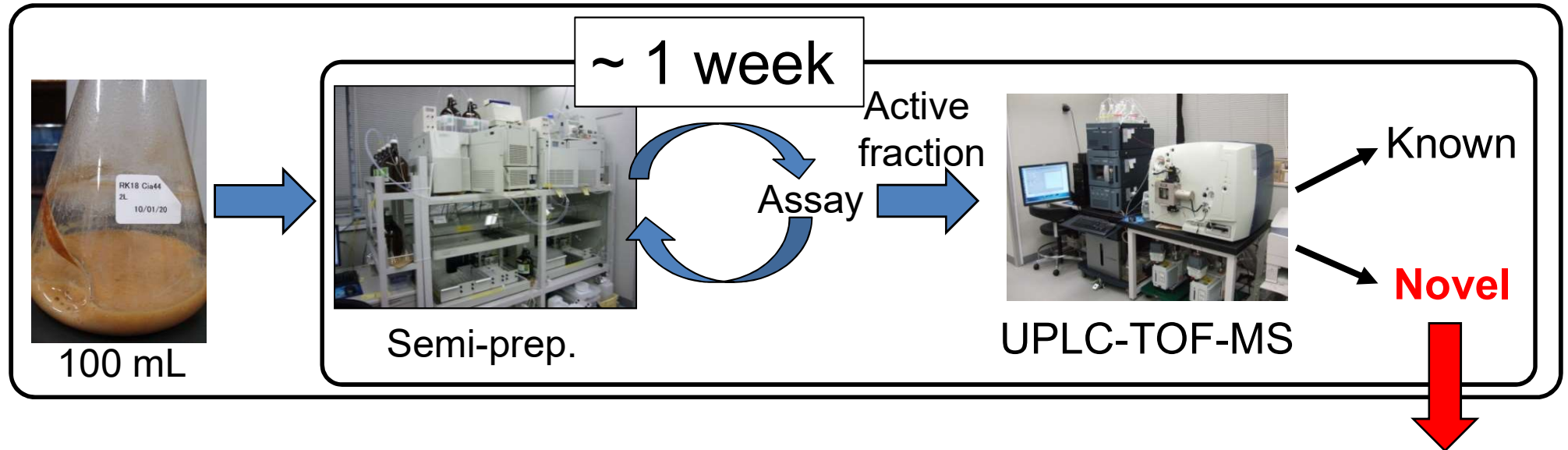
100 nL
30 nL
10 nL

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A																								
B																								
C																								
D																								
E																								
F																								
G																								
H																								
I																								
J																								
K																								
L																								
M																								
N																								
O																								
P																								

Hit samples

Saving sample consumption !

High-throughput isolation system is required for natural library !



Active compounds are isolated **within two weeks** from culture broth

Only limited laboratories can perform

Construction of mass spectral database (CB MS database)

CB library Including ~1,200 compounds isolated from actinomycetes, fungi, and plant in laboratory

Analyzed by **UPLC-TOF MS** (Acquity UPLC, LCT premier XE, Waters)

UPLC condition

Solvent: **5-100% CH₃CN-0.1% formic acid**

Measuring time: 7 min/sample



Extracted from MS chromatography

Registered on ChromaLynx (Waters)

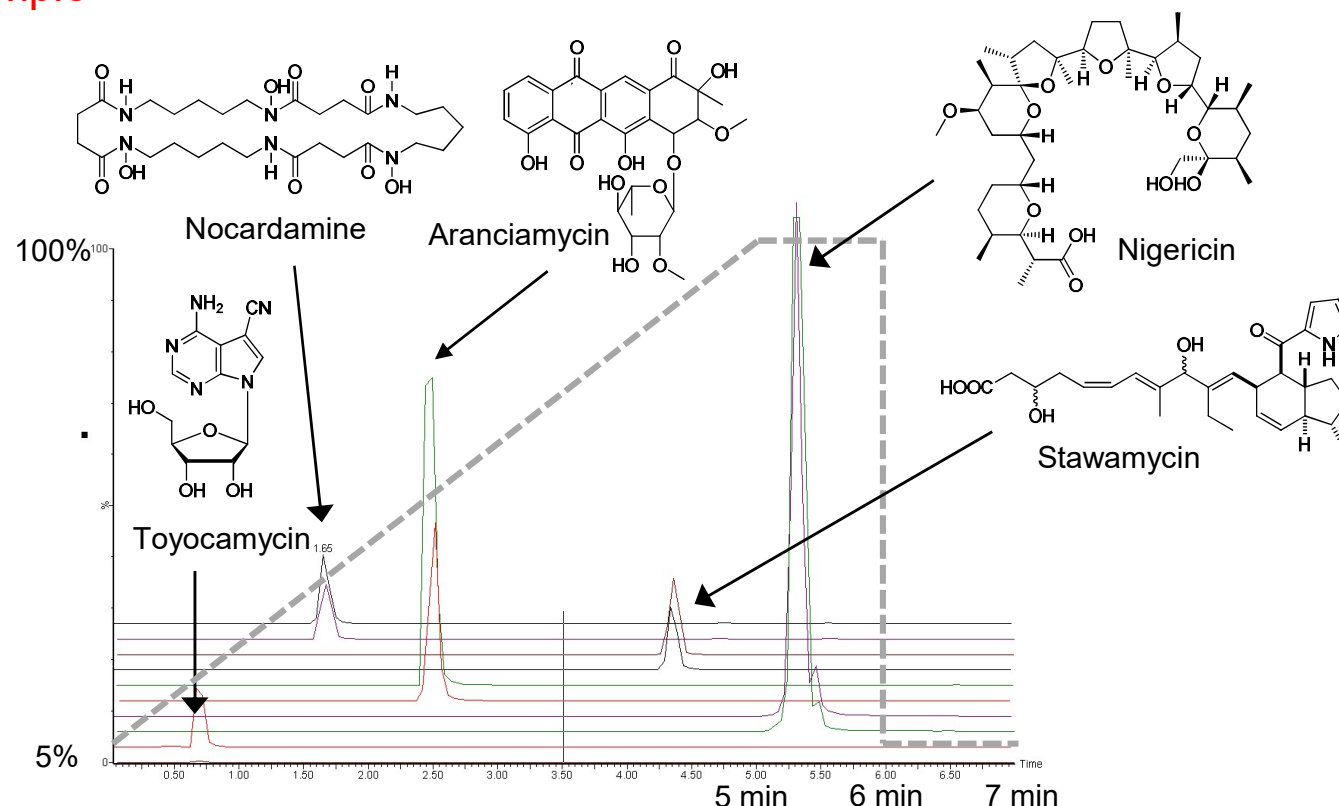
Retention time

high resolution mass

MS spectrum

100 analyses/hr.

CB MS library



This system also enables to identify the active compounds through random screening/
Usually determine active compounds within two weeks from culture broth

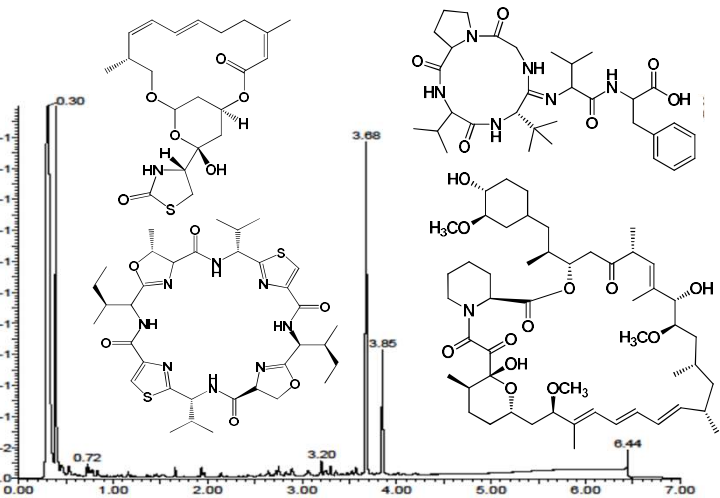
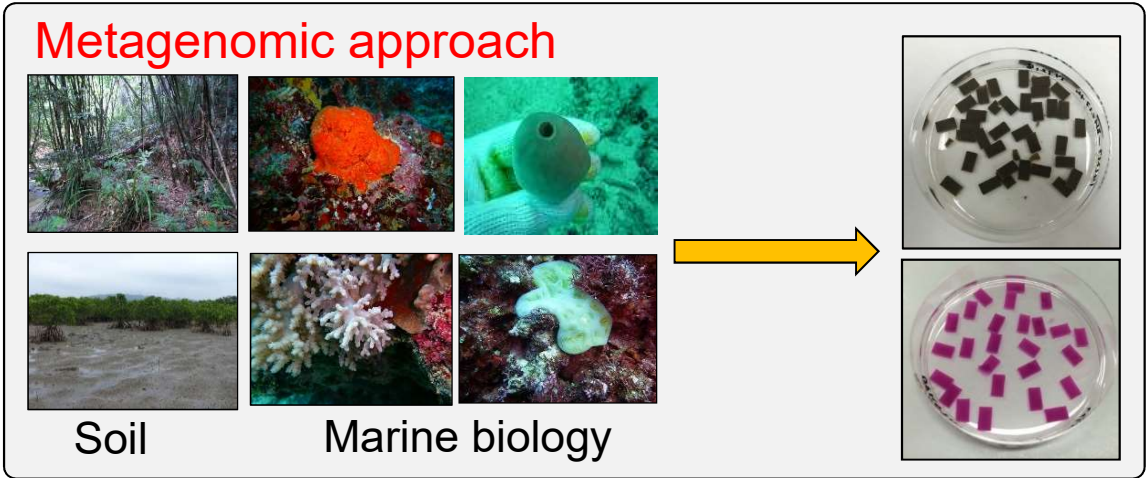
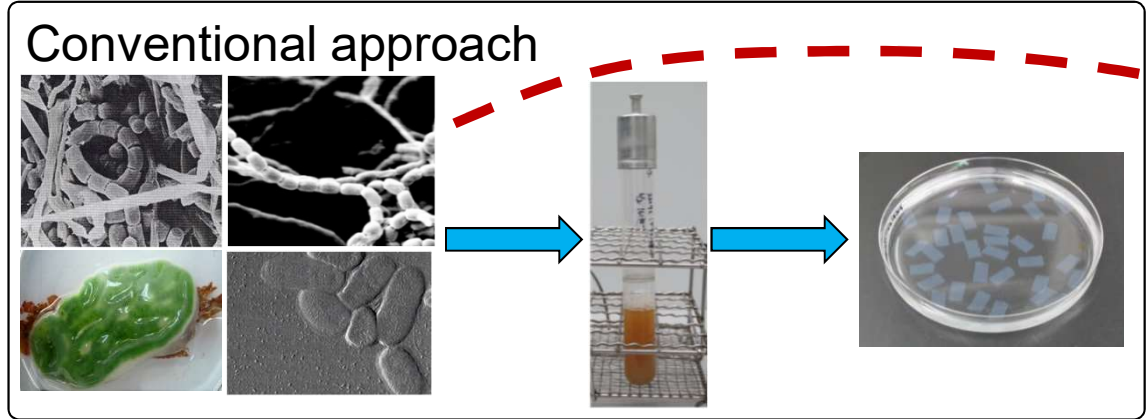
Southeast Asia has rich ocean (marine) resources

Japan is rich in ocean resources, but it is more abundant in Southeast Asia.

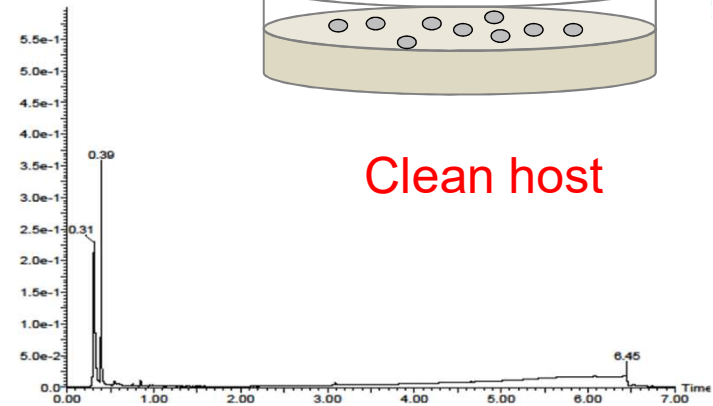
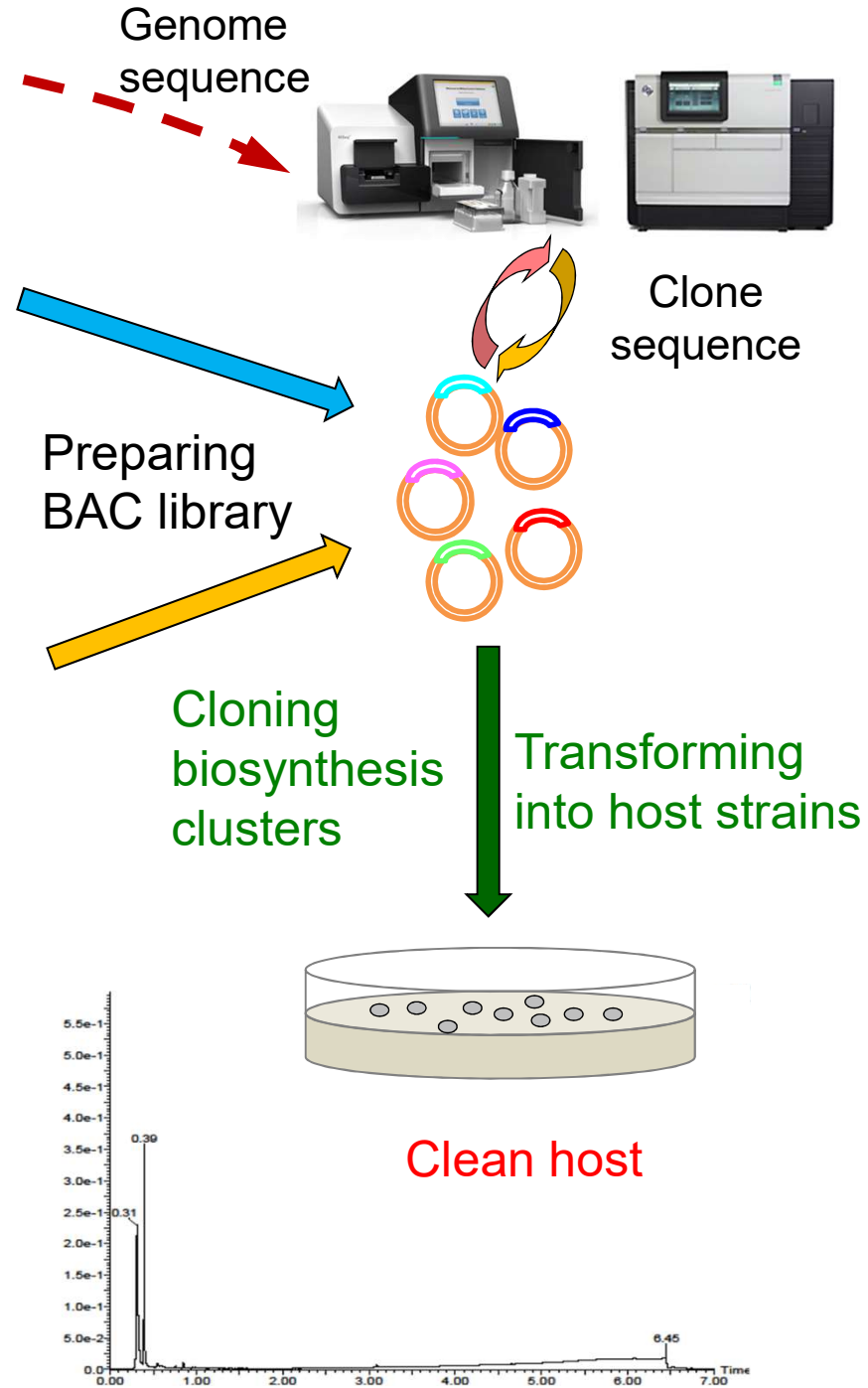


We should expand our search for new species of producers of microbial secondary metabolites

Heterologous expression of microbial secondary metabolites



Heterologous expression

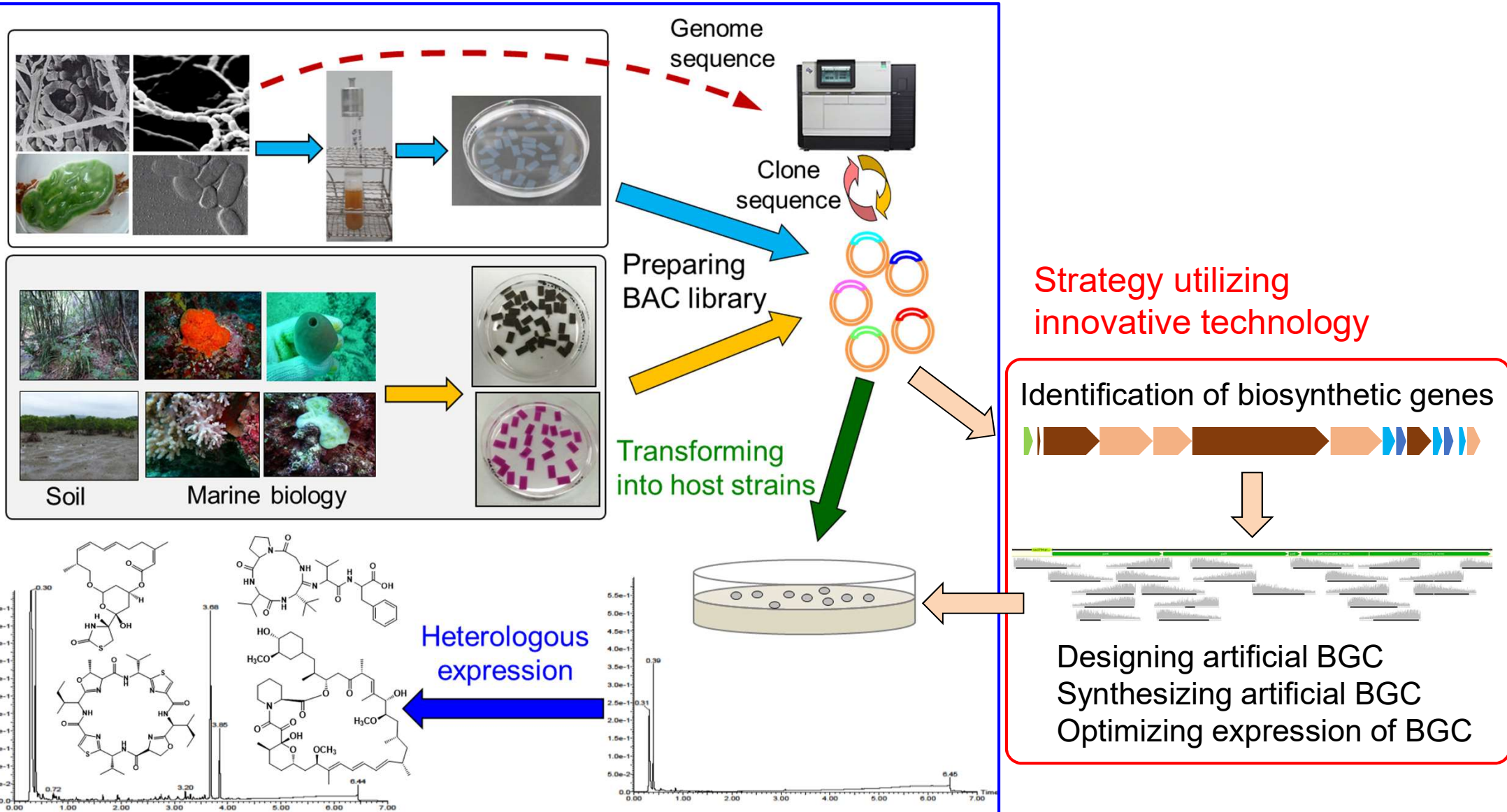


Strategy for the identification of biosynthetic gene cluster of unculturable microorganisms

1. Single cell analysis

Almost all new symbionts (like *Entotheonella*) are unculturable

Conventional and straight-forward strategy



Heterologous expression with artificial
synthetic biosynthetic gene cluster

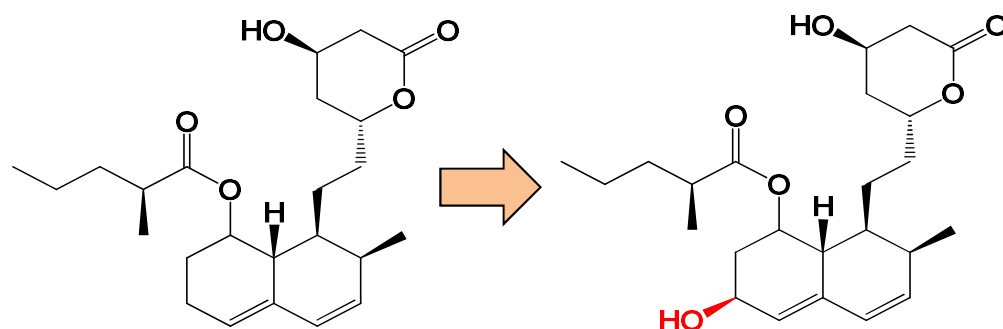
Derivatization of middle weight molecular natural compounds

Bottleneck of clinical drug development

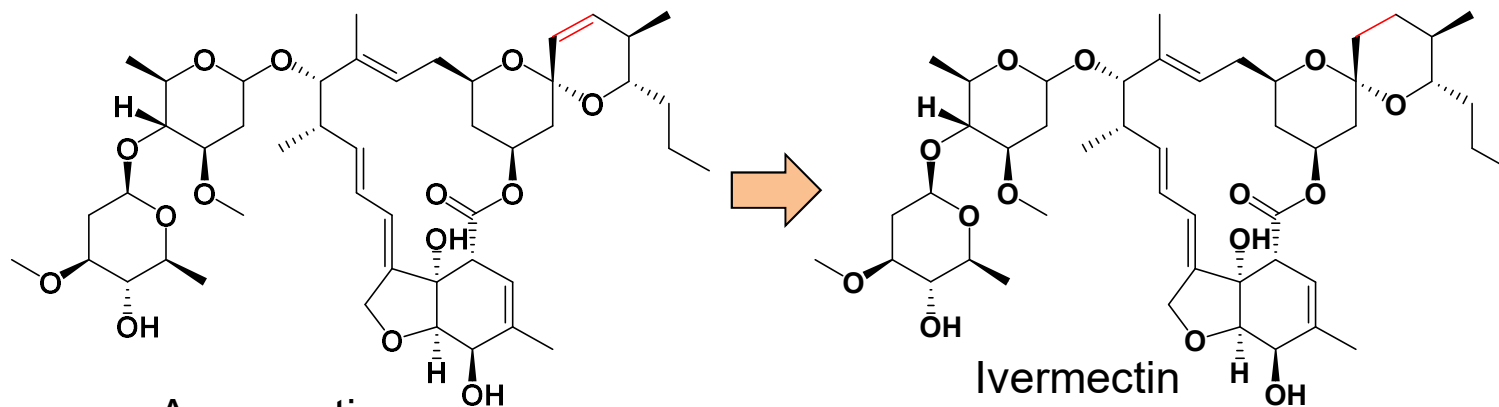
In clinical drug development, it is necessary to make fine tuning such as improvement of metabolism and metabolism in human

Bottleneck:

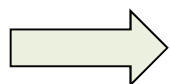
It is extremely difficult to derivatize natural products for fine tuning due to their complex structure



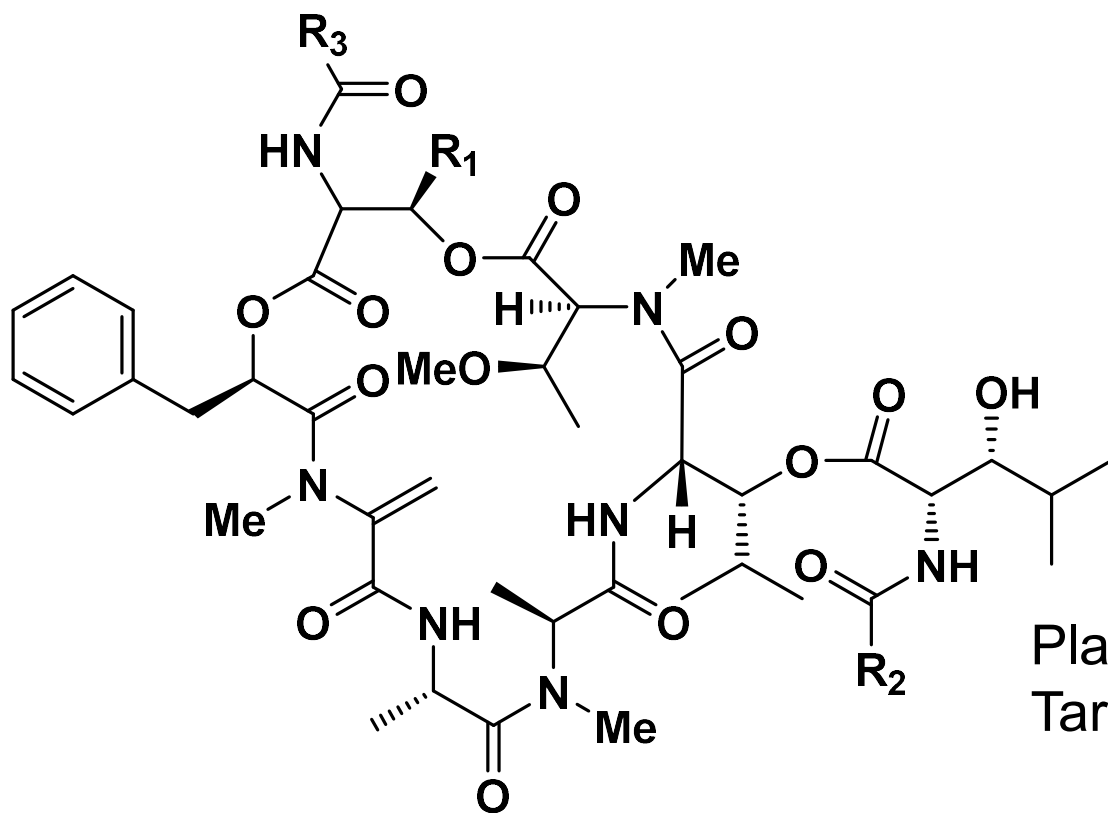
Mevalotin (Pravastatin)
Anti-hypercholesterolemia



Ivermectin
Anti-parasite



Developing new technology for the derivatization of natural products



Platelet aggregation inhibitor, Bronchodilator
Target: Trimer G protein Gp/11

J. Biol. Chem., **279**(46), 47438-47445 (2004).

Proc. Natl. Acad. Sci. USA., **107**(31), 13666-13671 (2010).

YM-254890 (*Chromobacterium* sp.):

$$R_1 = \text{Me}, R_2 = \text{Me}, R_3 = \text{Me}$$

FR900359 (Japanese name: 万両 (Manryo), scientific name: *Ardisia crenata*):

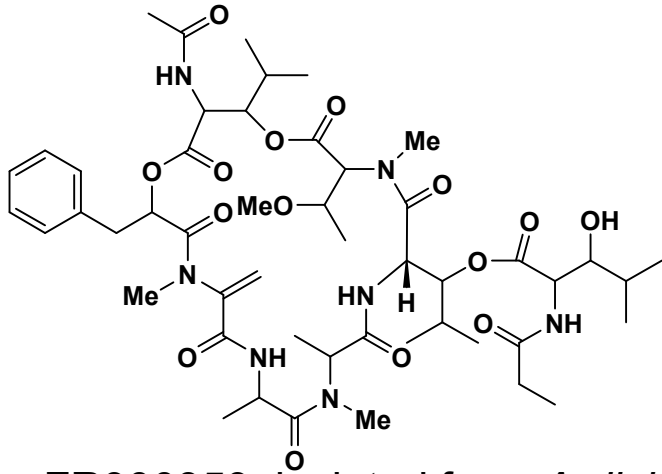
$$R_1 = iPr, R_2 = Et, R_3 = Me$$

(Produced by Symbiont *Burkholderia* sp.)

Sameuramide (Tunicate, ascidian):

$$R_1 = i\text{Pr}, R_2 = \text{Et}, R_3 = \text{Et}$$

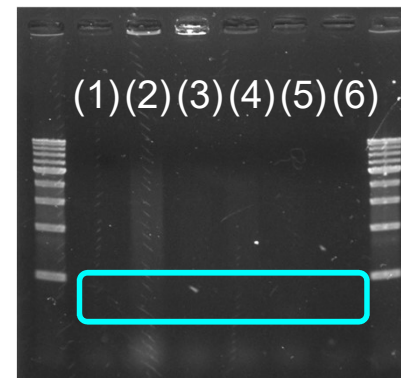
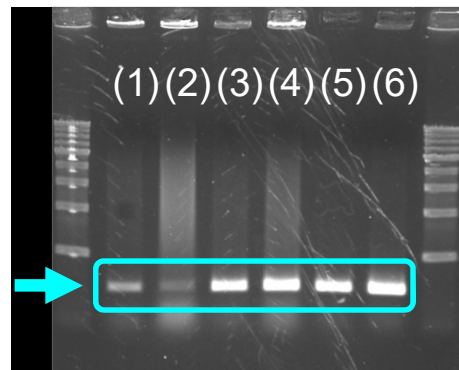
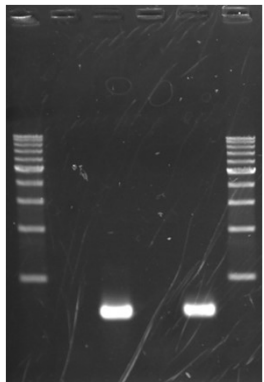
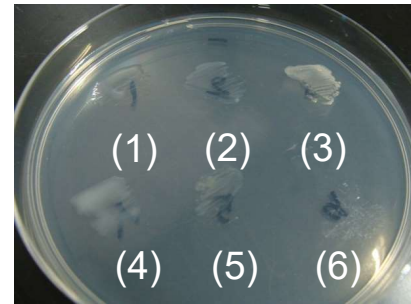
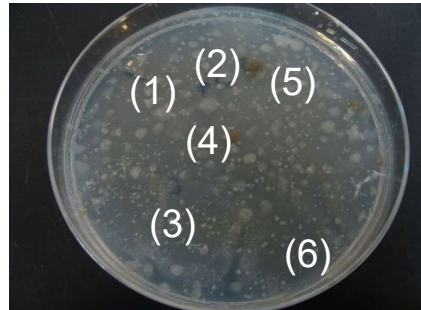
Heterologous expression of FR900359 from the plant symbiont



G($\alpha_{q/11}$) protein (GPCR) inhibitor
Nat. Commun., **6**, 10156 (2015).

FR900359: isolated from *Ardisia crenata*
(symbiotic *Burkholderia* sp. produces)

A. Carlier *et al.*, Environmental Microbiology, 18(8), 2507–2522 (2016).



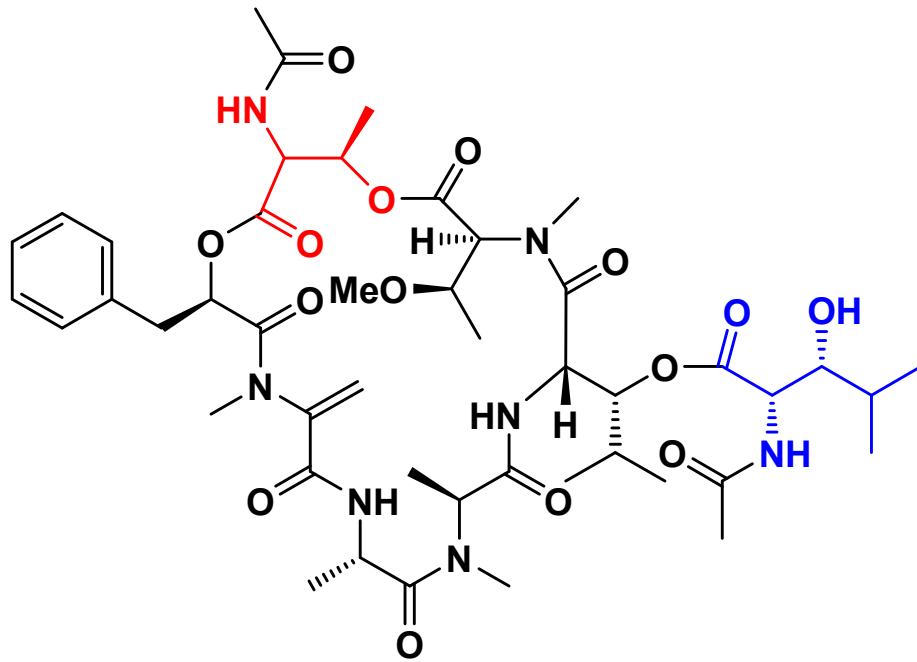
Cannot maintain the strain
possessing biosynthetic genes

Biosynthetic gene harboring microorganisms can be observed soon after preparation

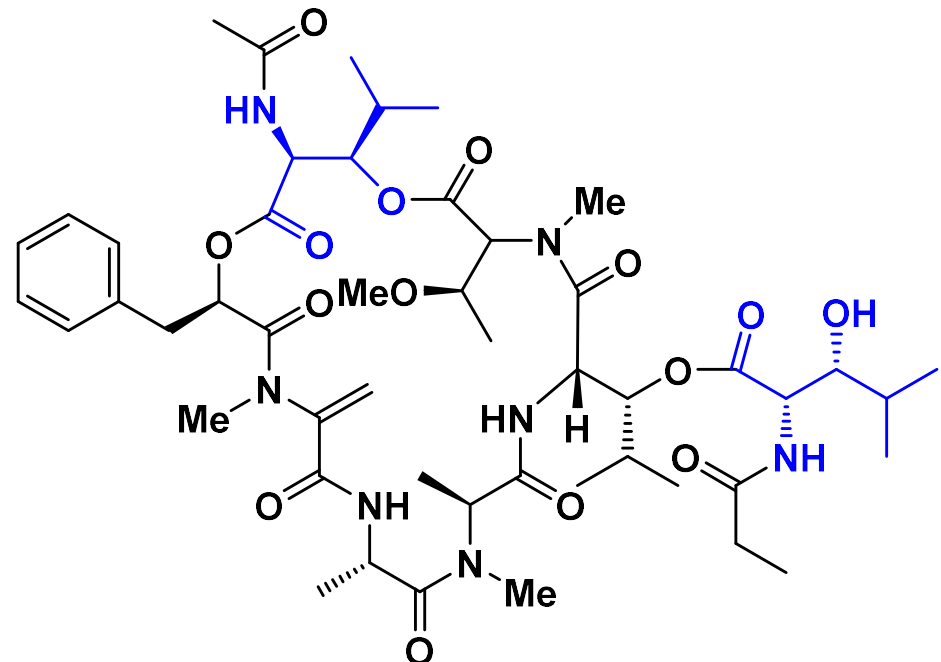


Ardisia crenata

Producing FR900359 by applying YM-254890 biosynthetic gene cluster

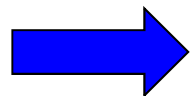


YM-254890



FR900359

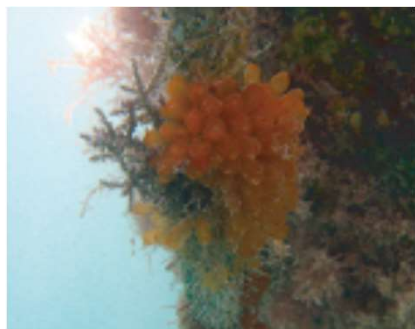
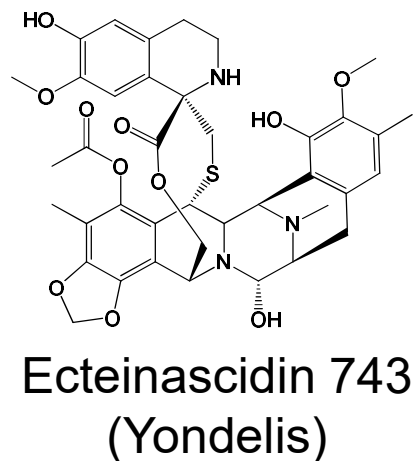
- FR900359 can be synthesized by exchanging the Thr by Leu in YM-254890
- Hydroxylation enzymes at the position of C-3 in Leu commonly exist in both biosynthetic gene clusters



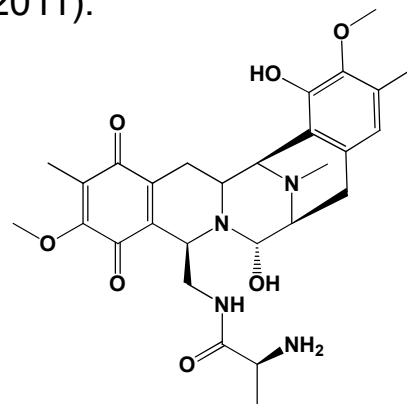
Changing A domain in YM-254890 biosynthetic gene cluster

Meta-omic characterization of the marine invertebrate microbial consortium that produces the chemotherapeutic natural product ET-743

ACS Chemical Biology, **6**, 1244 (2011).

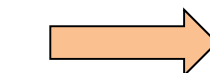


Ecteinascidia turbinata
1 g / 1 t

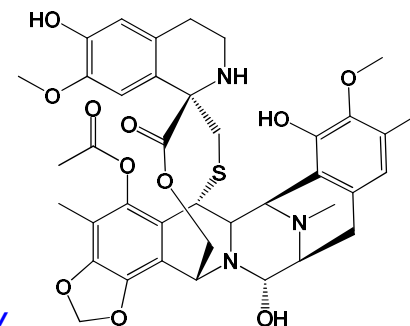


Safracin A

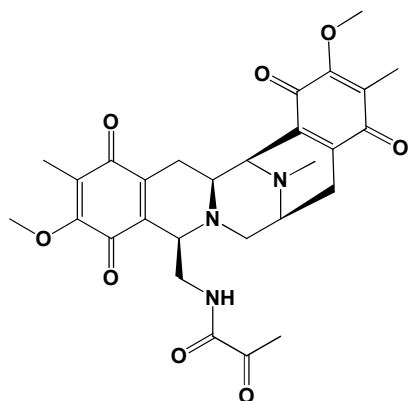
Semi-synthesis



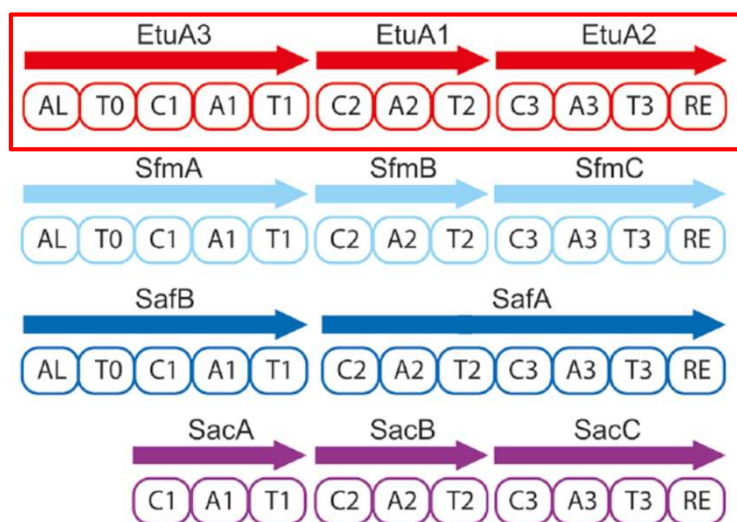
25 steps
Yield: 1.0%



ET-743 biosynthetic gene was discovered from meta genome sequence data based on the information of saframycin biosynthetic gene



Saframycin B



Candidatus Endoecteinascidia frumentensis

ET-743 will be synthesized by employing synthesized DNA optimized to *Streptomyces* host



経済産業省
Ministry of Economy, Trade and Industry

Project main member



国立研究開発法人 日本医療研究開発機構
Japan Agency for Medical Research and Development

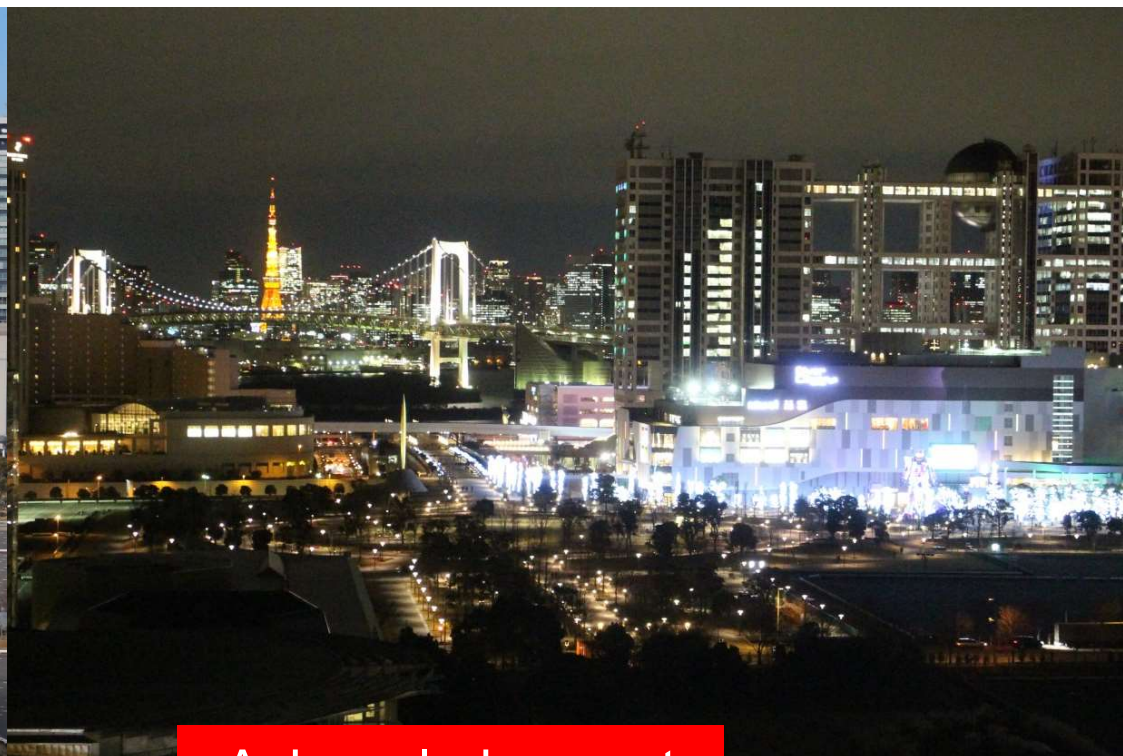


Laboratory staff

AIST, Tokyo Waterfront



Technology Research Association for Next generation natural products chemistry
<http://www.natprodchem.jp/>



Acknowledgement

Heterologous Expression
Kitasato University
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Genome Sequence
OIST
Prof. Noriyuki Sato
Dr. Manabu Fujie

RIKEN
Dr. Shunji Takahashi
Dr. Keita Amagai

OIAS
Dr. Takashi Hirano
Ms. Kunuko Teruya

The University of Tokyo
Prof. Tomohisa Kuzuyama