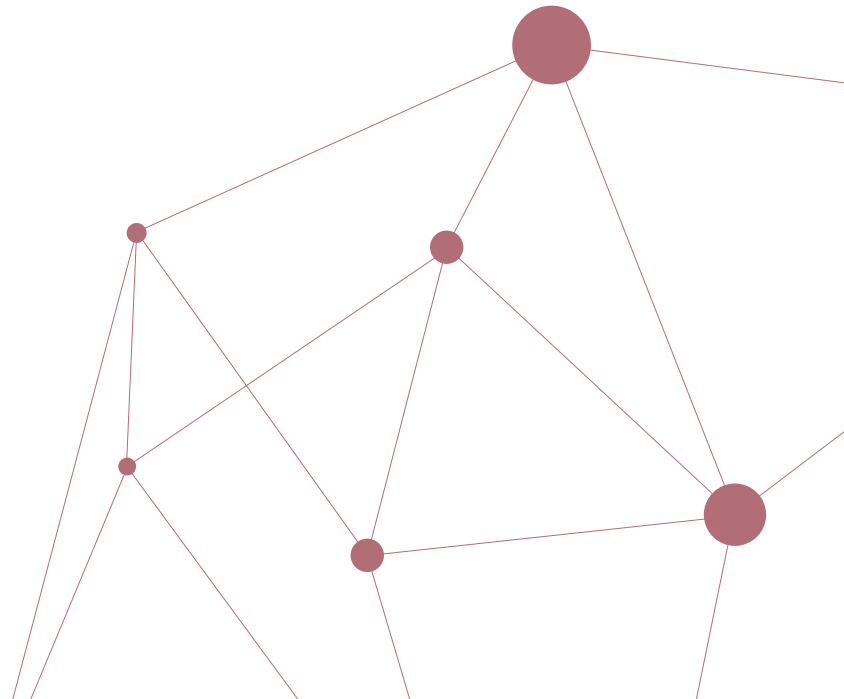
# H Genome-wide Functional Analysis

01. *S. pombe* Mutant Library
02. *S. cerevisiae* VN-Fusion Library
03. Genome-wide Drug On/Off-Target Identification Service: *GPScreen*™



# 01. *S. pombe* Mutant Library

# *S. pombe* Mutant Library

## Overview

Bioneer's exclusive *S. pombe* (*Schizosaccharomyces pombe*) Genome-wide Deletion Mutant Library is a powerful tool for large-scale genetic functional analysis, for identification and verification of drug targets, and for integrated systemic research of cell function. Co-developed by Bioneer and KRIBB (Korea Research Institute of Biotechnology and Bioscience) in collaboration with Dr. Paul Nurse of the CRC (Cancer Research Center) in the UK, the *S. pombe* Genome-wide Deletion Mutant Library (*S. pombe* Library) can be used for genetic and chemical screening such as drug target identification, gene expression profiling, and synthetic lethal profiling. *S. pombe* library offers higher homology with mammalian cells and human genes than those of *S. pombe*.

*S. pombe* Library targets every 4,914 ORF (Open Reading Frame) in the *S. pombe* genome via targeted mutagenesis. A total of 4,845 heterozygous diploid deletion mutants representing 98.6% of the organism genome and 3,420 haploid deletion mutants with 95.6% genome coverage are available. Since there are different tag sequences (barcode) for each individual mutant, the library provides an ideal way to approach research in gene function and drug target screening for large number of genes by using pools of mutants. It is also possible to analyze biological gene functions through phenotype research with the deletion mutant having specific genes absent.

### ○ Characteristics of *S. pombe* Deletion Mutant Library

| | Diploid | Haploid |
|---|---|---|
| No. of Mutants | 4,845 | 3,420 |
| Genotype | SP286 h+/h+ ade6-M210/ade6-M216 ura4-D18/ ura4-D18 leu1-32/leu1-32 | ED666 h+ ade6-M210/ ura4-D18/ leu1-32<br>ED668 h+ ade6-M216/ ura4-D18/ leu1-32 |
| Section Marker | KanMX4, G418 | |
| Culture Media | YES; for rich complete medium<br>EMM; for minimal medium | |
| Strain Verification | Check PCR, Sequencing | |
| Storage | Store at -70℃ (Glycerol type)<br>Store at 22℃ to 25℃ (Agar type) | |
| References | Kim DU., *et al. Nat Biotechnol.* 2010 Jun;28(6):617-23 | |

* Patent: 10-1098032 KR, 12/989, 192. New strains are being added on an ongoing basis.

# *S. pombe* Mutant Library

## *S. pombe* Deletion Mutant Construction

### *S. pombe* Diploid Deletion Mutant Construction:

Diploid deletion mutants in the *S.pombe* genome were systematically constructed with targeted mutagenesis at each target ORF. The chromosomal location of the ORFs and their DNA sequence information were obtained from the *S.pombe* database at the Welcome Trust Sanger institute (www.pombase.org). the deletion cassette module construct contains a selection marker (Figure 1), tag sequences (molecular barcodes), and the sequences for homologous recombination (Figure 2). The cassette for each gene was transformed into diploid host strain (SP286), and the deletion was confirmed by G418 antibiotic resistance (Figure 3). (when the cassette is normally inserted into the target ORF position, G418 appears colonized by antibiotic resistance resulting from the KanMX4 marker gene upon antibiotic treatment.)

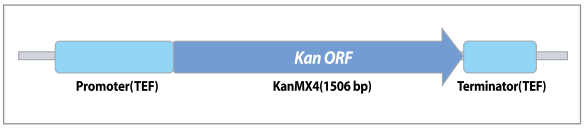



Figure 1. KanMX4 as a selection marker.

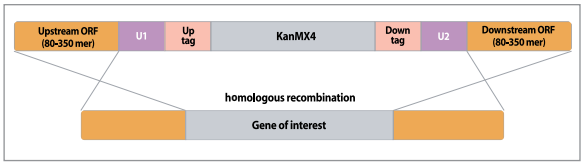



Figure 2. The scheme of a deletion cassette map.

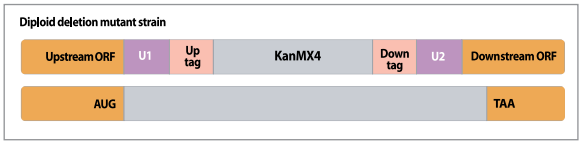



Figure 3. Diploid mutant strain construction.

### *S. pombe* Haploid Deletion Mutant Construction:

*S. pombe* haploid deletion mutants were produced from diploid deletion mutants by the following procedure.

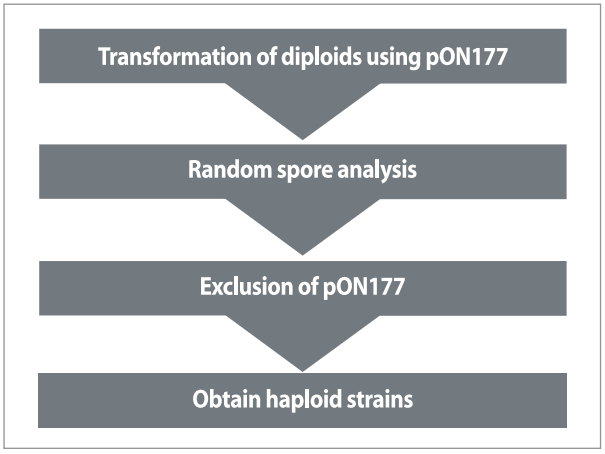



Figure 4. The scheme of haploid strains.

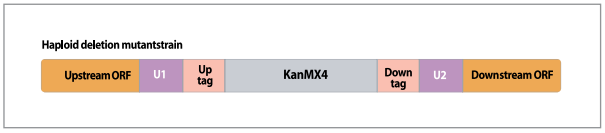



Figure 5. Haploid mutant strain construction.

## *S. pombe* Deletion Mutant Verification

Deletion mutants were confirmed by colony PCR with *AccuOligo*® *S. pombe* Validation Primer.
To know whether the mutant grown in G418 plate is the final deletion mutant of target gene, we performed PCR with two kinds of gene-specific primers(5' upstream & 3' downstream) of target gene. CP5-primers were used with N-terminus PCR with CPN1 and CPN10 primers, and CP3 primers with CPC1 and CPN3 primers were used for PCR at the C-terminus site. The presence and size of the PCR product were analyzed by electrophoresis to identify the mutants.

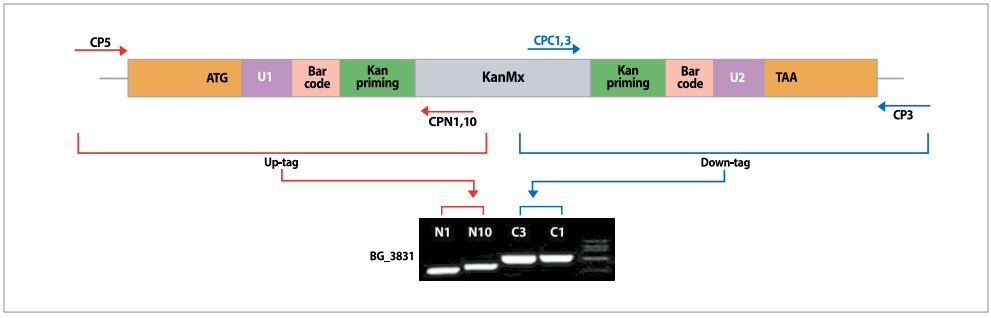



**Figure 6**. Example of strain verification using colony PCR.

To confirm deletion mutant for a target ORF, the tag sequence was amplified by colony PCR using a primer specific to the target gene and the common primer in KanMX module. The CPN1, CPN10, CPC1, and CPC3 sequences in the KanMX module are as follows.

**CPN1** 5-CGTCTGTGAGGGGAGCGTTT-3 **CPN10** 5-GATGTGAGAACTGTATCCTAGCAAG-3
**CPC1** 5-TGATTTTGATGACGAGCGTAAT-3 **CPC3** 5-GGCTGGCCTGTTGAACAAGTCTGGA-3

## Experimental Data

### Application 1 (Anti-fungal)

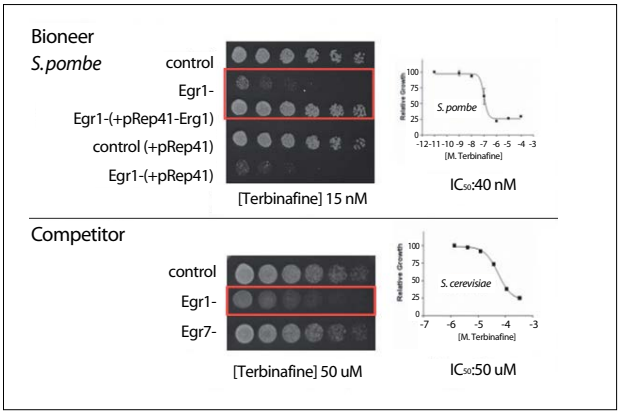



**Figure 7**. Screening of Terbinafine.

A diagram showing the sensitivity of *S. pombe* system to terbinafine being 1000-times more than that of *S. cerevisiae* system, also meaning more specificity. (*S. pombe* gene is more specific for this antimicrobial agent.)

### Application 2 (Anti-hyperlipidemic)

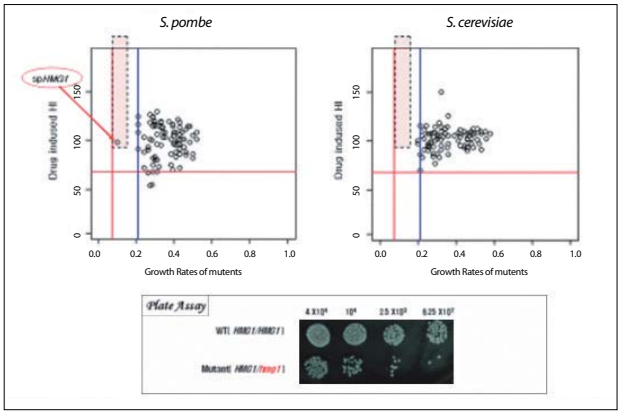



**Figure 8**. Screening of Simvastain.

The diagram showing that HMG1 gene, the action point of simvastatin, a therapeutic agent for the treatment of hyperlipidemia, was isolated from the fission yeast *S. pombe* and was not detected in the germination yeast *S. cerevisiae*. (*S. pombe* can be found to be more effective in screening for this hyperlipidemic drug.)

## Technical Support

- Tel. +82-42-930-8777
- E-mail: spombe-support@bioneer.com

For more information, please refer to bioneer website (mainpage>Life Science Research>Gene-function tab).

# *S. pombe* Individual Deletion Mutant Strains

## Description

*S. pombe* Library targets every 4,914 ORF (Open Reading Frame) in the *S. pombe* genome via targeted mutagenesis. A total of 4,845 heterozygous diploid deletion mutants representing 98.6% of the organism genome and 3,420 haploid deletion mutants with 95.6% genome coverage are available. Since there are different tag sequences (barcode) for each individual mutant, the library provides an ideal way to approach research in gene function and drug target screening for large number of genes by using pools of mutants. It is also possible to analyze biological gene functions through phenotype research with the deletion mutant having specific genes absent.

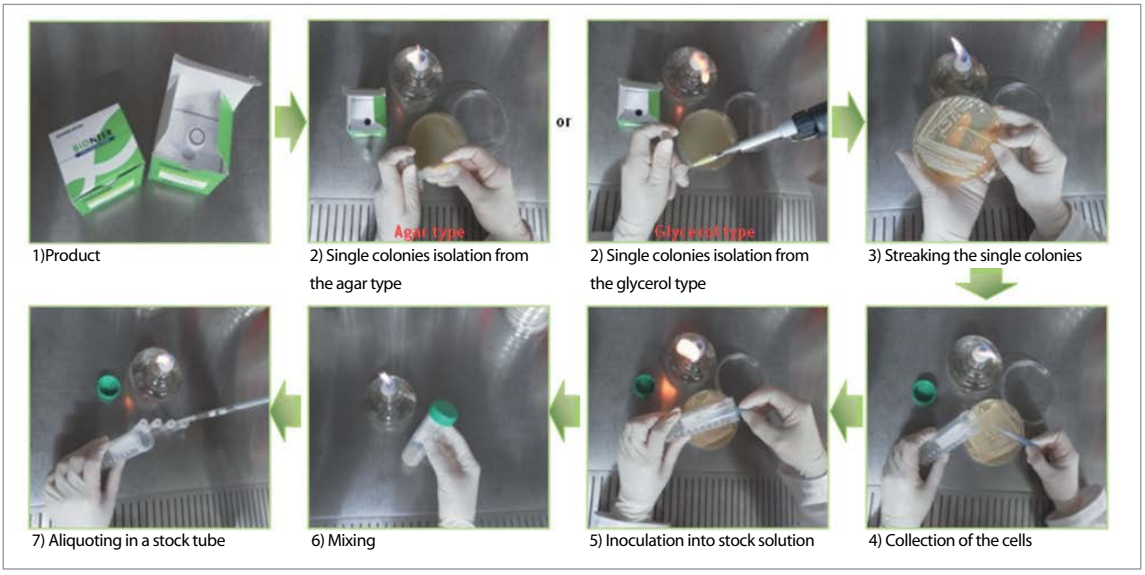
## Features and Benefits

- Deletion mutants of each *S. pombe* gene
- Similar physiological process with mammalian cells
- Human cancer-related genes with over 30% of homology
- Rapid cell cycle(~3h) with simplified analysis of molecular biological mechanism and pathway
- Phenotype analysis possible with its recessive mutant type
- Unknown genomic function analysis through functional complementation
- Drug target screening at the genomic level in living cells available

## Procedure

### Generation of *S. pombe* Culture

For long-term storage, keep your strain into glycerol stocks at -70℃ upon receipt. Refer to stocks procedure below.
(If you store the stock in duplicate and different deep freezer, it can increase the storage efficiency)




## Specifications

- Contents

| | |
|---|---|
| Individual Deletion Mutant Strain | 1 tube |
| Strain information sheet | 1 copy |
| Product manual | 1 copy |

- Note

- Agar Format

The individual Deletion Mutant Strain is supplied in a 2.0 ml tube containing 1 ml YES agar medium with Geneticin (G418) at a concentration of 100 μg/ml. The strain in agar type is shipped at room temperature and viable up to 2 weeks when stored at 4℃. For long-term storage, Keep your strain into glycerol stocks upon receipt.

- Glycerol Format

The individual Deletion Mutant Strain can be also supplied in a 2.0 ml tube containing 0.5 ml YES / 25% glycerol media with Geneticin (G418) at a concentration of 100 mg/L. The mutant in this glycerol type is shipped on dry ice and can be maintained at -70℃. Dry ice shipping and handling charge will be added.

# *S. pombe* Individual Deletion Mutant Strains

## Application

Haploinsufficient mutant library is used as a mechanism analysis in molecular level about natural materials that are difficult to explain the effect before. This type of library is applicable for high contents screening (HCS) for new drug production of metabolism diseases such as anticancer, hyperlipidemia, etc.

- Biological mechanism and toxicity research of drug candidate
- Discovery of target molecule and identification of drug candidate (Especially, it could establish bridge-head in the anticancer drug research)

## Ordering Information

| Cat. No. | Type | Ship Format | Product Description |
|---|---|---|---|
| M-1010D-A | Diploid | Agar | *S. pombe* Individual Heterozygous Diploid Deletion Mutant Strain, Agar |
| M-1010D-G | | Glycerol | *S. pombe* Individual Heterozygous Diploid Deletion Mutant Strain, Glycerol |
| M-1010H-A | Haploid | Agar | *S. pombe* Individual Haploid Deletion Mutant Strain, Agar |
| M-1010H-G | | Glycerol | *S. pombe* Individual Haploid Deletion Mutant Strain, Glycerol |

# *S. pombe* Deletion Mutant Sets

## Description

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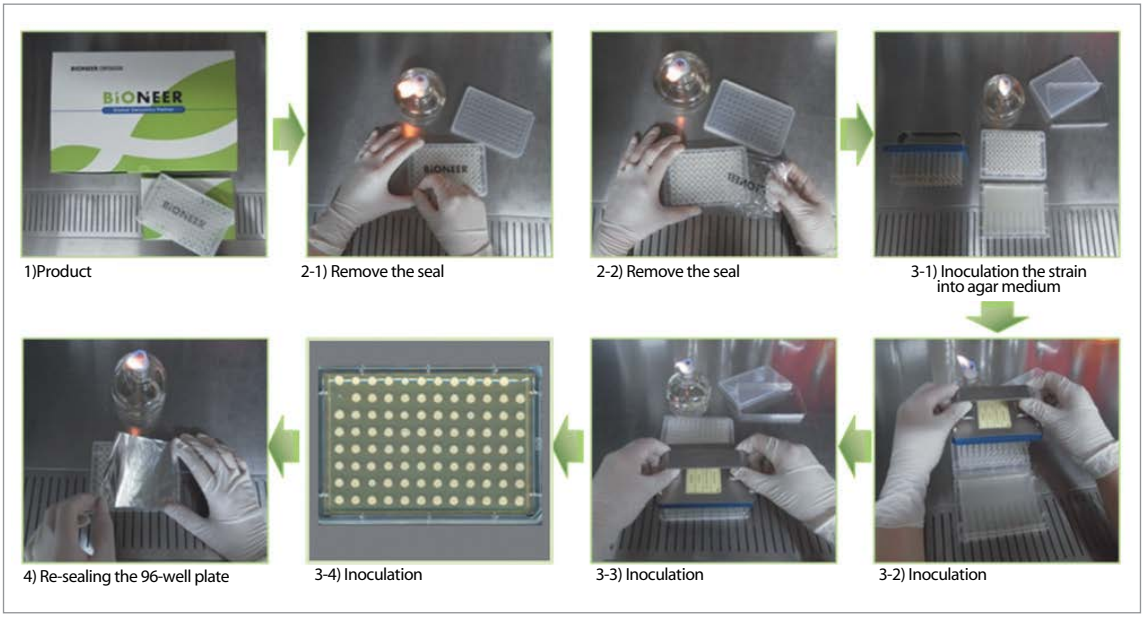
- Rapid cell cycle(~3h) with simplified analysis of molecular biological mechanism and pathway
- Phenotype analysis possible with it's recessive mutant type
- Unknown genomic function analysis through functional complementation
- Drug target screening at the genomic level in living cells available

## Procedure

### Culture of mutants in 96-well plate

For long-term storage, keep your strain into glycerol stocks at -70℃ upon receipt. Refer to stocks procedure below.
(If you store the stock in duplicate and different deep freezer, it can increase the storage efficiency)




## Specifications

### Contents

*S. pombe* Haploid Deletion Mutant Set Ver 5.0

| | |
|---|---|
| 3,420 strains supplied in 96-well plate | 36 plates total |
| Wild type Strain (ED666 and ED668) | 2 vials |
| List of haploid strains on CD | 1 copy |
| Product manual | 1 copy |

### Storage

The *S. pombe* Deletion Mutant Sets are supplied in 96-well plates containing 100 µl of YES media (25% glycerol & 100 mg/L Geneticin (G418)). The set is shipped on dry ice and is viable for a few years when stored at -70℃.

# *S. pombe* Deletion Mutant Sets

## Application

Haploinsufficient mutant library is used as a mechanism analysis in molecular level about natural materials that are difficult to explain the effect before. This type of library is applicable for high contents screening (HCS) for new drug production of metabolism diseases such as anticancer, hyperlipidemia, etc.

- Biological mechanism and toxicity research of drug candidate
- Discovery of target molecule and identification of drug candidate (Especially, it could establish bridge-head in the anticancer drug research)

## Ordering Information

| Cat. No. | Type | Product Description | Quantity |
|---|---|---|---|
| M-5030H-LT | Haploid | *S. pombe* Haploid Deletion Mutant Set Ver 5.0 | 36 plates (96-well) |
| M-5030H-5Y* | | *S. pombe* Haploid Deletion Mutant Set Ver 5.0 5Y License | 36 plates (96-well) |

*Up to 3 Sets of the M-5030H-5Y can be purchased and used by any scientist(s) at the purchaser(s)' organization or of purchaser's laboratory of the M-5030H-LT. For more information, please send an e-mail (pombeadmin@bioneer.com / spombe-support@bioneer.com).

# *AccuOligo*® *S. pombe* Validation Primer Set

## Description

*AccuOligo*® *S. pombe* Validation Primer Set is primer set for checking quality of *S. pombe* deletion mutants (diploids and haploids) by PCR. This primer set contains target gene specific primers and the KanMX module, which is the deletion cassette for the preparation of total PCR mixture. Bioneer also sells *AccuPower*® PCR PreMix for PCR as well as primer sets.

## Features and Benefits

- High quality primer: All primers are purified with Bio-RP purification system and checked with MALDI-TOF spectrometer QC
- Ease-of-use: It can be easily performed by one pipetting, in order to premix type is contained forward primer and reverse primer.

## Procedure

### Procedures for Verifying *S. pombe*

1. Genomic DNA extraction from mutant

After strains are cultured in liquid media, gDNA is extracted using *AccuPrep*® Genomic DNA Extraction Kit (K-3032 or K-3032-2). Alternatively, more robust purity and yield gDNA can be achieved using Automated nucleic acid extraction system such as *ExiPrep*™ 16 Plus or *ExiPrep*™96 Lite.

2. PCR mixture preparation

Reaction example

| *AccuPower*® PCR PreMix | Reaction Tube | Reaction Tube | - |
|---|---|---|---|
| *AccuPower*® PCR Master Mix | - | - | 10 µl |
| Genomic DNA | 1 µl | 1 µl | 1 µl |
| Primer set | 1 µl | 1 µl | 1 µl |
| D.W. | 18 µl | 48 µl | 8 µl |
| Total volume | 20 µl | 50 µl | 20 µl |

3. Vortex reaction tube(s) and Spin down.

4. Perform PCR with the cycling protocol described below

| Step 1 | 94℃ | 5 min |
|---|---|---|
| Step 2 | 94℃ | 30 sec |
| Step 3 | 55℃ | 30 sec |
| Step 4 | 72℃ | 1 min |
| Repeat step 2 through 4 for 30 cycles | | |
| Step 5 | 72℃ | 5 min |

Perform agarose gel electrophoresis with 8~10 µl of the PCR mixture for verification.

# *AccuOligo*® *S. pombe* Validation Primer Set

## Specifications

### Contents

| | |
|---|---|
| *AccuOligo*® primers in 96-well plate (CP3-CPC3) | 36 plates total |
| *AccuOligo*® primers in 96-well plate (CP5-CPN1) | 36 plates total |
| List of primers on CD | 1 copy |
| Product manual | 1 copy |

### Common primer sequences of KanMX module

| | |
|---|---|
| CPN1 | 5'-CGTCTGTGAGGGGAGCGTTT-3' |
| CPC3 | 5'-GGCTGGCCTGTTGAACAAGTCTGGA-3' |

### Storage

The validation primer set is lyophilized in 96-well plates and shipped at room temperature. Resuspend each primer in 10 mM Tris buffer (pH 8.0) or distilled $H_2O$ to the concentration of 10 pmoles/μl. The primer in Tris buffer or $dH_2O$ is stable for one year at -20 ℃.

## Experimental Data

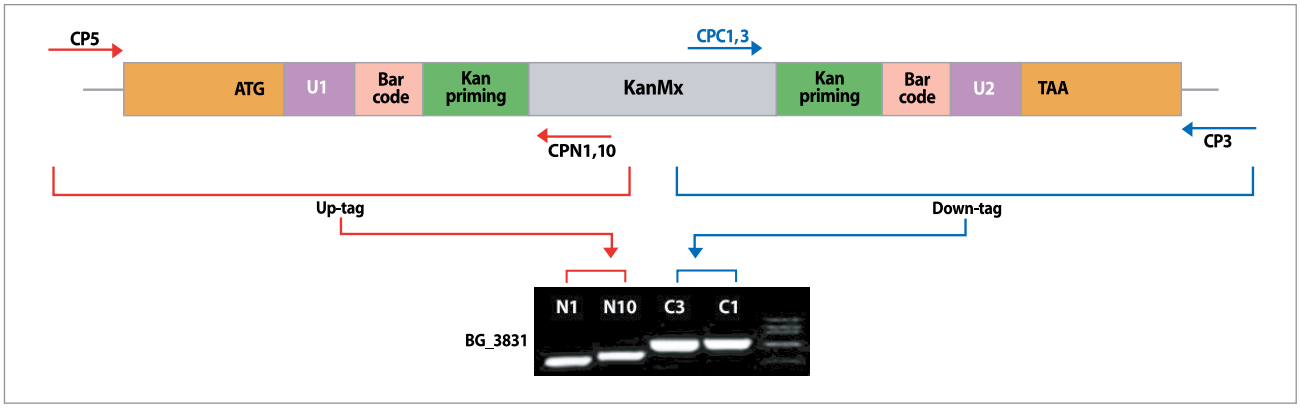



Figure 1. Data using *AccuOligo*® *S.pombe* Validation Primer

1.2% agarose gel Electrophoresis result after genomic DNA extraction from *S.pombe*

## Ordering Information

| Cat. No. | Product Description | Quantity |
|---|---|---|
| M-3030P | *AccuOligo*® *S. pombe* Validation Primer Set ver 3.0 | 72 plates (96-well) |
| M-3030P-U4 | *S. pombe* Validation Primer ver 3.0 to ver 4.0 Upgrade Pkg | 6 plates (96-well) |
| M-3030P-U5 | *S. pombe* Validation Primer ver 3.0 to ver 5.0 Upgrade Pkg | 20 plates (96-well) |

# *S. pombe* Genomic DNA

## Description

*S. pombe* Genomic DNA is extracted from *S. pombe* using *AccuPrep*® Genomic DNA Extraction Kit has high capacity of DNA binding. Extracted genomic DNA is used in many experiments as gene cloning, PCR, quantitative Real-Time PCR, southern blotting, and etc.

## Features and Benefits

- No additional work to extract the genomic DNA
- High yield, high purity genomic DNA
- Purified through silica-based DNA binding column with high binding capacity

## Specifications

- Contents

| | |
|---|---|
| *S. pombe* Genomic DNA in 2.0 ml tube | 1 tube |
| List of Genomic DNA on CD | 1 copy |
| Product manual | 1 copy |

- Storage

The *S. pombe* Genomic DNA is supplied in 2.0 ml tube and shipped on dry ice. The product should be stored at -20℃.

## Ordering Information

| Cat. No. | Product Description | Quantity |
|---|---|---|
| M-1030-D | *S. pombe* Genomic DNA | 2 μg |

# Media for the Fission Yeast *S. pombe*

▪ YES (Yeast Extract with supplements) for rich complete media

| Amount | Component | Final concentration |
|---|---|---|
| 5 g/L | Yeast extract | 0.5% w/v |
| 30 g/L | Glucose | 3.0% w/v |

Supplements: 225 mg/L adenine, histidine, leucine, uracil and lysine hydrochloride.
Solid media is made by adding 2% Difco Bacto Agar.

▪ EMM: Edinburgh Minimal Medium

| Amount | Component | Final concentration |
|---|---|---|
| 3 g/L | Potassium hydrogen phthalate | 14.7 mM |
| 5 g/L | $NH_4Cl$ | 15.5 mM |
| 2.2 g/L | $Na_2HPO_4$ | 93.5 mM |
| 20 g/L | Glucose | 2.0% w/v |
| 20 ml/L | Salts | |
| 1 ml/L | Vitamins | |
| 0.1 ml/L | Minerals | |

Soild media is made by adding 2% Difco Bacto Agar.

▪ Stock Solutions: Filter-sterilize and store at 4℃.

- 50x Salt Stock

| Amount | Component | Final concentration |
|---|---|---|
| 52.5 g/L | $MgCl_2 \cdot 6H_2O$ | 0.26 M |
| 0.735 g/L | $CaCl_2 \cdot 2H_2O$ | 4.99 mM |
| 50 g/L | KCl | 0.67 M |
| 2 g/L | $Na_2SO_4$ | 14.1 mM |

- 1000x Vitamin Stock

| Amount | Component | Final concentration |
|---|---|---|
| 1 g/L | Pantothenic acid | 4.2 mM |
| 10 g/L | Nicotinic acid | 81.2 mM |
| 10 g/L | Inositol | 55.5 mM |
| 10 mg/L | Biotin | 40.8 μM |

- 10,000x Mineral Stock

| Amount | Component | Final concentration |
|---|---|---|
| 5 g/L | Boric acid | 80.9 mM |
| 4 g/L | $MnSO_4$ | 23.7 mM |
| 4 g/L | $ZnSO_4 \cdot 7H_2O$ | 13.9 mM |
| 2 g/L | $FeCl_2 \cdot 6H_2O$ | 7.4 mM |
| 0.4 g/L | Molybdic acid | 2.47 mM |
| 1 g/L | KI | 6.02 mM |
| 0.4 g/L | $CuSO_4 \cdot 5H_2O$ | 1.6 mM |
| 10 g/L | Citric acid | 47.6 mM |

# 02. *S. cerevisiae* VN-Fusion Library

# *S. cerevisiae* VN-Fusion Library

## Overview

The yeast *S. cerevisiae* (*Saccharomyces cerevisiae*) is a widely used as a simple eukaryotic model system, where its genome can be easily manipulated. This enables researchers to study many biological processes, involving dynamic networks of protein-protein interactions. Thus, identification and visualization of protein-protein interactions provide significant insight into the individual roles of cellular proteins. Of many known technologies to study a large scale protein-protein interaction, Bimolecular Fluorescence Complementation (BiFC) assay is now regarded as one of the most advanced and effective tools for studying *in vivo* protein-protein interactions in several organisms.

The BiFC assay is based on the formation of a fluorescent complex by fragments of yellow fluorescent protein, based on association of two interacting partners fused to the fragments. This approach enables visualization of the subcellular localizations of specific protein complexes in the normal intracellular environment. To meet this need, Bioneer's *S. cerevisiae* VN-Fusion Library, which is created by Dr. Won-Ki Huh of Seoul National University (Korea), provides up to 5,809 VN-tagged Open Reading Frames (ORFs) covering 93% of the yeast proteome.

### ○ Characteristics of *S. cerevisiae* VN-Fusion Library

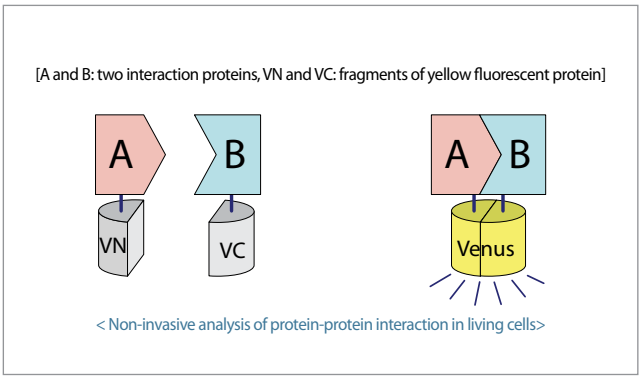
| | |
|---|---|
| No. of Strains | 5,809 strains |
| Selection Marker | KIURA3 |
| Genotype | All *S. cerevisiae* VN-Fusion strains were derived from BY4741<br>(*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*)<br>Haploid |
| Culture Media | YPD: for general culture and maintenance medium<br>SC-Ura or SC-His: for medium selection & counter selection (auxotrophic culture) |
| Strain Verification | Medium selection & Counter selection, Check PCR |
| Storage | Store at -70℃ (Glycerol type)<br>Store at 22℃ to 25℃ (Agar type) |
| References | 1) Sung MK., *et al.*, *J. Microbiol. Methods.*, 83(2): 194-201 (2010)<br>2) Sung MK., *et al.*, *Yeast*, 24: 767-775 (2007)<br>3) Huh, W., *et al.*, *Nature*, 425: 686-691 (2003) |
| Patent | 10-2009-0048746 |

* New strains are being added on an ongoing basis.

# *S. cerevisiae* VN-Fusion Library

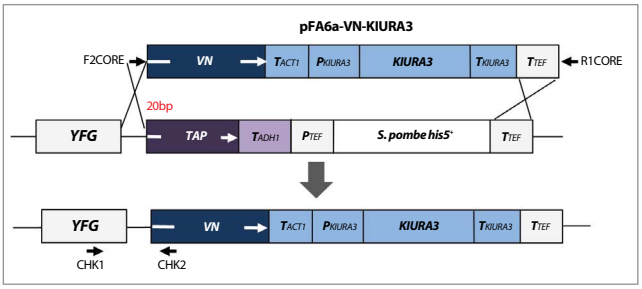
## Bimolecular fluorescence complementation (BiFC)

Most biological processes are carried out and regulated by dynamic networks of protein-protein interactions. Therefore, understanding and checking their interactions are necessary processes for understanding the cellular functions of proteins. Bimolecular fluorescence complementation (BiFC) assays are easy to use when studying protein interactions as their fluorescence signals can be directly measured through a microscope. In the BiFC assay, the protein that exhibits yellow fluorescence ('Venus' in the figure below) is divided into two sections ('VN' and 'VC' in the figure below) to utilize their characteristic which only produces illuminance when those are close to each other by forming a whole fluorescent protein complex.




## Construction of *S. cerevisiae* VN-Fusion Library

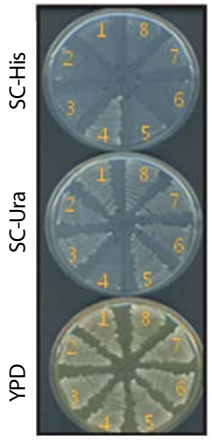
A ~2.5 kb DNA cassette including the VN and KlURA3 marker gene was amplified by PCR using pFA6a-VNKlURA3 as a template, and the "universal" F2CORE and R1CORE primers. The obtained DNA cassette was transformed into ~6,000 yeast strains from the TAP-tagged collection (Ghaemmaghami *et al.*, 2003). The transformed cells were spread on SC-Ura plates and incubated at 30℃ for 3 days. Among several colonies, 10 colonies were picked, streaked on fresh SC-Ura plates, and incubated at 30℃ for 24 hours. To check correct switching to the VN tag, cells grown on SC-Ura plates were replica-plated onto SC-His plates. Cells also growing on SC-His plates were discarded.



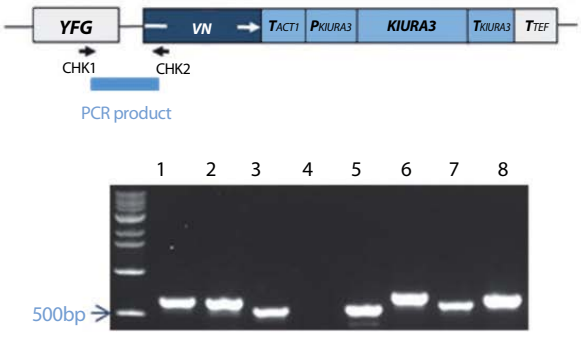

## Validation of *S. cerevisiae* VN-Fusion

To confirm that the TAP tag was successfully substituted to the VN tag, colony PCR was performed on the transformants using the CHK1 primer (gene-specific primer) and CHK2 primer (VN-specific primer).

| No. | ORF |
|---|---|
| 1 | YIL095W |
| 2 | YBR276C |
| 3 | YLR182W |
| 4 | YBR198C |
| 5 | YOR133W |
| 6 | YKR079C |
| 7 | YMR102C |
| 8 | YDR251W |




<Medium selection/counter-selection>




<Check PCR>

[Results: *S. cerevisiae* VN-Fusion strains- 1, 2, 3, 5, 6, 7, 8 / TAP-fusion strain-4]

## Experimental Data

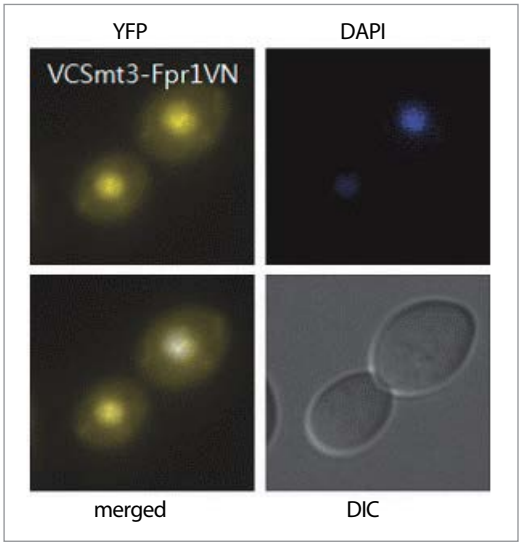



**Figure 1.** Visualization of subcellular location of protein-protein interaction.

The result is Smt3 protein-protein interaction with BiFC. Diploid cells expressing the VN-tagged Fpr1 and the VC-tagged Smt3 together were grown in medium, and analysed for BiFC. The BiFC signal (Fpr1-Smt3 interaction) was clearly detected in the nucleus.

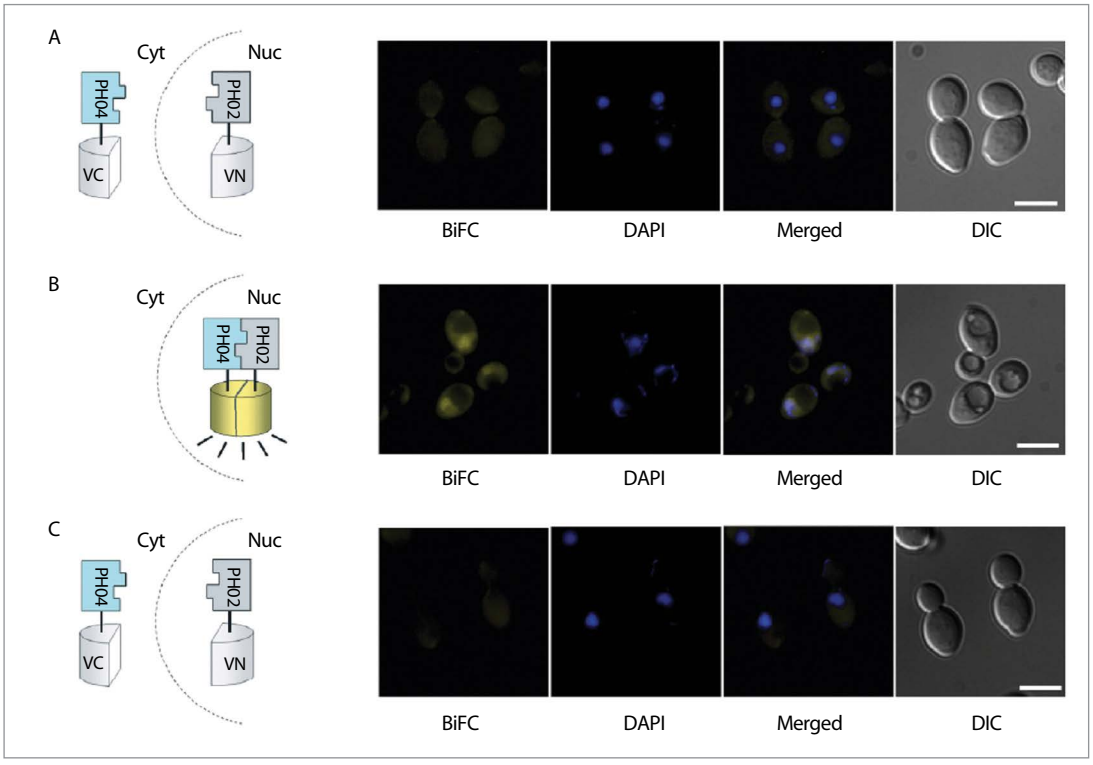



**Figure 2.** Visualization of induced protein-protein interaction (*Yeast* 2007; 24: 767-775.)

Pho2, Pho4: transcription factors involved in phosphate metabolism
(A) In medium containing a high concentration of phosphate: did not show any BiFC signal
(B) Phosphate starvation: detected BiFC signal accumulating in the nucleus (Pho2-Pho4 interaction in the nucleus)
(C) Medium lacking phosphate to medium containing a high concentration of phosphate: the BiFC signal disappeared from the nucleus

## Technical Support

- Tel. +82-42-930-8777
- E-mail: vn-support@bioneer.com

For more information, please refer to bioneer website (mainpage>Life Science Research>Gene-function tab).

# *S. cerevisiae* Individual VN-Fusion Strains

## Description

*S. cerevisiae* VN-fusion library is a system that allows the interaction of proteins *in vivo* to be more easily detected through fluorescent signals. It consists of 5,809 VN-tagged Open Reading Frames (ORFs) and 93% of the entire genome of *S. cerevisiae*. While the VN-fusion library is developed by Dr. Won-Ki Huh of Seoul National University (Korea), Bioneer owns its exclusive business license.

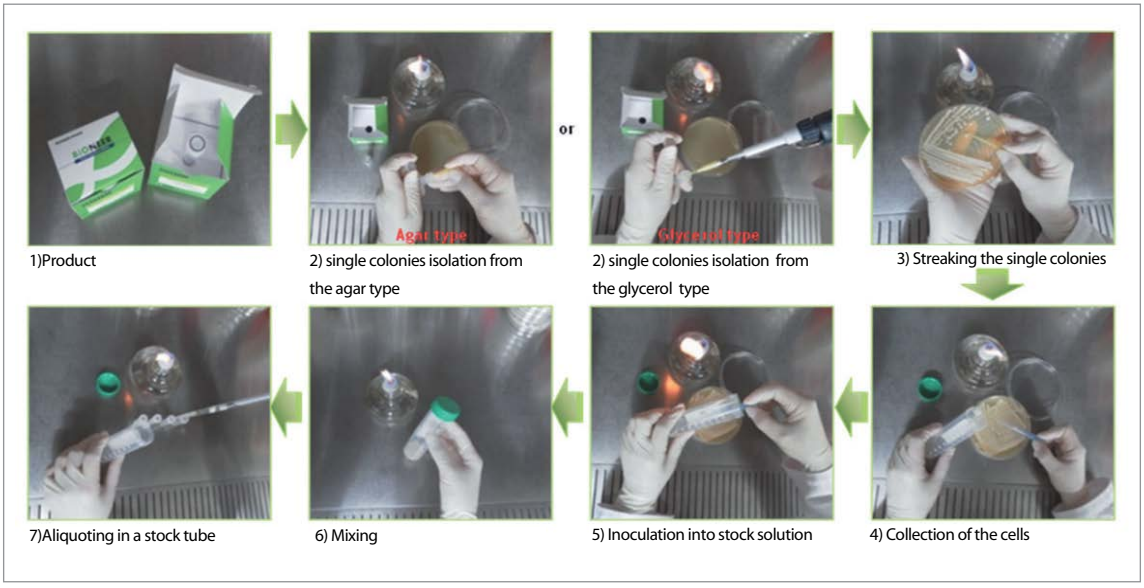
## Features and Benefits

- A powerful tool for studying protein-protein interactions in living cells and analyzing intra-cellular location
- Simple observation by fluorescence microscope with strong fluorescence emitted during the formation of fluorescence complex
- Analysis of protein interactions at the genomic level (Capable of covering 93% of the yeast proteome)
- Easy study of unknown functions and interactions of new proteins
- Proteinization analysis possible (ubiquitination, sumoylation, neddylation, etc.)

## Procedure

### Generation of *S. cerevisiae* Culture

For long-term storage, keep your strain into glycerol stocks at -70℃ upon receipt. Refer to stocks procedure below (If you store the stock in duplicate and different deep freezer, it can increase the storage efficiency)




## Specifications

### Contents

| | |
|---|---|
| Individual strain in 2.0 ml tube | 1 tube |
| Strain information sheet | 1 copy |

### Note

- Agar Format

The individual strain is supplied in a 2.0 ml tube containing 1ml YPD agar medium. The strain in agar type is shipped at room temperature and is viable up to 2 weeks when stored at 4℃.

- Glycerol Format

The individual strain is supplied in a 2.0 ml tube containing 0.5 ml YPD / 25% glycerol media. The strain in glycerol type is shipped on dry ice and can be stored at -70 ℃. Dry ice shipping and handling charge will be added.

# *S. cerevisiae* Individual VN-Fusion Strains

## Applications

▪ The field of basic biological research

- Dynamic analysis of protein-protein interaction in various conditions
- Identification of cell signal transduction for induction or inhibition of protein-protein interaction
- Database for protein mathematization information in various conditions

▪ The field of basic Medicine and Pharmacy

- Drug discovery, target validation, genome-wide high-throughput screening, pathway mapping, drug mechanism-of-action studies, and diagnostics.
- Validation of disease diagnosis and drug target candidate caused by protein-protein interaction problem
- Comprehension in molecular level for mechanism of drugs that influence protein-protein interaction

## Ordering Information

| Cat. No. | Ship Format | Product Description |
|---|---|---|
| V-1010VN-A | Agar | *S. cerevisiae* Individual VN-Fusion Strain, Agar |
| V-1010VN-G | Glycerol | *S. cerevisiae* Individual VN-Fusion Strain, Glycerol |

# *S. cerevisiae* VN-Fusion Set

## Description

This product is a yeast strain library product for analyzing protein interactions in yeast cells at the genome-wide level by applying bimolecular fluorescence complementation technique. It divides the fluorescent protein into N-terminus and C-terminus fragments and attaches them to two proteins to be tested for interaction. And it was developed using a method of analyzing the fluorescence that appears when two fragments of a fluorescent protein are combined to form a complete fluorescent protein. Thus, it is accepted that there are many advantages and potentials of success that can be applied to the dynamic analysis of protein interactions in living cells. *S. cerevisiae* VN-Fusion Set products, unlike conventional methods of analyzing protein interactions through in vitro analysis or artificial protein expression, can quickly analyze the interactions between proteins that are expressed *in vivo* from their unique promoters in nature state as whole proteins of the yeast. In addition, about 93% of the yeast proteome is obtained and 5,809 VN-tagged Open Reading Frames (ORFs) are constructed. Therefore, protein interactions can be analyzed at the genomic level.

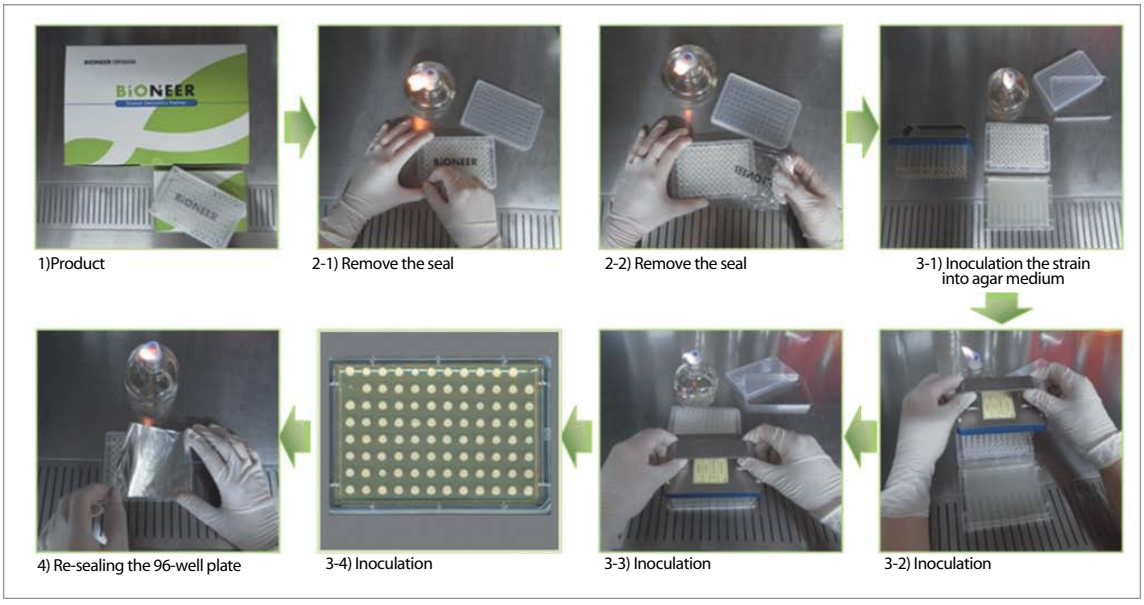
## Features and Benefits

- A powerful tool for studying protein-protein interactions in living cells and analyzing intra-cellular location
- Simple observation by fluorescence microscope with strong fluorescence emitted during the formation of fluorescence complex
- Analysis of protein interactions at the genomic level (Capable of covering 93% of the yeast proteome)
- Easy study of unknown functions and interactions of new proteins
- Proteinization analysis possible
  (ubiquitination, sumoylation, neddylation, etc.)

## Procedure

### Culture of *S. cerevisiae* stains in 96-well plate

For long-term storage, keep your strain into glycerol stocks at -70℃ upon receipt. Refer to stocks procedure below
(If you store the stock in duplicate and different deep freezer, it can increase the storage efficiency)




## Specifications

### Contents

| | |
|---|---|
| 5,809 strains supplied in 96-well plate | 63 plates total |
| List of strains on CD | 1 copy |
| Product manual | 1 copy |

### Storage

*S. cerevisiae* VN-Fusion Sets are supplied in 96-well plates containing 100 µl of YPD/25% glycerol media. The set is shipped on dry ice and is viable for a few years when stored at -70℃.

# *S. cerevisiae* VN-Fusion Set

## Applications

▪ The field of basic biological research

- Identification of binding proteins, investigation of novel functional roles of proteins, global dynamic analysis of protein-protein interaction under the various conditions, and creating database for post-translational modifications' information of proteins under the various conditions.
- Dynamic analysis of protein-protein interaction in various conditions
- Identification of cell signal transduction for induction or inhibition of protein-protein interaction
- Database for protein mathematization information in various conditions

▪ The field of basic Medicine and Pharmacy

- Drug discovery, target validation, genome-wide high-throughput screening, pathway mapping, drug mechanism-of-action studies, and diagnostics.
- Validation of disease diagnosis and drug target candidate caused by protein-protein interaction problem
- Comprehension in molecular level for mechanism of drugs that influence protein-protein interaction

## Ordering Information

| Cat. No. | Product Description | Quantity |
|---|---|---|
| V-1030VN | *S. cerevisiae* VN-Fusion Set (5,809 strains) | 63 plates (96-well) |

# *AccuOligo*® *S. cerevisiae* Validation Primer Set

## Description

This product is a primer product used to confirm the quality of *S. cerevisiae* VN-Fusion Library by PCR. The mixture of the specific primer for the target gene and the common primer for the VN module can be easily prepared PCR mixture by one pipetting. Bioneer also provides *AccuPower*® PCR PreMix for PCR as well as primer sets.

## Features and Benefits

- Providing high quality primers completed QC test using Bio-RP or MALDI-TOF spectrometer.
- PreMix type primer configuration allows forward, reverse-primer to be used in one pipetting.
- The locations of the sets and their respective primers are the same, providing convenience for checking large quantities of quality.

## Procedure

### Procedures for Verifying *S. cerevisiae*

1. Genomic DNA extraction from *S. cerevisiae*

After strains are cultured in liquid media, gDNA is extracted using the *AccuPrep*® Genomic DNA Extraction Kit (Cat. No. K-3032 or K-3032-2, Bioneer). Alternatively, more robust purity and yield gDNA can be achieved using automated nucleic acid extraction system such as *ExiPrep*™16 Plus or *ExiPrep*™96 Lite.

2. Preparing of PCR mixture

Reaction example

| *AccuPower*® PCR PreMix | Reaction Tube | Reaction Tube | - |
|---|---|---|---|
| *AccuPower*® PCR Master Mix | - | - | 10 µl |
| Genomic DNA | 1 µl | 1 µl | 1 µl |
| Primer set | 1 µl | 1 µl | 1 µl |
| D.W. | 18 µl | 48 µl | 8 µl |
| Total volume | 20 µl | 50 µl | 20 µl |

3. Vortex reaction tube(s) and spin down.

4. Perform PCR with the cycling protocol described below

| Step 1 | 94℃ | 3 min |
|---|---|---|
| Step 2 | 94℃ | 30 sec |
| Step 3 | 50℃ | 30 sec |
| Step 4 | 72℃ | 40 sec |
| Repeat step 2 through 4 for 35 cycles | | |
| Step 5 | 72℃ | 10 min |

Perform agarose gel electrophoresis with 8-10 µl of the PCR mixture for verification.

# *AccuOligo*® *S. cerevisiae* Validation Primer Set

## Specifications

### Contents

| | |
|---|---|
| *AccuOligo*® primers in 96-well plate | 63 plates total |
| List of primers on CD | 1 copy |
| Product manual | 1 copy |

### Primer sequences

| | |
|---|---|
| CHK1 | gene-specific primer |
| CHK2 | 5'-CACCATGGTGGCGATGGATC-3' |

### Storage

The validation primer set is lyophilized in 96-well plates and shipped at room temperature. Resuspend each primer in 10 mM Tris buffer (pH 8.0) or distilled $H_2O$ to the concentration of 10 pmoles/50 µl when it is used. The primer in Tris buffer or $dH_2O$ is stable for one year at -20 ℃.

## Experimental Data

| No. | ORF |
|---|---|
| 1 | YIL095W |
| 2 | YBR276C |
| 3 | YLR182W |
| 4 | YBR198C |
| 5 | YOR133W |
| 6 | YKR079C |
| 7 | YMR102C |
| 8 | YDR251W |


**Figure 1.** Example of strain verification using *AccuOligo*® *S. cerevisiae* Validation Primer Set.
The results are performed using electrophoresis in 1.2% agarose gel after gDNA was extracted from *S. cerevisiae*.

## Ordering Information

| Cat. No. | Product Description | Quantity |
|---|---|---|
| V-1030VN-P | *AccuOligo*® *S. cerevisiae* VN-Fusion Validation Primer Set | 63 plates (96-well) |

# *S. cerevisiae* Protein Tagging Vectors for BiFC Analysis

## Description

*S. cerevisiae* protein tagging vectors for BiFC analysis are used to create the VN- or VC-fusion proteins. The plasmids contain one of three selectable markers - the kanMX6 module, the His3MX6 module and the *S. cerevisiae* TRP1 gene, for selection with G418, growth media lacking histidine or tryptophan, respectively. And high purity and yield plasmid DNA is extracted using *AccuPrep*® Nano-Plus Plasmid Midi Extraction Kit(Cat. No. K-3122, Bioneer).

## Features and Benefits

- Plasmid DNA purified with the *AccuPrep*® Plasmid Extraction Kit (Cat. No. K-3030-1,Bioneer)
- BiFC vector system is useful for the development of a high-throughput platform to study protein-protein interactions in living yeast cells.

## Specifications

### Contents

| | |
|---|---|
| Vector | 1 tube |
| Vector information sheet | 1 copy |

### Storage

The individual vector is supplied in a 2.0 ml tube and shipped at room temperature. The dry vector pellet can be dissolved in Tris buffer (pH 8.0) or distilled $H_2O$. Store the dissolved vector at -20 ℃.

## Ordering Information

| Cat. no. | Product Description | Quantity |
|---|---|---|
| V-1010-V1 | pFA6a-VN173-HIS3MX6 | 5 μg |
| V-1010-V2 | pFA6a-VC155-HIS3MX6 | 5 μg |
| V-1010-V3 | pFA6a-VN173-TRP1 | 5 μg |
| V-1010-V4 | pFA6a-VC155-TRP1 | 5 μg |
| V-1010-V5 | pFA6a-VN173-KanMX6 | 5 μg |
| V-1010-V6 | pFA6a-VC155-KanMX6 | 5 μg |
| V-1010-V7 | pFA6a-HIS3MX6-PGAL1-VN173 | 5 μg |
| V-1010-V8 | pFA6a-HIS3MX6-PGAL1-VC155 | 5 μg |
| V-1010-V9 | pFA6a-TRP1-PGAL1-VN173 | 5 μg |
| V-1010-V10 | pFA6a-TRP1-PGAL1-VC155 | 5 μg |
| V-1010-V11 | pFA6a-KanMX6-PGAL1-VN173 | 5 μg |
| V-1010-V12 | pFA6a-KanMX6-PGAL1-VC155 | 5 μg |
| V-1010-V13 | pFA6a-HIS3MX6-PCET1-VN173 | 5 μg |
| V-1010-V14 | pFA6a-HIS3MX6-PCET1-VC155 | 5 μg |
| V-1010-V15 | pFA6a-TRP1-PCET1-VN173 | 5 μg |
| V-1010-V16 | pFA6a-TRP1-PCET1-VC155 | 5 μg |
| V-1010-V17 | pFA6a-KanMX6-PCET1-VN173 | 5 μg |
| V-1010-V18 | pFA6a-KanMX6-PCET1-VC155 | 5 μg |

# Media for the Budding Yeast *S. cerevisiae*

▪ YPD (Yeast Peptone Dextrose) for routine growth

| Amount | Component | Final concentration |
|---|---|---|
| 10g/L (±0.2g) | Yeast extract | 1% w/v |
| 20g/L (±0.4g) | Peptone | 2% w/v |
| 20g/L (±0.4g) | Dextrose (D-glucose) | 2% w/v |

▪ SC media: for Medium selection/Counter-selection

- SD: Synthetic Minimal Glucose Medium

| Amount | Component | Final concentration |
|---|---|---|
| 6.7g/L (±0.13g) | Bacto-yeast nitrogen base (w/o a.a) | 0.67% w/v |
| 20g/L (±0.4g) | Dextrose (D-glucose) | 2% w/v |

- SC (Synthetic Complete Media): SD + Various Supplements

| Constituent | Final conc. (mg/L) | Stock per 100 ml (g) | Amount of stock (ml) for 1 L |
|---|---|---|---|
| Adenine sulfate | 20 | 0.2 (±0.004)* | 10 |
| Uracil | 20 | 0.2 (±0.004)* | 10 |
| L-Tryptophan | 20 | 1 (±0.02) | 2 |
| L-Histidine-HCl | 20 | 1 (±0.02) | 2 |
| L-Arginine-HCl | 20 | 1 (±0.02) | 2 |
| L-Methionine | 20 | 1 (±0.02) | 2 |
| L-Tyrosine | 30 | 0.2 (±0.004) | 15 |
| L-Leucine | 60 | 1 (±0.02) | 6 |
| L-Isoleucine | 30 | 1 (±0.02) | 3 |
| L-Lysine-HCl | 30 | 1 (±0.02) | 3 |
| L-Phenylalanine | 50 | 1 (±0.02)* | 5 |
| L-Glutamic acid | 100 | 1 (±0.02)* | 10 |
| L-Aspartic acid | 100 | 1 (±0.02)*# | 10 |
| L-Valine | 150 | 3 (±0.06) | 5 |
| L-Threonine | 200 | 4 (±0.08)*# | 5 |
| L-Serine | 400 | 8 (±0.16) | 5 |

**Note:** The synthetic medium is based on media described by Wickerham. SC contains synthetic minimal medium (SD) with various supplements.
* Store at room temperature.
# Add after autoclaving the media.

**SC-Ura:** Prepare a separate stock solution of various supplements. After the SD broth has been autoclaved, add various supplements (Ade, Trp, His, Arg, Met, Tyr, Leu, Ile, Lys, Phe, Glu, Asp, Val, Thr, Ser) to the medium.

**SC-His:** Prepare a separate stock solution of various supplements. After the SD broth has been autoclaved, add various supplements (Ade, Ura, Trp, Arg, Met, Tyr, Leu, Ile, Lys, Phe, Glu, Asp, Val, Thr, Ser) to the medium.

**Optional:** If necessary, the media were solidified by including 2% agar.

# 03. Genome-wide Drug On/Off-Target Identification Service: *GPScreen*™

# Primary Activity Test Service for *GPScreen*™

## Description

Primary activity test is the first step to determine the growth-inhibitory activity ($GI_{50}$*) of customer-provided compounds in wild type *S. pombe* cells (SP286). The test can be used as the first step of *GPScreen*™ services. *GPScreen*™ will be performed only to the compounds that show growth-inhibitory activity in wild-type *S. pombe* in this primary activity test.

Following information will be provided as the results.

1. Solubility of compounds in cultured –media of *S. pombe*.
2. Growth-inhibitory activity ($GI_{50}$) of compounds in wild type *S. pombe*.

* $GI_{50}$ means the concentration of drugs for 50% inhibition of cell growth.

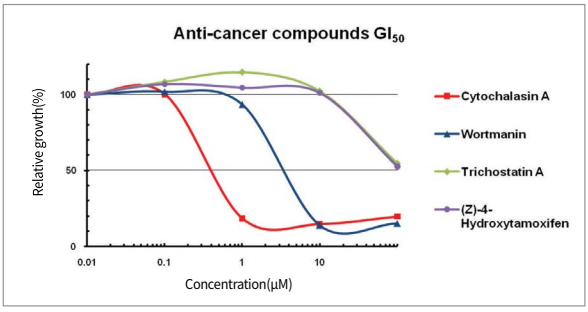



**Figure 1.** Determination of Growth-Inhibitory Activity ($GI_{50}$) of compounds.

## Ordering Information

| Cat. No. | Product Description | Cell type |
|---|---|---|
| GPS-00 | Primary Activity Test Service in Wild Type *S. pombe* | SP286 (Wild-type *S. pombe* ) |

- Price of primary test can be discounted when the number of compounds is ≥ 2.
- Please let us (gpscreen@bioneer.com) know the details about your compounds/ candidate drugs, so that we can provide you with a more accurate quotations and timeline estimate.

# *GPScreen*™ Services Using *S. pombe* Genome-wide Deletion Mutant Library

## Description

Precise Drug Target Identification is the first step for improving efficacy, tracing and avoiding side-effects as well as understanding the mode-of-actions of drug candidates. However, until now, effective systematic approaches for the precise drug target identification at genome levels have not been commercially available. *GPScreen*™ is a genome-wide HTS drug target screening system using drug-induced haploinsufficiency (DIH) in the World's first *S. pombe* genome-wide heterozygous deletion mutant library (kim DU *et al.*,*Nat. Biotech*.2010). Fission yeast *S. pombe* is considered a superior model organism of mammalian cells as its cell division pattern is similar to that of mammalian cells.

Bioneer's *GPScreen*™ Custom Service analyzes the drug effects on 4,845 genes individually, which covers almost 98% of genome of *S. pombe*, thereby providing a systemic screening solution for drug target identification of drug candidates which would accommodate customer's drug discovery and development in a quick and cost effective way. More detailed information is described in bioneer website.

## Features and Benefits

- The most advanced genome-wide drug On/Off- target identification technology
- Almost all types of drug targets possible to be screened at the genome level
- Live cell-based screening
- Fast and accurate high-throughput screening (HTS)
- Applicable to drug repositioning, natural drug target discovery, drug toxicity evaluation.

## Applications

- Drug target identification and drug toxicity evaluation
- Drug prioritization
- Drug repositioning and drug efficacy improvement
- Natural drug target discovery and mechanism of action (MOA) study
- Chemogenomic profiling

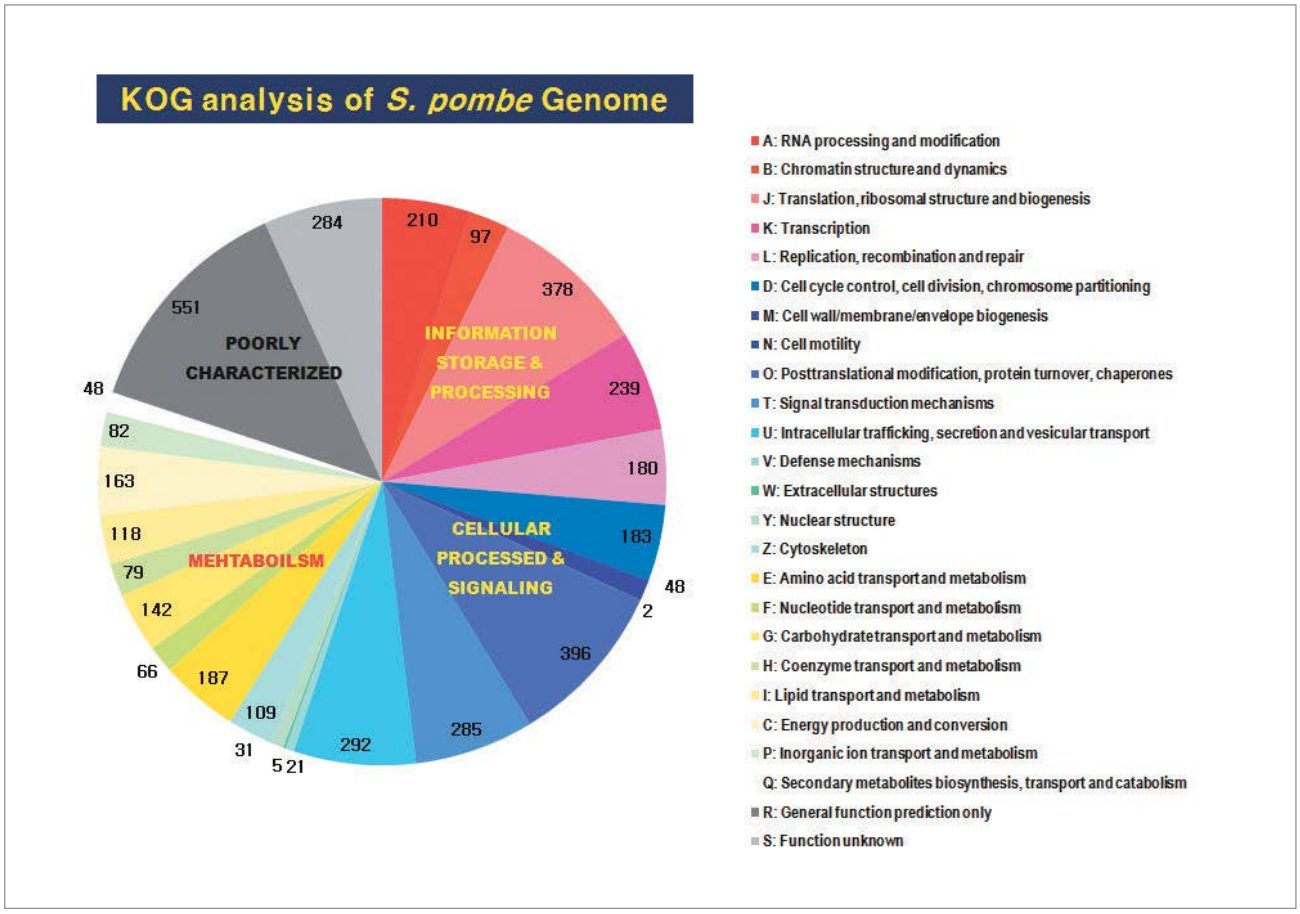
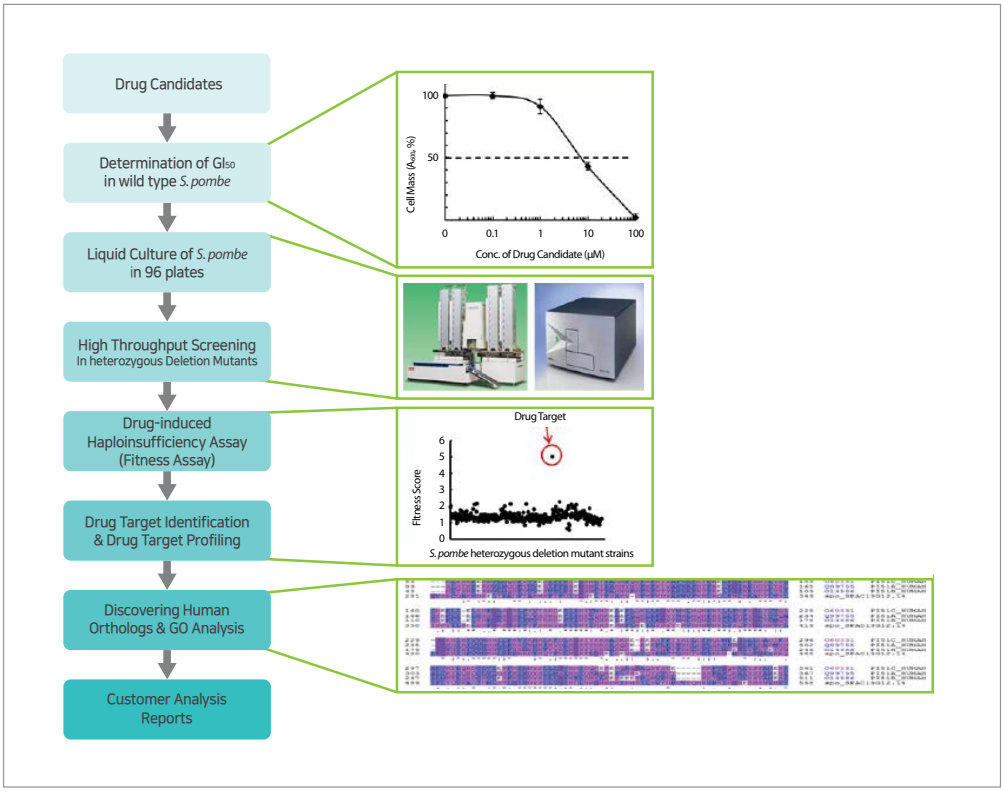



Figure 1. KOG-based Analysis of *S. pombe* Genome.

## Procedure




## Experimental Data

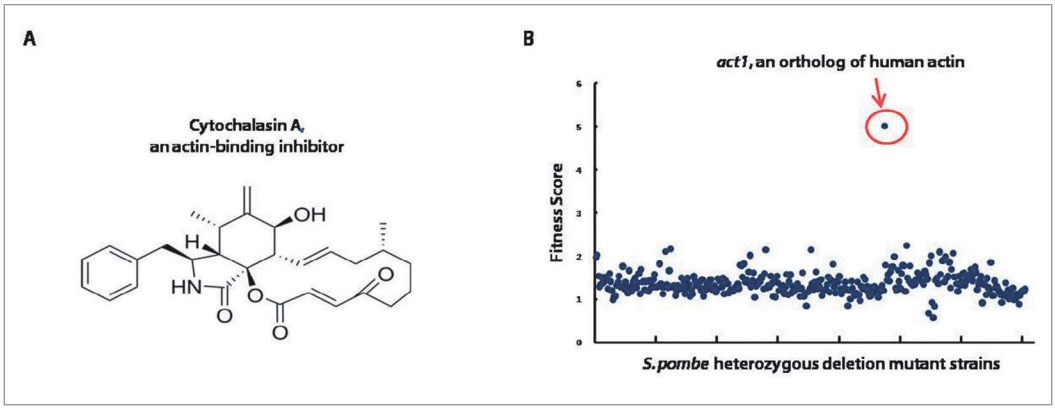



**Figure 2.** Drug On-Target Identification using *GPScreen*™.

In the presence of cytochalasin A, a heterozygous deletion mutant of *act1* showed more potent growth defect than those of wild type *S. pombe* cells and other mutants as a result of a decrease in "functional" Act1 protein. *act1* was the only gene in the genome-wide screen to show this effect, demonstrating that *act1* is a target of cytochalasin A.

# *GPScreen*™ Services using *S. pombe* Genome-wide Deletion Mutant Library

## Ordering Information

*GPScreen*™ Service Using *S. pombe* Genome-wide Mutant Set

| Cat. No. | Full Screening Service | No. of genes |
|---|---|---|
| GPS-01-GW | *S. pombe* Genome-wide Heterozygous Deletion Mutant Screening Service | 4,845 |

*GPScreen*™ Service Using *S. pombe* Essential Gene Mutant Set

| Cat. No. | Essential Gene Screening Service | No. of genes |
|---|---|---|
| GPS-02-ESS | *S. pombe* Essential Gene Heterozygous Deletion Mutant Screening Service | 1,277 |

*GPScreen*™ Services Using KOG Analysis-based Functional Group Subsets

| Cat. No. | Functional Group-based Subset Services | No. of genes |
|---|---|---|
| Information Storage and Processing | | |
| GPS-03K-A | RNA Processing and Modification Screening Service | 210 |
| GPS-03K-B | Chromatin Structure and Dynamics Screening Service | 97 |
| GPS-03K-J | Translation, Ribosomal Structure and Biosis Screening Service | 378 |
| GPS-03K-K | Transcription Screening Service | 239 |
| GPS-03K-L | Replication, Recombination and Repair Screening Service | 180 |
| Cellular Processes and Signaling | | |
| GPS-03K-D | Cell Cycle Control, Cell Division, Chromosome Partitioning Screening Service | 183 |
| GPS-03K-M | Cell Wall/Membrane/Envelope Biosis Screening Service | 48 |
| GPS-03K-N | Cell Motility Service | 2 |
| GPS-03K-O | Post-translational Modification, Protein Turnover, Chaperones Screening Service | 396 |
| GPS-03K-T | Signal Transduction Mechanisms Screening Service | 285 |
| GPS-03K-U | Intracellular Trafficking, Secretion, and Vesicular Transport Screening Service | 292 |
| GPS-03K-V | Defense Mechanisms Screening Service | 21 |
| GPS-03K-W | Extracellular Structures Screening Service | 5 |
| GPS-03K-Y | Nuclear Structure Screening Service | 31 |
| GPS-03K-Z | Cytoskeleton Screening Service | 109 |
| Metabolism | | |
| GPS-03K-E | Amino Acid Transport and Metabolism Screening Service | 187 |
| GPS-03K-F | Nucleotide Transport and Metabolism Screening Service | 66 |
| GPS-03K-G | Carbohydrate Transport and Metabolism Screening Service | 142 |
| GPS-03K-H | Coenzyme Transport and Metabolism Screening Service | 79 |
| GPS-03K-I | Lipid Transport and Metabolism Screening Service | 118 |
| GPS-03K-C | Energy Production and Conversion Screening Service | 163 |
| GPS-03K-P | Inorganic Ion Transport and Metabolism Screening Service | 82 |
| GPS-03K-Q | Secondary Metabolites Biosynthesis, Transport and Catabolism Screening Service | 48 |
| Poorly Characterized | | |
| GPS-03K-R | General Function Prediction Only Screening Service | 551 |
| GPS-03K-S | Function Unknown Screening Service | 284 |

※ Please inquire about *GPScreen*™ service price separately.

## Technical Support

If you have further questions about the *GPScreen*™ Service course or price, please contact "*S.pombe* GPS Team" and we will discuss them in more detail.

- Tel. +82-42-930-8570
- E-mail: gpscreen@bioneer.com