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GPScreen™

Genome-wide Drug Target Identification & Validation Services

Innovative Technology for **Drug Target Discovery**

BIONEER
Innovation • Value • Discovery

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GPScreen™ :
Innovative Genome-wide Drug Target Screening Technology

GPScreen™(Genome-wide *S. pombe* Screening) is an innovative high throughput drug target screening system using drug-induced haploinsufficiency (DIH) in the *S. pombe* genome-wide deletion mutant library. Through the genome-wide screening on about 5,000 genes in the library at each gene level, *GPScreen*™ provides the reliable drug targets, outperforming traditional drug target discovery technologies such as genome sequencing, SNP chip, microarray and protein chip which have limitations in presenting drug targets.
As of Jan 2017, 4,845 heterozygous deletion mutants are prepared and used for *GPScreen*™ service, which is responsible for 98.6% genome coverage.
We also provide drug target validation service in human cells using Bioneer's proprietary human siRNA library and *in vivo* delivery platform technology (SAMiRNA™) to verify the function of target genes identified from *GPScreen*™.

Applications

- Drug target identification
- Natural drug target discovery
- Mode of action study of small molecules
- Genome-wide drug toxicity profiling for drug prioritization at an early stage of drug discovery
- Drug repurposing

Features & Benefits

- The most advanced genome-wide drug on/off- target identification technology
- Based on World's-unique *S. pombe* genome-wide deletion library
- Almost all types of drug targets possible to be screened at the genome level
- Live cell-based screening
- Possible to be screened at the specific functional group subsets

Step 1

01 Drug Candidates

02 Determination of GI_{50} in wild type *S. pombe*

03 Preliminary Test Report

Step 2

04 Drug Treatment on individual mutant strains

05 Drug-induced Haploinsufficiency Assay (Fitness Assay)

06 Drug Target Identification & Drug Target Profiling

07 Discovering Human Orthologs & GO Analysis

08 Customer Analysis Report

Step 3

09 Drug Target Validation in Human cells (Optional)

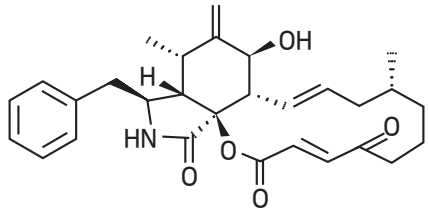
* GI_{50} : the concentration for 50% inhibition in cell proliferation

Genome-wide Drug Target Identification & Profiling with *GPScreen*™

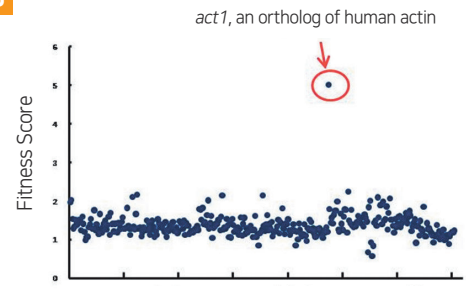
Drug target identification with *GPScreen*™

A

Cytochalasin A,
an actin-binding inhibitor



B



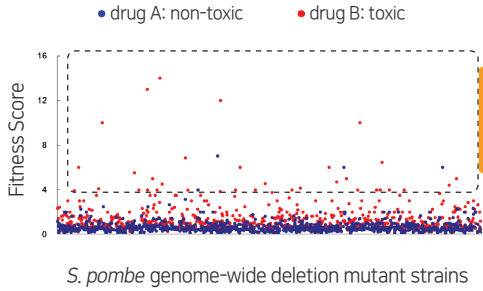
A Chemical structure of cytochalasin A

B In the presence of cytochalasin A, a heterozygous deletion mutant of *act1* shows decreased growth 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. This result shows that ***GPScreen***™ can be used as a powerful tool for precise drug target identification of drug candidates.

Drug toxicity profiling for drug prioritization with *GPScreen*™

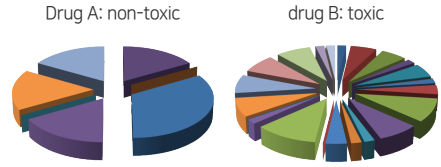
A

< Genome-wide drug target profiles >



B

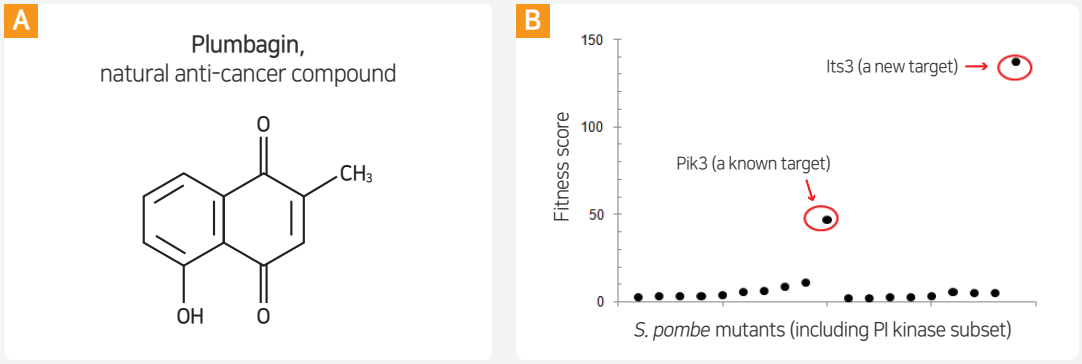
< Drug Target Spectrum >



A Two known drugs with distinct *in-vivo* toxicity were treated to *S. pombe* genome-wide deletion mutant library pool at the GI_{50} concentration of each compound for 15 h, and their genome-wide drug target profiles were obtained by analyzing the barcode frequency of each mutant in comparison with that of DMSO control.

B Next, the promising targets (fitness value; > 4; plotted box area of A) of the two drugs were classified into gene functional groups in which non-toxic drug A showed more selective target profile than that of toxic drug B, which is well correlated with their known *in-vivo* toxicity. These results suggest that ***GPScreen***™ can be applied to drug toxicity evaluation at preclinical and early stages in drug R&D process.

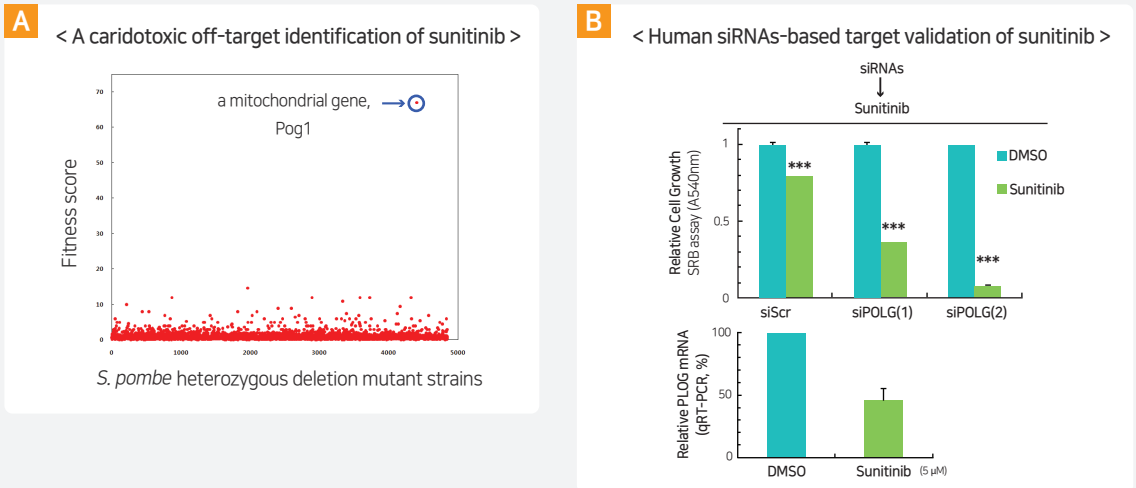
Natural drug target discovery with *GPScreen*™



A Chemical structure of Plumbagin

B Because PI3K is a known target of plumbagin in human breast cancer cells (*Mol Cancer Ther* 5: 3209-3221, 2006), we compared the effect of DIH among various PIK-deletion mutants in *S. pombe*. The potency of DIH in a PI5K its3-deleted mutant was comparable to that in pik3 (*S. pombe* ortholog of human PI3K)-deleted mutant, but not found in other types of PIK-deletion mutants such as Fab1, Tra1, and Ptn1. This result shows that ***GPScreen***™ can be used as a powerful tool for discovery a new molecular target of drugs and also applied to drug repurposing.

Off-target identification of drug with *GPScreen*™



A Identification of Pog1 as a potential target hit of sunitinib from genome-wide drug target screening with ***GPScreen***™ using *S. pombe* genome-wide deletion library.

B POLG, a mitochondrial DNA polymerase subunit gamma-1, is human ortholous of Pog1. POLG-knockdown affects the cytotoxic response and expression at a transcriptional level by sunitinib in human cell line (Hela), and also was already known to induce mitochondrial damage of cardiomyocytes, causing cardiotoxicity. These results indicate that POLG might play a crucial role in mitochondrial damage as a new toxicity target of sunitinib for cardiotoxicity and that drug target screening with ***GPScreen***™ can be applied to drug toxicity target discovery via genome-wide drug on/off-target identification.

Drug Target Validation in Human Cells using SAMiRNA™ & Human siRNA Library

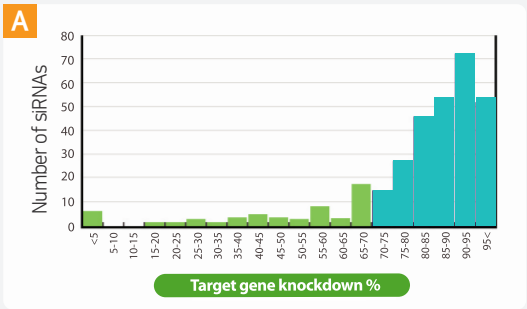
SAMiRNA™ (Self-Assembled-Micelle-inhibitory-RNA) is a novel siRNA molecule conjugated with lipid and hydrophilic polymer, which spontaneously forms stable nanoparticles and optimized for systemic delivery without any other reagent and formulation process. With this feature, SAMiRNA™ nanoparticles incorporate siRNA, miRNA or even antisense oligonucleotide, with the ideal size for selective localization in either vascularized tumors via Enhanced Permeability and Retention (EPR) effects or for targeting tissues through the addition of ligand moieties on the surface of SAMiRNA™.

The therapeutic potential of SAMiRNA™ is highlighted by virtue of its negligible toxicity and superb *in vivo* serum stability as well as its long lasting target gene silencing efficacy proven in various cancer and lung fibrosis animal disease models. In addition, SAMiRNA™ has been shown to induce no innate immune response in human PBMC test and high dose-administrated rodent models. These data indicate SAMiRNA™'s exceptional therapeutic potential as an RNAi platform targeting for various diseases *in vivo* .

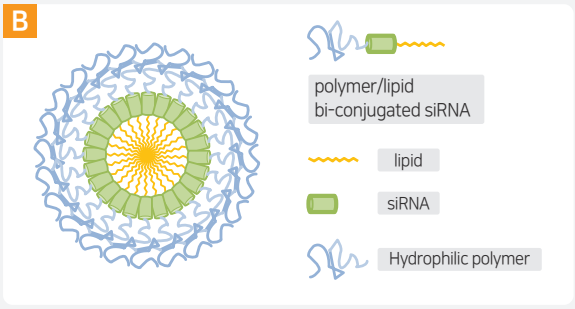
In vivo siRNA delivery challenges are overcome by SAMiRNA™ technology.

Challenges	SAMiRNA™
Rapid clearance and degradation in serum	Improved serum stability
Toxicity of delivery systems	No-detectable liver toxicity or innate immune response
Limited tissue specificity	Tumor tissue targeting capabilities
Low silencing potency	Long lasting *in vivo* silencing efficiency

Bioneer's *AccuTarget*™ Human Pre-designed siRNA Library designed by Turbo si-Designer consists of 55,386 siRNAs for 18,462 human genes. Considering several important parameters including base composition, the number of repetitive bases in a row, thermodynamic instability, energy profiling and base preference, Turbo si-Designer identifies highly effective siRNA target sites with exceptional success rates. Bioneer's *AccuTarget*™ Human Pre-designed siRNAs demonstrate maximized knockdown efficiency for target genes and minimized off-target effect.



High efficiency of Bioneer's *AccuTarget*™ Human Pre-designed siRNA Libraries



Structure of SAMiRNA™ (*J Biol Chem*. 2016 Mar 18;291(12):6433-46)

References

(The list below is representative.)

List of articles using *GPScreen*™ service

Publication year	Title	Author	Journal
2020	The Hsp90 Inhibitor, Monorden, Is a Promising Lead Compound for the Development of Novel Fungicides	Hang T. T. Nguyen, Soyoung Choi, Soonok Kim, Ju-Hee Lee, Ae Ran Park, Nan Hee Yu, Hyeokjun Yoon, Chang-Hwan Bae, Joo Hong Yeo, Gyung Ja Choi, Hokyoung Son, Jin-Cheol Kim	Frontiers in Plant Science 2020 Apr;11:371
2018	Genome-wide evidences of bisphenol a toxicity using *Schizosaccharomyces pombe*	Dong-Myung Kim, Jeonghoon Heo, Dong Woo Lee, Mayumi Tsuji, Mihi Yang	Archives of Pharmacal Research 2018 Aug;41(8):830-837
2016	Identification of a Mitochondrial DNA Polymerase Affecting Cardiotoxicity of Sunitinib Using a Genome-Wide Screening on *S. pombe* Deletion Library	Dong-Myung Kim, Hanna Kim, Ji-Hyun Yeon, Ju-Hee Lee, Han-Oh Park	TOXICOLOGICAL SCIENCES 2016 Jan;149(1): 4–14.
2012	The Natural Anticancer Agent Plumbagin Induces Potent Cytotoxicity in MCF-7 Human Breast Cancer Cells by Inhibiting a PI-5 Kinase for ROS Generation	Ju-Hee Lee, Ji-Hyun Yeon, Hanna Kim, Whijae Roh, Jeiwook Chae, Han-Oh Park, Dong-Myung Kim	PLoS ONE 2012 Sep;7(9): e45023

List of articles using *S. pombe* knockout library (haploid set) for drug screening

Publication year	Title	Author	Journal
2019	A Genome-Wide Screen for Wortmannin-Resistant Mutants in *Schizosaccharomyces pombe*: The Phosphorylation-Impaired Mutants Are Resistant to Signaling Defect	Merve Yılmazer, Burcu Kartal, Cxag˘ atay Tarhan, Ilayda O¨ zarabacı, Sedef Akc¸ aalan, Egemen O¨ zkan, Semian Karaer Uzuner, Ercan Arıcan, Bedia Palabıyık	DNA AND CELL BIOLOGY 2019 Dec;38(12): 1427-1436
2017	A genome-wide screen for FTY720-sensitive mutants reveals genes required for ROS homeostasis	Kanako Hagihara, Kanako Kinoshita, Kouki Ishida, Shihomi Hojo, Yoshinori Kameoka, Ryosuke Satoh, Teruaki Takasaki, Reiko Sugiura	Microb Cell 2017 Dec 4; 4(12): 390–401
2016	Calcium modulation of doxorubicin cytotoxicity in yeast and human cells	Thi Thuy Trang Nguyen, Ying Jun Lim, Melanie Hui Min Fan, Rebecca A. Jackson, Kim Kiat Lim, Wee Han Ang, Kenneth Hon Kim Ban, Ee Sin Chen	Genes to Cells 2016 Mar;21(3):226-40
2015	Chromosome segregation and organization are targets of 5′-Fluorouracil in eukaryotic cells	Laura Mojardín, Javier Botet, Sergio Moreno, and Margarita Salas	Cell Cycle 2015; 14(2): 206–218

* If you question about *S. pombe* knockout library (haploid set), please e-mail us at pombe-support@bioneer.com

Accomplishments of *GPScreen*™

(The list below is representative.)

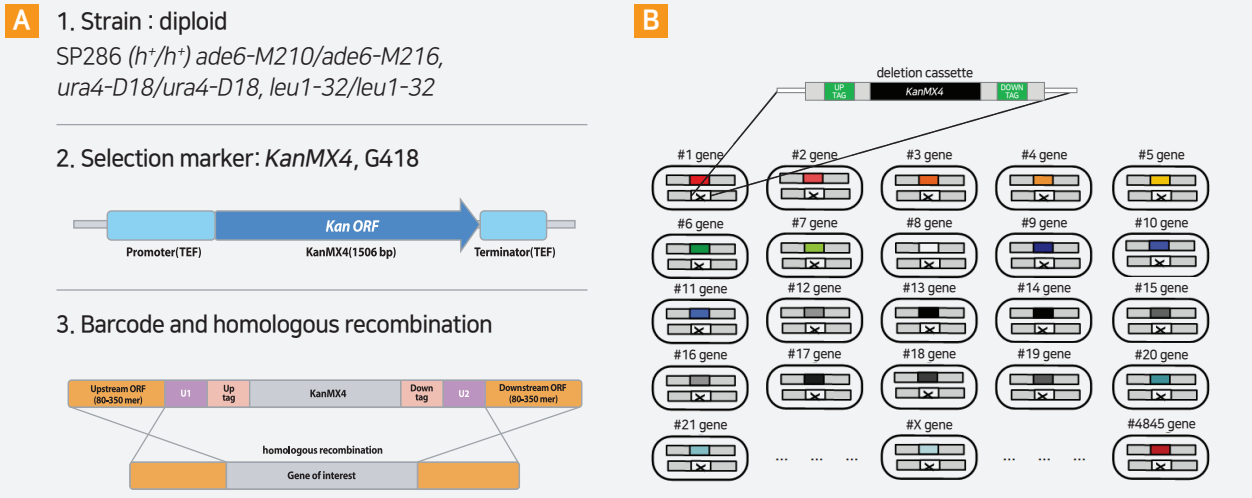
Pharmacy	○ DONG-A PHARM (KOREA) ○ LG Life Sciences (KOREA) ○ SK BIOPHARMACEUTICALS (KOREA)	○ JEILPHARM (KOREA) ○ HANALL BIOPHARMA (KOREA) ○ Two global pharmaceutical companies (JAPAN & USA)
University or Research institution	○ KRIBB (KOREA) ○ KRICT (KOREA) ○ KyungHee University (KOREA)	○ Sungkyunkwan University (KOREA) ○ Sookmyung Women's University (KOREA) ○ Chonnam National University (KOREA)

Genome-wide Drug Target Screening using *S. pombe* genome-wide deletion mutant library

S. pombe* genome-wide deletion mutant library** was developed by Bioneer and KRIBB in Korea, by collaborating with Dr. Paul Nurse of Cancer Research Center in UK as reported in ***Nat. Biotech 28, 617-623 (2010). Fission yeast is the simplest cell division model organism. The library covering almost all genes/ORF (total of 4,914 different types) in genome was constructed by homologous recombination in which each ORF is replaced with a deletion cassette tagged with unique DNA sequence barcode (See the figure A). The library is available from Bioneer exclusively and can be used for genetic and chemical screening such as drug target identification, gene expression profiling and synthetic lethal profiling. *S. pombe* has a higher homology with human genes than those of *S. cerevisiae*; Moreover, it contains 3,500 homologs to human genes and 500 unique genes not in budding yeast, *S. cerevisiae*.

Drug-induced Haploinsufficiency (DIH) is a phenomena in nature that reducing the copy number of a drug target gene from two copies to one often results in a strain that is sensitized to drugs that act on the product of the gene. DIH in yeast has now been considered as a valuable tool for drug target identification. Heterozygous diploid strain, which lack a copy of a gene, is significantly sensitive to drugs that act on the product of the gene while wild type strain is modestly affected by the drugs. Strains lacking one copy of the target gene grow significantly slow.
By comparing the growth rate of clones, the drug targets are identified clearly. Using this principle, previous report has provided identifications of drug targets from budding yeast *S. cerevisiae*.[1]

1. Lee AY *et al*. Mapping the Cellular Response to Small Molecules Using Chemogenomic Fitness Signatures. ***Science,*** *344, 208-211 (2014).*



***S. pombe* genome-wide deletion mutant library** (*Nature Biotech*., 2010)

A Construction of *S. pombe* deletion mutant.

B Genetic status of *S. pombe* heterozygous deletion mutant library.

Ordering Information

*Price and timeline can be varied according to the number of compound.
Please e-mail us at gpscreen@bioneer.com for details.

Step 1 Primary Test Service for Determination of Growth-inhibitory Concentration (GI_{50}) in Wild Type *S. pombe*

Cat. No.	Service	No. of Genes
GPS-00	Primary Test Service in Wild Type *S. pombe*	Wild type 1 strain

※ This test will be performed to all compounds, and must be requested in advance of *GPScreen*™.

Step 2 *GPScreen*™ Service using *S. pombe* Mutant Set

Cat. No.	Service	No. of Genes
GPS-01-GW	*S. pombe* Genome-wide Heterozygous Deletion Mutant Screening Service	4,845
GPS-02-ESS	*S. pombe* Essential Gene Heterozygous Deletion Mutant Screening Service	1,277

※ Genes to be *GPScreen*™ can either be customized or selected from the GO, KEGG or KOG analysis-based functional group subsets.

Step 3 Drug Target Validation in Human Cells

Cat. No.	Service	No. of Genes
GPS-03-HC	Drug target validation in human cells	Inquired genes

※ Target validation will be performed using Bioneer's proprietary human siRNA library.

※ *In vivo* delivery platform technology (SAMiRNA™) consisting of siRNA sequence are available at a competitive price with comprehensive services. Moreover, SAMiRNA™ can be labeled with a variety of fluorescent dyes to detect the location of SAMiRNA™ in animal models.

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