# Recombinant protein production system having excellent performance

Technology / Recombinant protein manufacturing system

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**Patent title** Vector for expressing recombinant antigen using CRISPR editing technology, and method for simultaneous multi-insertion thereof

**Inventor** Korea Research Institute of Chemical Technology / Kim Cheon-saeng

**Patent application No.** KR 10-2018-0108713 (2018.09.12)

**Authority status** Registered

## Technicality

### Technology overview

- A technology in which multiple vectors expressing recombinant antigens at multiple locations can be simultaneously inserted into eukaryotic cell chromosomes through a homology directed recombination scheme, which is a CRISPR technology
- The technology is a technology for expressing a recombinant protein that can be used as an immunogen by using cells after selecting a human cell line capable of stably overexpressing immunogens by inserting the same into two places at the same time. The technology can be usefully used for the development of recombinant immunogens and vaccines against various viruses.

### Development background and problem to be solved

- When expression is performed in prokaryotic cells, glycosylation different from the case of protein expression in eukaryotic cells such as human cells is induced, and thus there is a limitation in producing proteins that require excessive glycosylation.
- As a countermeasure, a method of purifying transiently-expressed proteins after transfection of a recombinant immunogen expression plasmid into eukaryotic cells has been mainly used so far, but this has a limitation in producing a large amount of recombinant immunogens.
- Therefore, there is a need for a technology by which various recombinant antigens can be stably secured.

### Excellence and discrimination of technology

#### ▶ Excellence of technology

- High-efficiency recombinant protein stably expressing cells are produced by using a CRISPR multi-insertion editing technology.
- Recombinant protein properties are maintained, and expression increases.
- Efficient production of high-purity immunogens is possible.

#### ▶ Discrimination of technology

- Excellent performance can be secured compared to proteins produced through existing E.coli (expression amounts, multi-expression of the same gene, multi-expression of heterogeneous genes, and the like)
- The unique properties of recombinant proteins are maintained, and stable overexpression is induced.
- High-yield constant cell lines can be easily produced.

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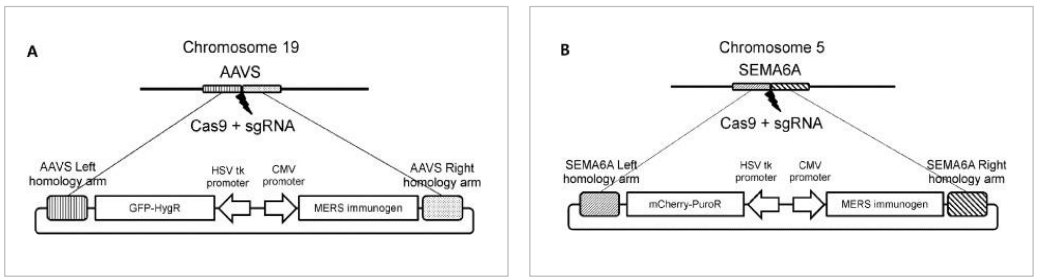
## Implementation method

- The technology is a method of expressing recombinant antigens at multiple locations in eukaryotic chromosomes through a homology-directed repair scheme, which is a CRISPR gene editing technology, so as to induce stable overexpression of recombinant antigens in eukaryotic cells.
- The technology is a method for expressing a recombinant antigen by simultaneous multi-insertion using a vector for expressing a recombinant antigen, a vector for expressing sgRNAs respectively targeting AAVS and SEMA6A sites, and a Cas 9 protein expression vector.
- The antigen is a MERS virus antigen and is a receptor binding site (RBD) located in a spike protein, and six histidine amino acid tags required for protein purification are fused to a C-terminal.




**Picture** Mimetic diagram of stably-expressing stable cell line production

## Degree of technology completion (TRL)

Degree of technology completion: TRL4 (Lab Scale prototype development stage)

| TRL1 | TRL2 | TRL3 | TRL4 | TRL5 | TRL6 | TRL7 | TRL8 | TRL9 |
|---|---|---|---|---|---|---|---|---|
| Technical principle presentation | Technology concept setting | Technology concept verification | Lab Scale prototype development | Implementation environment application experiment | Full Scale prototype development | Quasi-commercial product development | Commercial product development | Commercial product implementation |

# Utilization

## Utilization field and applied product

### Utilization field

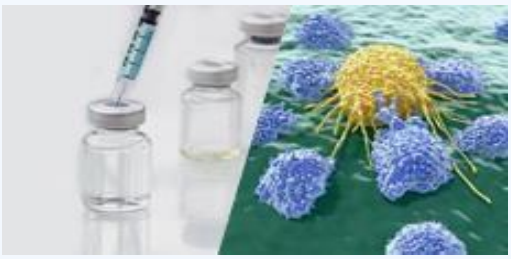
- Therapeutic antibody
- Treatment vaccine
- Preventive vaccine
- Disease diagnosis

**Picture 1** Protein medical product

<Data: Dr.Ignacio Dallo>

### Applied product

- Immune anticancer agent
- Stem cell treatment agent
- Gene treatment agent



**Picture 2** Immunotherapy agent

<Data: Heavy particle therapy partner >

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## Technology trend

- According to the order of development of gene scissor technologies, there are 3rd-generation CRISPR (CRISPR/Cas9), including 1st-generation zinc finger nucleases (ZFNs) and 2nd-generation transcription activator-like effector nucleases (TALENs). Among these, researches based on CRISPR, the 3rd-generation gene scissor technology, are being actively conducted.
- Gene scissor technologies include a non-homologous end-joining (NHEJ) method or a homology-directed repair (HDR) method using the principles of homologous recombination. Yet, researches on how to inactivate a target gene through NHEJ rather than HDR of inserting a specific gene or repair DNA are in progress for gene correction or gene addition.

## Family patent status

| Application nation | Application No. (Application date) / Registration No. | Title of the invention |
|---|---|---|
| KOR | KR 10-2018-0108713 (2018.09.12)/ 10-2121817 | Vector for expressing recombinant antigen using CRISPR editing technology, and method for simultaneous multi-insertion thereof |
| PCT | KR 2019-011830 (2019.09.11)/ - | VECTOR EXPRESSING RECOMBINANT ANTIGEN BY USING CRISPR EDITING TECHNOLOGY, AND SIMULTANEOUS MULTIPLE INSERTION METHOD THEREFOR |

# Market prospect

## Target market size and prospect

- The global vaccine market size is expected to grow at a CAGR of 11% from USD 33.57 billion in 2017 to USD 103.57 billion in 2028. Regarding transboundary outbreaks of infectious diseases, in addition to preventive vaccines which prevent infectious diseases in advance, recently therapeutic vaccines which can treat already occurred diseases (viruses, cancer, etc.) are being actively developed. Thus, steady growth is expected.


Table 1 **Global vaccine market status and prospect [billion dollar]**

<Data: BIS Research / Biotech Policy Research Center Reconfiguration>

| Division | 2017 | 2018 | 2019 | 2020 | 2021 | 2022 | 2023 | 2024 | 2025 | 2026 | 2027 | 2028 | CAGR (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Subunit and bonding | 12.94 | 14.02 | 15.25 | 16.65 | 18.26 | 20.1 | 22.22 | 24.66 | 27.47 | 30.73 | 34.51 | 38.91 | **10.8** |
| Attenuation | 7.15 | 7.81 | 8.55 | 9.37 | 10.28 | 11.31 | 12.46 | 13.73 | 15.16 | 16.76 | 18.57 | 20.6 | **10.2** |
| Inactivation | 5.94 | 6.42 | 6.97 | 7.62 | 8.36 | 9.24 | 10.24 | 11.42 | 12.8 | 14.42 | 16.32 | 18.57 | **11.2** |
| Recombination | 4.08 | 4.42 | 4.81 | 5.27 | 5.81 | 6.45 | 7.19 | 8.07 | 9.1 | 10.33 | 11.8 | 13.55 | **11.9** |
| Toxoid | 3.46 | 3.73 | 4.03 | 4.34 | 4.69 | 5.06 | 5.47 | 5.92 | 6.4 | 6.93 | 7.51 | 8.14 | **8.1** |
| Next generation | 0 | 0 | 0 | 0 | 1.08 | 1.75 | 1.91 | 2.12 | 2.39 | 2.74 | 3.19 | 3.79 | **11.7** |

Table 2 **Market status and prospect by vaccine types [billion dollar]**

<Data: BIS Research / Biotech Policy Research Center Reconfiguration >

## Technology transfer query

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## Technology transfer process