(Note: Found any ambiguity of interpretation in this provisional translation, the Japanese text shall prevail.)

Chapter 2 Biological Inventions

8. Examples Relating to Judgment of Necessity for Deposit of Microorganisms, etc. (Draft)

This section explains about the judgment on whether microorganisms, etc. ("microorganisms, etc." here include microorganisms, plants and animals) are necessary to be deposited or not prior to filing of the application, based on specific cases.

For information on general matters relating to the judgment of necessity for Deposit, see "5. Deposit".

The present Examples include the following Cases.

8.1 Inventions Relating to Bacteria
Case 1-1 A case where the bacteria are readily available to a person skilled in the art (No need to deposit)
Case 1-2 A case where the bacteria are not readily available to a person skilled in the art (Need to deposit)
Case 1-3 A case of the invention relating to a DNA derived from bacteria (No need to deposit)

8.2 Inventions Relating to Antibodies
Case 2-1 A case where the hybridoma can be prepared by a person skilled in the art on the basis of the description in the specification (No need to deposit)
Case 2-2 A case where the hybridoma can be prepared by a person skilled in the art on the basis of the description in the specification (No need to deposit)
Case 2-3 A case where the hybridoma is not readily available to a person skilled in the art (Need to deposit)

8.3 Inventions Relating to Cells
Case 3-1 A case where the cells can be produced by a person skilled in the art on the basis of the description in the specification (No need to deposit)
Case 3-2 A case where the cells are not readily available to a person skilled in the art (Need to deposit)

8.4 Inventions Relating to Animals
Case 4-1 A case where the animal can be produced by a person skilled in the art on the basis of the description in the specification (No need to deposit)
Case 4-2 A case where the animal is not readily available to a person skilled in the art (Need to deposit)

(Points of concern)
In the present Examples, each case shall not mean that there are no other reasons for refusal such as lack of novelty/inventive step.

8.1 Inventions Relating to Bacteria

Case 1-1 A case where the bacteria are readily available to a person skilled in the art (No need to deposit)

[Claims]
1. A β-galactosidase derived from a Streptomyces lividans strain xyz-1; ATCC ******, having the following physicochemical properties:
(a) function and substrate specificity: to hydrolyze a substrate having β-D-galactoside bond and to release a D-galactoside group;
(b) optimum pH: 4.5;
(c) stable pH: 3.0 to 5.5;
(d) optimum temperature: 55°C;
(e) stable temperature: 50°C; and
(f) molecular weight: 200 kD as measured by gel filtration chromatography.

[Outline of Description of the invention]
Since neutral or acidic material such as milk, cheese whey, lactose solution and the like is the subject of processing by a β-galactosidase, the β-galactosidase with sufficient enzyme activity in an acidic condition is required. But a microorganism that produces the β-galactosidase with sufficient enzyme activity in an acidic condition was not known at the filing.
The inventors isolated the β-galactosidase described in claim 1 from a *Streptomyces lividans* strain xyz-1 with a specific technique. Further, the *Streptomyces lividans* strain xyz-1 is listed in a catalog issued by ATCC with the storage number ATCC ******, and freely accessible prior to the filing.

[Explanation on judgment of necessity for deposit of microorganisms, etc.]
In the present case, based on the Description of the invention, it was confirmed prior to the filing that the *Streptomyces lividans* strain xyz-1 was a microorganism stored in ATCC that is a reliable culture collection and freely accessible from the catalog issued by ATCC. Further, the storage ATCC number of xyz-1 was also described in the Description of the invention.
Therefore, the *Streptomyces lividans* strain xyz-1 is readily available to a person skilled in the art, and any person skilled in the art can isolate the β-galactosidase described in claim 1 with the specific technique described in the specification.
Accordingly, the *Streptomyces lividans* strain xyz-1 is not required to be deposited.

Case 1-2 A case where the bacteria are not readily available to a person skilled in the art (Need to deposit)

[Claims]
1. A Bacillus subtilis strain T-169 capable of decomposing dioxin.

[Outline of Description of the invention]

A *Bacillus subtilis* strain T-169 was isolated from the sample, which was collected from muddy sediment of seabed of Toyama Bay in Japan, with a method known to a person skilled in the art. The taxonomic characteristic of T-169 was analyzed in detail, and the difference between T-169 and any other strains in the same species was examined. The *Bacillus subtilis* strain T-169 proved to be a new strain, and with further experiments, it was verified that T-169 has an ability to decompose dioxin with high efficiency.

[Explanation on judgment of necessity for deposit of microorganisms, etc.]

In general, variety and quantity of microorganisms inhabiting soil, seawater or the like are not always stable even though the soil and seawater were obtained from a certain area.

Therefore, even when a novel microorganism is isolated from a sample of the soil, seawater or the like from a certain area, it is difficult to obtain the same novel microorganism reproducibly in the absence of a reasonable ground that the novel microorganism is present in the sample collected again from the soil, seawater or the like.

In the present case, there is no description about the reasonable ground that the *Bacillus subtilis* strain T-169 is present in the sample collected again from muddy sediment of seabed of Toyama Bay in the Description of the invention.

Consequently, the *Bacillus subtilis* strain T-169 is not reproducible in confirmatory studies conducted by a person skilled in the art. Therefore, the *Bacillus subtilis* strain T-169 is not a microorganism that can be prepared by a person skilled in the art on the basis of the description in the specification.

Accordingly, the *Bacillus subtilis* strain T-169 is required to be deposited, since the *Bacillus subtilis* strain T-169 is not a microorganism readily available to a person skilled in the art.

Case 1-3 A case of the invention relating to DNA derived from bacteria (No need to deposit)

[Claims]
1. A DNA comprising the nucleic acid sequence represented by SEQ ID NO: 1, encoding an argininosuccinate synthase derived from a coryneform bacterium strain K-336.
2. An expression vector containing the DNA described in claim 1.
3. A transformant, retaining the vector described in claim 2 in a state that the vector is capable of expression.

[Outline of Description of the invention]
A coryneform bacterium strain K-336 producing L-arginine was isolated from soil based on the drug tolerance. Its taxonomic characteristic was analyzed in detail, and the difference between K-336 and the similar species was examined. The coryneform bacterium strain K-336 proved to be a new species.

It had been known at the filing that ArgA to ArgH genes are involved in an L-arginine biosynthetic pathway of the coryneform bacterium. The inventors for the first time identified an ArgG gene comprising the nucleic acid sequence represented by SEQ ID NO: 1 from the coryneform bacterium strain K-336, made to express the ArgG gene with a known genetic technology, and verified that the protein encoded by the ArgG gene is an argininosuccinate synthase.

[Explanation on judgment of necessity for deposit of microorganisms, etc.]
In the present case, the invention described in claim 1 is not a coryneform bacterium strain K-336 but a DNA. Further, since the nucleic acid sequence of the DNA is specifically described in the specification, the DNA can be obtained based on the nucleic acid sequence by a person skilled in the art through an artificial synthesis method and the like. In addition, a person skilled in the art can incorporate the DNA into a suitable expression vector and prepare a transformant retaining the expression vector in a state capable of expressing.

Accordingly, the coryneform bacterium strain K-336 is not required to be deposited.

8.2 Inventions Relating to Antibodies

Case 2-1 A case where the hybridoma can be prepared by a person skilled in the art on the basis of the description in the specification (No need to deposit)

[Claims]
1. An antigenic protein A consisting of the amino acid sequence represented by SEQ ID NO: 1.
2. A monoclonal antibody against the antigenic protein A described in claim 1.
3. A hybridoma producing the monoclonal antibody described in claim 2.

[Outline of Description of the invention]

A novel antigenic protein A was isolated and purified from the outer membrane of virus X. Since the antigenic protein A was found to react only with a serum derived from a person infected with virus X, the antigenic protein A is useful for identifying a person infected with virus X.

Further, a partial amino acid sequence of the antigenic protein A was determined, and a gene encoding the antigenic protein A consisting of the amino acid sequence represented by SEQ ID NO: 1 was cloned by a known genetic engineering technique based on the partial amino acid sequence.

[Note]

There is no example in the specification that a monoclonal antibody specifically reacting with the antigenic protein A was prepared.

[Explanation on judgment of necessity for deposit of microorganisms, etc.]

In the present case, the monoclonal antibody described in claim 2 is a monoclonal antibody specified only by an antigen.

Generally, there is common general knowledge that, when a protein having immunogenicity is obtained, a monoclonal antibody binding to the protein can be obtained by using the protein as an immunogen based on a known hybridoma method.

Further, a person skilled in the art can prepare an antigenic protein A consisting of the amino acid sequence represented by SEQ ID NO: 1 by means of obtaining a gene encoding the antigenic protein A and expressing the gene using a known genetic technology, on the basis of the description in the specification. In addition, it is obvious that the antigenic protein A has immunogenicity.

Consequently, a person skilled in the art can obtain the monoclonal antibody described in claim 2 and a hybridoma producing the monoclonal antibody by the means of preparing the antigenic protein A and using the antigenic protein A as an immunogen by a known hybridoma method, on the basis of the description in the specification.

Therefore, the hybridoma described in claim 3 is a microorganism that can be prepared by a person skilled in the art on the basis of the description in the specification.

Accordingly, the hybridoma described in claim 3 is not required to be deposited, since the hybridoma described in claim 3 is a microorganism readily available to a person skilled in the art.

Case 2-2 A case where the hybridoma can be prepared by a person skilled in the art on the basis of the description in the specification (No need to deposit)

[Claims]
1. A monoclonal antibody of IgM isotype, reacting with a surface antigen P of a virus Y with an association constant of $10^{10}M^{-1}$ or more.
2. A hybridoma producing the monoclonal antibody described in claim 1.

[Outline of Description of the invention]

The surface antigen P of a virus Y was already isolated and purified, and the antibody detecting the surface antigen P was publicly known at the filing. However, the monoclonal antibody of IgM isotype had not been considered suitable for detection of the surface antigen P due to its properties of easy aggregation and the other. The inventors obtained for the first time a monoclonal antibody of IgM isotype capable of detecting the surface antigen P of a virus Y with high sensitivity.

The inventors selected a specific partial amino acid sequence from an amino acid sequence encoding the surface antigen P, and prepared a polypeptide consisting of the specific partial amino acid sequence, and confirmed that the polypeptide functions as an immunogen. Further, using the polypeptide as an immunogen, they prepared a hybridoma producing the monoclonal antibody based on a known hybridoma method. As a result, there were obtained 149 lines of the hybridoma in which the antibody production was confirmed. From these, 10 lines were selected, an association constant of the antibody produced by the selected hybridoma was measured, and only 3 lines of the hybridoma were confirmed to satisfy the conditions of being an IgM isotype and having an association constant of $10^{10}M^{-1}$ or more. However, when a set of these experiments was performed three times repeatedly for preparing the hybridoma in a similar manner, at least one line of the hybridoma producing an antibody, which satisfies the conditions of being an IgM isotype and having an association constant of $10^{10}M^{-1}$ or more, was obtained in every experiment.

[Explanation on judgment of necessity for deposit of microorganisms, etc.]

In the present case, the monoclonal antibody described in claim 1 is a monoclonal antibody satisfying limitative conditions, "reacting with a surface antigen P of a virus Y with an association constant of $10^{10}M^{-1}$ or more".

Generally, there is common general knowledge that it is in many cases not reproducible to obtain a hybridoma producing a monoclonal antibody that satisfies limitative conditions.

However, in the Description of the invention, there is a description that plural lines of a hybridoma producing a monoclonal antibody of IgM isotype that satisfy the limitative conditions, "reacting with a surface antigen P of a virus Y with a association constant of $10^{10}M^{-1}$ or more" can be obtained by selecting a certain specific partial amino acid sequence from the amino acid sequence encoding a surface antigen P of a virus Y. Further, there is also a description that a hybridoma producing the monoclonal antibody of IgM isotype that satisfies the limitative conditions can be reproducible by repeatedly performing the experiments of preparing the hybridoma in a similar manner using a polypeptide consisting of the specific partial amino acid sequence as an immunogen.

Consequently, the monoclonal antibody described in claim 1 and the hybridoma producing the monoclonal antibody are reproducible in confirmatory studies conducted by a person skilled in the art.

.

Therefore, the hybridoma described in claim 2 is a microorganism that can be prepared by a person skilled in the art on the basis of the description in the specification.

Accordingly, the hybridoma described in claim 2 is not required to be deposited, since the hybridoma described in claim 2 is a microorganism readily available to a person skilled in the art.

Case 2-3 A case where the hybridoma is not readily available to a person skilled in the art (Need to deposit)

[Claims]
1. A monoclonal antibody ABC-1, suppressing cell proliferation by binding to receptor Z.
2. A hybridoma H- ABC-1 producing the monoclonal antibody described in claim 1.

[Outline of Description of the invention]

Receptor Z was already isolated and purified, and it was publicly known at the filing that cell proliferation was suppressed by agonist binding to receptor Z. Further, an attempt to prepare a monoclonal antibody that suppresses the cell proliferation by binding to receptor Z was performed prior to the filing. However, an antibody suppressing the cell proliferation by binding to receptor Z was not obtained prior to the filing.

When the inventors prepared a monoclonal antibody based on a known hybridoma method using receptor Z as an immunogen, they obtained numerous hybridomas producing a monoclonal antibody that binds to receptor Z. Among them, however, only one line was the hybridoma producing the monoclonal antibody that suppresses the cell proliferation. Further, the monoclonal antibody suppressing the cell proliferation was named "monoclonal antibody ABC-1" and the hybridoma producing the "monoclonal antibody ABC-1" was named "hybridoma H-ABC-1".

[Explanation on judgment of necessity for deposit of microorganisms, etc.]

In the present case, the monoclonal antibody ABC-1 described in claim 1 is a monoclonal antibody produced by the specific hybridoma line, hybridoma H-ABC-1.

Generally, there is common general knowledge that it is difficult to obtain a specific hybridoma line by design based on a known hybridoma method.

Further, in the Description of the invention, there is only a description that one line of the hybridoma H-ABC-1 producing the monoclonal antibody ABC-1 was obtained based on a known hybridoma method, and there is no description of a method for obtaining the hybridoma H-ABC-1 reproducibly.

Consequently, the monoclonal antibody ABC-1 or hybridoma H-ABC-1 is not reproducible in confirmatory studies conducted by a person skilled in the art. Therefore, the hybridoma H-ABC-1 is not a microorganism that can be prepared by a person skilled in the art on the basis of the description in the specification.

Accordingly, the hybridoma H-ABC-1 is required to be deposited, since the hybridoma H-ABC-1 is not a microorganism readily available to a person skilled in the art.

8.3 Inventions Relating to Cells

Case 3-1 A case where the cells can be produced by a person skilled in the art on the basis of the description in the specification (No need to deposit)

[Claims]
1. A method for isolating mouse lung cancer cells from a heterogeneous cell population containing the mouse lung cancer cells, comprising:
(1) a step of preparing a vector ligated to a nucleic acid molecule encoding a fluorescent protein under the control of a lung cancer cell-specific promoter consisting of the nucleic acid sequence represented by SEQ ID NO: 1;
(2) a step of introducing the vector into the cell population; and
(3) a step of identifying and isolating mouse lung cancer cells as cells generating fluorescence from the cell population.
2. The mouse lung cancer cells, isolated by the method described in claim 1.

[Outline of Description of the invention]
A novel promoter that functions specifically in lung cancer cells was cloned from a mouse. The nucleic acid sequence of the promoter is represented by SEQ ID NO: 1. In addition, based on a known technique, a heterogeneous cell population containing lung cancer cells was prepared from a mouse. Further, a vector ligated to a nucleic acid molecule encoding GFP, that is well-known as a kind of fluorescent protein, under the control of the promoter was introduced into the cell population, GFP was expressed only in lung cancer cells in the cell population, and mouse lung cancer cells were identified and isolated as fluorescing cells among the cell population.

[Explanation on judgment of necessity for deposit of microorganisms, etc.]
In the present case, in the Description of the invention, the nucleic acid sequence of a promoter specifically functioning in lung cancer cells is disclosed. It is also disclosed that mouse lung cancer cells were identified and isolated from a heterogeneous cell population by using a vector ligated to a nucleic acid molecule encoding GFP under the control of the promoter.

Consequently, the mouse lung cancer cells can be identified/isolated reproducibly in confirmatory studies conducted by a person skilled in the art.

Therefore, the mouse lung cancer cells described in claim 2 are microorganisms that can be produced by a person skilled in the art on the basis of the description in the specification.

Accordingly, the mouse lung cancer cells described in claim 2 are not required to be deposited, since the mouse lung cancer cells are microorganisms readily available to a person skilled in the art.

Case 3-2 A case where the cells are not readily available to a person skilled in the art (Need to deposit)

[Claims]
1. A mesenchymal stem cell line H-01, derived from a mouse mesenchymal stem cell, capable of subculturing in a medium containing 1% or less of serum, exhibiting fibrous form when cultured in the medium containing low serum, and being induced to differentiate into target cells at a rate of 80 % or more by culturing in a medium containing a conditioned medium of the target cells.

[Outline of Description of the invention]
Mesenchymal stem cells obtained from mouse bone marrow were cultured for three weeks in a serum-free medium and dead cells were removed. Subsequently, by repeatedly subculturing the remaining cells for examining the differentiation potential, a mutant cell line differentiating into astrocyte-like cells was fortuitously obtained by culturing in a medium containing an astrocyte-conditioned medium. Further, the mutant cell line was named "mesenchymal stem cell line H-01". Here, by performing a further analysis of the differentiation potential of the mesenchymal stem cell line H-01, the mesenchymal stem cells were induced to differentiate into adipocytes, smooth muscle cells, fibroblasts and the like respectively at the rate of nearly 100% by culturing in medium each containing a conditioned medium of the corresponding cells.

[Explanation on judgment of necessity for deposit of microorganisms, etc.]
Generally, there is common general knowledge that it is difficult to obtain a specific mutant cell line intentionally during the cell culture since the mutation in a genome of a cell occurs randomly during the cell culture.
In the present case, in the Description of the invention, there is only a description that the mesenchymal stem cell line H-01 was established from a mutant cell line that was obtained fortuitously in the process of subculturing the mesenchymal stem cells obtained from mouse bone marrow, and there is no description about a method for obtaining the mesenchymal stem cell line H-01 reproducibly.
Consequently, the mesenchymal stem cell line H-01 is not reproducible in confirmatory studies conducted by a person skilled in the art. Therefore, the mesenchymal stem cell line H-01 is not a microorganism that can be produced by a person skilled in the art on the basis of the description in the specification.
Accordingly, the mesenchymal stem cell line H-01 is required to be deposited, since the mesenchymal stem cell line H-01 is not a microorganism readily available to a person skilled in the art.

8.4 Inventions Relating to Animals

Case 4-1 A case where the animal can be produced by a person skilled in the art on the basis of the description in the specification (No need to deposit)

[Claims]
1. A transgenic mouse introduced with a proto-oncogene comprising the base sequence represented by SEQ ID NO: 1.

[Outline of Description of the invention]
A novel proto-oncogene consisting of the base sequence represented by SEQ ID NO: 1 was cloned from human. Further, a plurality of transgenic mice were prepared based on a known gene transfer method by introducing the gene into a commercially available fertilized BALB/c mouse ovum to create transgenic mice. These mice developed tumors at an average age of 5 months old.

[Explanation on judgment of necessity for deposit of microorganisms, etc.]
In the present case, in the Description of the invention, a novel proto-oncogene consisting of the nucleic acid sequence SEQ ID NO: 1 is described. The description also describes that transgenic mice were prepared based on a known gene transfer method, using said proto-oncogene and a commercially available mouse.

Consequently, a transgenic mouse introduced with a proto-oncogene consisting of the base sequence represented by SEQ ID NO: 1 is reproducible in confirmatory studies conducted by a person skilled in the art.

Therefore, the transgenic mouse described in claim 1 is an animal that can be produced by a person skilled in the art on the basis of the description in the specification.

Accordingly, the prepared transgenic mouse (a fertilized egg thereof and the like) is not required to be deposited, since the transgenic mouse described in claim 1 is an animal readily available to a person skilled in the art.

Case 4-2 A case where the animal is not readily available to a person skilled in the art (Need to deposit)

[Claims]
1. An RFG mouse spontaneously developing dermatitis, developing periocular edema as an incipient lesion at 3 weeks old.

[Outline of Description of the invention]
In the process of maintaining the strain of BALB/c mouse, a mutant individual which developed periocular edema as an incipient lesion at 3 weeks old and spontaneously developing dermatitis under a clean condition was fortuitously obtained. Subsequently, an inbred mouse strain was established from the mutant individual and named "RFG mouse". After establishing the inbred mouse strain, in the process of 25 generations, an RFG mouse spontaneously developed dermatitis while maintaining a characteristic of developing periocular edema as an incipient lesion at 3 weeks old.

[Explanation on judgment of necessity for deposit of microorganisms, etc.]
Generally, it is common general knowledge that it is difficult to obtain a specific mutant individual reproducibly in a process of maintaining a mouse strain, since the mutation occurring in a genome of a mouse randomly occurs in a process of maintaining the mouse strain.

In the present case, in the Description of the invention, there is only a description that the RFG mouse is an inbred mouse established from a mutant individual obtained fortuitously in the process of maintaining the strain of BALB/c mouse, and there is no description about a method for obtaining the RFG mouse with repeatability.

Consequently, the RFG mouse is not reproducible in confirmatory studies conducted by a person skilled in the art.. Therefore, the RFG mouse is not an animal that can be produced by a person skilled in the art on the basis of the description in the specification.

Accordingly, the RFG mouse (a fertilized egg thereof and the like) is required to be deposited, since the RFG mouse is not an animal readily available to a person skilled in the art.