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(54) Titre : MARQUEUR 65B13 DE CELLULES PROGENITRICES DE NEURONES GABA
(54) Title: GABA NEURON PROGENITOR CELL MARKER 65B13

(57) **Abrégé/Abstract:**

The present inventors identified a selective marker 65B13 for GABA neuron progenitor cells of the spinal dorsal horn and cerebellum, and successfully isolated GABA neuron progenitor cells using antibodies that bind to a protein encoded by the gene. 65B13 was demonstrated to be useful as a marker to isolate GABA-producing neuron progenitor cells in the spinal dorsal horn and cerebellum. GABA neuron progenitor cells can be efficiently identified or isolated by using the identified marker as an indicator.

ABSTRACT

The present inventors identified a selective marker 65B13 for GABA neuron progenitor cells of the spinal dorsal horn and cerebellum, and successfully isolated GABA neuron progenitor cells using antibodies that bind to a protein encoded by the gene. 65B13 was demonstrated to be useful as a marker to isolate GABA-producing neuron progenitor cells in the spinal dorsal horn and cerebellum. GABA neuron progenitor cells can be efficiently identified or isolated by using the identified marker as an indicator.

DESCRIPTION

GABA NEURON PROGENITOR CELL MARKER 65B13

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Technical Field

The present invention provides a 65B13 gene as a marker for GABA neuron progenitor cells, and relates to the use of the gene in identifying GABA neuron progenitor cells.

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Background Art

The brain functions by forming a complex network from a great variety of neurons. Its failure may result in various neurological diseases. To treat such diseases, transplantation and regeneration therapies are currently investigated. The most important thing in these therapeutic methods is to correctly identify various types of neurons in transplantation materials. Furthermore, from the viewpoint of improvement of safety and therapeutic effect, it is desirable to isolate only the type of cells that are needed for transplantation.

The cerebellum works on smooth motor functions such as regulation of balance, posture, and voluntary movement. The failure of cerebellar function due to cerebellar tumor, cerebellar vermis degeneration caused by chronic alcoholism, spinocerebellar degeneration, or such results in dynamic ataxia and balance disorder. Functional recovery can be achieved by replenishing lost neurons and reconstituting the network. There are about five types of neurons in the cerebellum including Purkinje cell, and formation of a proper network of the respective neurons according to organogenic program enables neurotransmission.

There is an area called "dorsal horn" in the dorsal spinal cord. Dorsal root ganglion neurons that detect stimuli from the periphery transmit signals to the dorsal horn interneurons, and the signals are further transmitted to the brain. The dorsal horn contains excitatory glutamatergic neurons and inhibitory GABA neurons. The balance between the two adequately regulates the signal transmission. Inactivation of GABA neurons results in chronic pain, etc.

The development of spinal cord and cerebellar GABA neurons is being studied, and their origin is nearly elucidated. However, there are few markers to identify their progenitor cells, and no cell-surface marker has been identified. Thus, techniques for isolating viable progenitor cells have not yet been developed.

The 65B13 gene is known to be transiently expressed in dopamine-producing neuron progenitor cells after the termination of cell division (see Patent Document 1); however, there is no report published on the connection between the gene and GABA neuron. Furthermore, it has

been reported that the types of spinal cord interneurons and Purkinje cells can be identified by using the expression of the Corl1 or Corl2 gene as an indicator, respectively (see Patent Documents 2 and 3). However, to date there is no known marker that can selectively identify GABA neuron progenitor cells. The transcription factor Ptf1a is known to be expressed in GABA progenitor cells; however, it is a transcription factor, and there is no known membrane protein that is useful as a selection marker (Non-Patent Documents 1 and 2).

Patent Document 1: WO2004/038018

Patent Document 2: WO2006/022243

Patent Document 3: WO2006/082826

Non-Patent Document 1: Glasgow SM, Henke RM, Macdonald RJ, Wright CV, Johnson JE. Ptf1a determines GABAergic over glutamatergic neuronal cell fate in the spinal cord dorsal horn. Development. 2005 Dec;132(24):5461-9.

Non-Patent Document 2: Fuse T, Matsuo N, Sone M, Watanabe M, Bito H, Terashima T, Wright CV, Kawaguchi Y, Nakao K, Nabeshima Y. Ptf1a, a bHLH transcriptional gene, defines GABAergic neuronal fates in cerebellum. Neuron. 2005 Jul 21;47(2):201-13.

Disclosure of the Invention

[Problems to be Solved by the Invention]

The present invention was achieved in view of the above circumstances. An objective of the present invention is to provide markers that enable selective identification of GABA neuron progenitor cells. Another objective of the present invention is to provide methods of using the markers as an indicator to identify GABA neuron progenitor cells, and reagents for use in these methods.

[Means for Solving the Problems]

The present inventors identified a selective marker, 65B13, for GABA neuron progenitor cells in the spinal dorsal horn and cerebellum, and successfully isolated GABA neuron progenitor cells using antibodies that bind to the protein encoded by the gene. Specifically, 65B13 was demonstrated to be useful as a marker to isolate GABA-producing neuron progenitor cells in the spinal dorsal horn and cerebellum.

GABA neuron progenitor cells can be efficiently identified or isolated by using the marker identified by the present inventors as an indicator.

The present invention relates to markers that enable selective identification of GABA neuron progenitor cells, methods of using the markers as an indicator to identify GABA neuron progenitor cells, and reagents for use in the methods. More specifically, the present invention provides:

[1] a method for detecting a GABA-producing neuron progenitor cell, which comprises the step of detecting the expression of a polynucleotide that can hybridize to a polynucleotide selected from (i), (ii), (iii), and (iv) below, or to a complementary sequence thereof:

(i) a polynucleotide comprising the nucleotide sequence of positions 178 to 2280 in SEQ ID

5 NO: 1; the nucleotide sequence of positions 127 to 2079 in SEQ ID NO: 3; the nucleotide sequence of positions 668 to 2770 in SEQ ID NO: 33; the nucleotide sequence of positions 130 to 2232 in SEQ ID NO: 35; the nucleotide sequence of positions 199 to 2100 in SEQ ID NO: 37; the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39; the nucleotide sequence of positions 15 to 2325 in SEQ ID NO: 41; the nucleotide sequence of positions 15
10 to 1916 in SEQ ID NO: 43; the nucleotide sequence of positions 199 to 1950 in SEQ ID NO: 45; the nucleotide sequence of positions 15 to 1766 in SEQ ID NO: 47; or the nucleotide sequence of positions 196 to 2262 in SEQ ID NO: 49;

(ii) a polynucleotide that is selectively expressed in a GABA-producing neuron progenitor cell, which is an amino acid sequence encoded by a nucleotide sequence comprising an insertion,
15 substitution, deletion of one or more nucleotides, and/or addition of one or more nucleotides to either or both ends thereof, in the nucleotide sequence of positions 178 to 2280 in SEQ ID NO: 1; the nucleotide sequence of positions 127 to 2079 in SEQ ID NO: 3; the nucleotide sequence of positions 668 to 2770 in SEQ ID NO: 33; the nucleotide sequence of positions 130 to 2232 in SEQ ID NO: 35; the nucleotide sequence of positions 199 to 2100 in SEQ ID
20 NO: 37; the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39; the nucleotide sequence of positions 15 to 2325 in SEQ ID NO: 41; the nucleotide sequence of positions 15 to 1916 in SEQ ID NO: 43; the nucleotide sequence of positions 199 to 1950 in SEQ ID NO: 45; the nucleotide sequence of positions 15 to 1766 in SEQ ID NO: 47; or the nucleotide sequence of positions 196 to 2262 in SEQ ID NO: 49;

25 (iii) a polynucleotide that is selectively expressed in a GABA-producing neuron progenitor cell and hybridizes under stringent conditions to a polynucleotide comprising the nucleotide sequence of positions 178 to 2280 in SEQ ID NO: 1; the nucleotide sequence of positions 127 to 2079 in SEQ ID NO: 3; the nucleotide sequence of positions 668 to 2770 in SEQ ID NO: 33; the nucleotide sequence of positions 130 to 2232 in SEQ ID NO: 35; the nucleotide
30 sequence of positions 199 to 2100 in SEQ ID NO: 37; the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39; the nucleotide sequence of positions 15 to 2325 in SEQ ID NO: 41; the nucleotide sequence of positions 15 to 1916 in SEQ ID NO: 43; the nucleotide sequence of positions 199 to 1950 in SEQ ID NO: 45; the nucleotide sequence of positions 15 to 1766 in SEQ ID NO: 47; or the nucleotide sequence of positions 196 to 2262 in SEQ ID
35 NO: 49; and

(iv) a polynucleotide that is selectively expressed in a GABA-producing neuron progenitor

cell and has 70% or higher sequence identity to a polynucleotide comprising the nucleotide sequence of positions 178 to 2280 in SEQ ID NO: 1; the nucleotide sequence of positions 127 to 2079 in SEQ ID NO: 3; the nucleotide sequence of positions 668 to 2770 in SEQ ID NO: 33; the nucleotide sequence of positions 130 to 2232 in SEQ ID NO: 35; the nucleotide sequence of positions 199 to 2100 in SEQ ID NO: 37; the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39; the nucleotide sequence of positions 15 to 2325 in SEQ ID NO: 41; the nucleotide sequence of positions 15 to 1916 in SEQ ID NO: 43; the nucleotide sequence of positions 199 to 1950 in SEQ ID NO: 45; the nucleotide sequence of positions 15 to 1766 in SEQ ID NO: 47; or the nucleotide sequence of positions 196 to 2262 in SEQ ID NO: 49;

[2] the method of [1], wherein the step of detecting the expression of a polynucleotide comprises the steps of:

(a) contacting a test cell sample with a polynucleotide that can hybridize to the polynucleotide selected in [1] or to a complementary sequence thereof, or with a probe comprising the polynucleotide; and

(b) detecting reactivity;

[3] the method of [2], wherein the probe is contacted with mRNA prepared from the test cell sample or a complementary DNA (cDNA) transcribed from the mRNA in step (a);

[4] the method of [1], wherein the step of detecting the expression of a polynucleotide that can hybridize to the polynucleotide selected in [1] or to a complementary sequence thereof comprises the steps of:

(a-1) conducting gene amplification using a polynucleotide derived from the test cell sample as a template, and a primer comprising a polynucleotide that can hybridize to the polynucleotide selected in [1] or to a complementary sequence thereof, or a set of primers comprising a polynucleotide that can hybridize to the polynucleotide selected in [1] or to a complementary sequence thereof; and

(b-1) detecting the resulting amplification product;

[5] the method of [4], wherein mRNA prepared from the test cell sample or a complementary DNA (cDNA) transcribed from the mRNA is used as a template in step (a-1);

[6] the method of any one of [1] to [5], wherein the detection step is followed by the step of separating a GABA-producing neuron progenitor cell from the detected sample;

[7] the method of any one of [1] to [6], which further comprises the step of detecting or selecting a GABA-producing neuron progenitor cell, using as an indicator the expression of a gene selected from the group consisting of the Corl1, Pax2, Lim1/2, Lbx1, and Corl2 genes;

[8] a method for detecting or selecting a GABA-producing neuron progenitor cell, which comprises the step of detecting a protein selected from:

(v) a protein comprising the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50;

(vi) a protein that is selectively expressed in a GABA-producing neuron progenitor cell and comprises an amino acid sequence comprising an insertion, substitution, deletion of one or more amino acids in the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50, and/or addition of one or more amino acids to either or both ends thereof;

(vii) a protein that is selectively expressed in a GABA-producing neuron progenitor cell and encoded by a polynucleotide that hybridizes under stringent conditions to a polynucleotide encoding the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50;

and

(viii) a protein that is selectively expressed in a GABA-producing neuron progenitor cell and comprises an amino acid sequence having 70% or higher identity to the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50;

[9] a method for detecting or selecting a GABA-producing neuron progenitor cell, which comprises the step of detecting a marker protein translated from a marker protein mRNA transcribed under the control of a promoter linked to a polynucleotide encoding the marker protein to express the mRNA, wherein the protein to be translated from the mRNA is selected from:

(v) a protein comprising the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50;

(vi) a protein that is selectively expressed in a GABA-producing neuron progenitor cell and comprises an amino acid sequence comprising an insertion, substitution, deletion of one or more amino acids in the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50, and/or addition of one or more amino acids to either or both ends thereof;

(vii) a protein that is selectively expressed in a GABA-producing neuron progenitor cell and encoded by a polynucleotide that hybridizes under stringent conditions to a polynucleotide encoding the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50; and

(viii) a protein that is selectively expressed in a GABA-producing neuron progenitor cell and comprises an amino acid sequence having 70% or higher identity to the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50;

[10] the method of [8] or [9], wherein the step of detecting the protein comprises the steps of: (d) contacting a test cell sample with an antibody that binds to the protein selected in [8] or [9]; and

(e) detecting reactivity;

[11] the method of any one of [8] to [10], wherein the detection step is followed by the step of

separating a GABA-producing neuron progenitor cell from the detected sample;

[12] the method of any one of [8] to [11], wherein the GABA-producing neuron progenitor cell marker protein other than the protein selected in [8] is a protein encoded by a gene selected from the group consisting of the Corl1, Pax2, Lim1/2, Lbx1, and Corl2 genes;

5 [13] a kit for detecting a GABA-producing neuron progenitor cell, which comprises a probe, a primer, or a set of primers that enable detection of the expression of a polynucleotide that can hybridize to a polynucleotide selected from (i), (ii), (iii), and (iv) below, or to a complementary sequence thereof:

- 10 (i) a polynucleotide comprising the nucleotide sequence of positions 178 to 2280 in SEQ ID NO: 1; the nucleotide sequence of positions 127 to 2079 in SEQ ID NO: 3; the nucleotide sequence of positions 668 to 2770 in SEQ ID NO: 33; the nucleotide sequence of positions 130 to 2232 in SEQ ID NO: 35; the nucleotide sequence of positions 199 to 2100 in SEQ ID NO: 37; the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39; the nucleotide sequence of positions 15 to 2325 in SEQ ID NO: 41; the nucleotide sequence of positions 15
15 to 1916 in SEQ ID NO: 43; the nucleotide sequence of positions 199 to 1950 in SEQ ID NO: 45; the nucleotide sequence of positions 15 to 1766 in SEQ ID NO: 47; or the nucleotide sequence of positions 196 to 2262 in SEQ ID NO: 49;
- 20 (ii) a polynucleotide encoding a protein that is selectively expressed in a GABA-producing neuron progenitor cell and which comprises an amino acid sequence encoded by a nucleotide sequence comprising an insertion, substitution, deletion of one or more nucleotides, and/or addition of one or more nucleotides to either or both ends thereof, in the nucleotide sequence of positions 178 to 2280 in SEQ ID NO: 1; the nucleotide sequence of positions 127 to 2079 in
25 SEQ ID NO: 3; the nucleotide sequence of positions 668 to 2770 in SEQ ID NO: 33; the nucleotide sequence of positions 130 to 2232 in SEQ ID NO: 35; the nucleotide sequence of positions 199 to 2100 in SEQ ID NO: 37; the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39; the nucleotide sequence of positions 15 to 2325 in SEQ ID NO: 41; the nucleotide sequence of positions 15 to 1916 in SEQ ID NO: 43; the nucleotide sequence of positions 199 to 1950 in SEQ ID NO: 45; the nucleotide sequence of positions 15 to 1766 in
30 SEQ ID NO: 47; or the nucleotide sequence of positions 196 to 2262 in SEQ ID NO: 49;
- 35 (iii) a polynucleotide encoding a protein that is selectively expressed in a GABA-producing neuron progenitor cell, which hybridizes under a stringent condition to a polynucleotide comprising the nucleotide sequence of positions 178 to 2280 in SEQ ID NO: 1; the nucleotide sequence of positions 127 to 2079 in SEQ ID NO: 3; the nucleotide sequence of positions 668 to 2770 in SEQ ID NO: 33; the nucleotide sequence of positions 130 to 2232 in SEQ ID NO: 35; the nucleotide sequence of positions 199 to 2100 in SEQ ID NO: 37; the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39; the nucleotide sequence of positions 15

to 2325 in SEQ ID NO: 41; the nucleotide sequence of positions 15 to 1916 in SEQ ID NO: 43; the nucleotide sequence of positions 199 to 1950 in SEQ ID NO: 45; the nucleotide sequence of positions 15 to 1766 in SEQ ID NO: 47; or the nucleotide sequence of positions 196 to 2262 in SEQ ID NO: 49; and

- 5 (iv) a polynucleotide encoding a protein that is selectively expressed in a GABA-producing neuron progenitor cell, which has 70% or higher sequence identity to a polynucleotide comprising the nucleotide sequence of positions 178 to 2280 in SEQ ID NO: 1; the nucleotide sequence of positions 127 to 2079 in SEQ ID NO: 3; the nucleotide sequence of positions 668 to 2770 in SEQ ID NO: 33; the nucleotide sequence of positions 130 to 2232 in SEQ ID NO: 35; the nucleotide sequence of positions 199 to 2100 in SEQ ID NO: 37; the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39; the nucleotide sequence of positions 15 to 2325 in SEQ ID NO: 41; the nucleotide sequence of positions 15 to 1916 in SEQ ID NO: 43; the nucleotide sequence of positions 199 to 1950 in SEQ ID NO: 45; the nucleotide sequence of positions 15 to 1766 in SEQ ID NO: 47; or the nucleotide sequence of positions 196 to 2262 in SEQ ID NO: 49;

[14] the kit of [13] for detecting or selecting a GABA-producing neuron progenitor cell, which further comprises a polynucleotide that hybridizes to the transcript of one or more genes selected from the group consisting of the Lbx1, Pax2, Lim1/2, Corl1, and Corl2 genes;

[15] the kit of [13] or [14], which further comprises a cerebellar cell or spinal cord cell;

- 20 [16] a kit for detecting or selecting a GABA-producing neuron progenitor cell, which comprises an antibody that binds to a protein selected from:

(v) a protein comprising the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50;

- 25 (vi) a protein that is selectively expressed in a GABA-producing neuron progenitor cell and comprises an amino acid sequence comprising an insertion, substitution, deletion of one or more amino acids in the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50, and/or addition of one or more amino acids to either or both ends thereof;

- 30 (vii) a protein that is selectively expressed in a GABA-producing neuron progenitor cell and encoded by a polynucleotide that hybridizes under stringent conditions to a polynucleotide encoding the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50; and

(viii) a protein that is selectively expressed in a GABA-producing neuron progenitor cell and comprises an amino acid sequence having 70% or higher identity to the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50;

- 35 [17] a kit for detecting or selecting a GABA-producing neuron progenitor cell, which comprises a polynucleotide comprising a polynucleotide encoding a marker protein linked to a

promoter to express the mRNA translated into a protein selected from:

(v) a protein comprising the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50;

(vi) a protein that is selectively expressed in a GABA-producing neuron progenitor cell and comprises an amino acid sequence comprising an insertion, substitution, deletion of one or more amino acids in the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50, and/or addition of one or more amino acids to either or both ends thereof;

(vii) a protein that is selectively expressed in a GABA-producing neuron progenitor cell and encoded by a polynucleotide that hybridizes under stringent conditions to a polynucleotide encoding the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50; and

(viii) a protein that is selectively expressed in a GABA-producing neuron progenitor cell and comprises an amino acid sequence having 70% or higher identity to the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50;

[18] the kit of [16] or [17] for detecting or selecting a GABA-producing neuron progenitor cell, which further comprises in combination an antibody that binds to a protein encoded by one or more genes selected from the group consisting of the Lbx1, Pax2, Lim1/2, Corl1, and Corl2 genes;

[19] the kit of any one of [16] to [18], which further comprises a cerebellar cell or spinal cord cell;

[20] the kit of [15] or [19], wherein the target cerebellar cell for detection is a Purkinje cell;

[21] the kit of [15] or [19], wherein the target spinal cord cell for detection is dI4 or dILA;

[22] a method of screening for a substance that is effective for differentiating a GABA-producing neuron progenitor cell, which comprises the steps of:

(I) contacting a test compound with a cell that can differentiate into a GABA-producing neuron progenitor cell; and

(II) detecting the expression of the polynucleotide of a GABA-producing neuron progenitor cell, which can hybridize to a nucleotide sequence selected from (i), (ii), (iii), and (iv) below, or to a complementary sequence thereof:

(i) a polynucleotide comprising the nucleotide sequence of positions 178 to 2280 in SEQ ID NO: 1; the nucleotide sequence of positions 127 to 2079 in SEQ ID NO: 3; the nucleotide sequence of positions 668 to 2770 in SEQ ID NO: 33; the nucleotide sequence of positions 130 to 2232 in SEQ ID NO: 35; the nucleotide sequence of positions 199 to 2100 in SEQ ID NO: 37; the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39; the nucleotide sequence of positions 15 to 2325 in SEQ ID NO: 41; the nucleotide sequence of positions 15 to 1916 in SEQ ID NO: 43; the nucleotide sequence of positions 199 to 1950 in SEQ ID NO:

45; the nucleotide sequence of positions 15 to 1766 in SEQ ID NO: 47; or the nucleotide sequence of positions 196 to 2262 in SEQ ID NO: 49;

(ii) a polynucleotide encoding a protein that is selectively expressed in a GABA-producing neuron progenitor cell and which comprises an amino acid sequence encoded by a nucleotide sequence comprising an insertion, substitution, deletion of one or more nucleotides, and/or addition of one or more nucleotides to either or both ends thereof, in the nucleotide sequence of positions 178 to 2280 in SEQ ID NO: 1; the nucleotide sequence of positions 127 to 2079 in SEQ ID NO: 3; the nucleotide sequence of positions 668 to 2770 in SEQ ID NO: 33; the nucleotide sequence of positions 130 to 2232 in SEQ ID NO: 35; the nucleotide sequence of positions 199 to 2100 in SEQ ID NO: 37; the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39; the nucleotide sequence of positions 15 to 2325 in SEQ ID NO: 41; the nucleotide sequence of positions 15 to 1916 in SEQ ID NO: 43; the nucleotide sequence of positions 199 to 1950 in SEQ ID NO: 45; the nucleotide sequence of positions 15 to 1766 in SEQ ID NO: 47; or the nucleotide sequence of positions 196 to 2262 in SEQ ID NO: 49;

(iii) a polynucleotide encoding a protein that is selectively expressed in a GABA-producing neuron progenitor cell, which hybridizes under a stringent condition to a polynucleotide comprising the nucleotide sequence of positions 178 to 2280 in SEQ ID NO: 1; the nucleotide sequence of positions 127 to 2079 in SEQ ID NO: 3; the nucleotide sequence of positions 668 to 2770 in SEQ ID NO: 33; the nucleotide sequence of positions 130 to 2232 in SEQ ID NO: 35; the nucleotide sequence of positions 199 to 2100 in SEQ ID NO: 37; the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39; the nucleotide sequence of positions 15 to 2325 in SEQ ID NO: 41; the nucleotide sequence of positions 15 to 1916 in SEQ ID NO: 43; the nucleotide sequence of positions 199 to 1950 in SEQ ID NO: 45; the nucleotide sequence of positions 15 to 1766 in SEQ ID NO: 47; or the nucleotide sequence of positions 196 to 2262 in SEQ ID NO: 49; and

(iv) a polynucleotide encoding a protein that is selectively expressed in a GABA-producing neuron progenitor cell, which has 70% or higher sequence identity to a polynucleotide comprising the nucleotide sequence of positions 178 to 2280 in SEQ ID NO: 1; the nucleotide sequence of positions 127 to 2079 in SEQ ID NO: 3; the nucleotide sequence of positions 668 to 2770 in SEQ ID NO: 33; the nucleotide sequence of positions 130 to 2232 in SEQ ID NO: 35; the nucleotide sequence of positions 199 to 2100 in SEQ ID NO: 37; the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39; the nucleotide sequence of positions 15 to 2325 in SEQ ID NO: 41; the nucleotide sequence of positions 15 to 1916 in SEQ ID NO: 43; the nucleotide sequence of positions 199 to 1950 in SEQ ID NO: 45; the nucleotide sequence of positions 15 to 1766 in SEQ ID NO: 47; or the nucleotide sequence of positions 196 to 2262 in SEQ ID NO: 49;

[23] the method of [22], which further comprises step (III) of selecting a compound which detects the expression of the polynucleotide in step (II);

[24] a method of screening for a substance that is effective for differentiating a GABA-producing neuron progenitor cell, which comprises the steps of:

- 5 (IV) contacting a test compound with a cell that can differentiate into a GABA-producing neuron progenitor cell; and
- (V) detecting a protein selected from:
 - (v) a protein comprising the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50;
 - 10 (vi) a protein that is selectively expressed in a GABA-producing neuron progenitor cell and comprises an amino acid sequence comprising an insertion, substitution, deletion of one or more amino acids in the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50, and/or addition of one or more amino acids to either or both ends thereof;
 - (vii) a protein that is selectively expressed in a GABA-producing neuron progenitor cell and
15 encoded by a polynucleotide that hybridizes under stringent conditions to a polynucleotide encoding the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50; and
 - (viii) a protein that is selectively expressed in a GABA-producing neuron progenitor cell and comprises an amino acid sequence having 70% or higher identity to the amino acid sequence
20 of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50;
- [25] a method of screening for a substance that is effective for differentiating a GABA-producing neuron progenitor cell, which comprises the steps of:
 - (IV-1) contacting a test compound with a cell that can differentiate into a GABA-producing neuron progenitor cell; and
 - 25 (V-1) detecting a marker protein translated from a marker protein mRNA transcribed under the control of a promoter linked to a polynucleotide encoding the marker protein to express the mRNA, wherein the protein to be translated from the mRNA is selected from:
 - (v) a protein comprising the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50;
 - 30 (vi) a protein that is selectively expressed in a GABA-producing neuron progenitor cell and comprises an amino acid sequence comprising an insertion, substitution, deletion of one or more amino acids in the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50, and/or addition of one or more amino acids to either or both ends thereof;
 - (vii) a protein that is selectively expressed in a GABA-producing neuron progenitor cell and
35 encoded by a polynucleotide that hybridizes under stringent conditions to a polynucleotide encoding the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50;

and

(viii) a protein that is selectively expressed in a GABA-producing neuron progenitor cell and comprises an amino acid sequence having 70% or higher identity to the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50;

5 [26] the method of [24] or [25], which further comprises step (VI) of selecting a compound which detects the protein in step (V);

[27] a method for producing a GABA-producing neuron progenitor cell, which comprises the steps of:

10 (VII) obtaining a cell population potentially containing a GABA-producing neuron progenitor cell;

(VIII) detecting a GABA-producing neuron progenitor cell using the method of any one of [1] to [12]; and

(IX) growing the cell detected or selected in step (VIII);

15 [28] the production method of [27], wherein the GABA-producing neuron progenitor cell is used to treat spinal cord injury or cerebellar degeneration;

[29] a GABA-producing neuron progenitor cell population obtained by the steps of:

(VII) obtaining a cell population potentially containing a GABA-producing neuron progenitor cell;

20 (VIII) detecting or selecting a GABA-producing neuron progenitor cell using the method of any one of [1] to [12]; and

(IX) growing the cell detected in step (VIII);

[30] a reagent for detecting or selecting a GABA-producing neuron progenitor cell, which comprises a probe, a primer, or a set of primers that enable to detect the expression of a polynucleotide that can hybridize to a polynucleotide selected from (i), (ii), (iii), and (iv)

25 below, or to a complementary sequence thereof:

(i) a polynucleotide comprising the nucleotide sequence of positions 178 to 2280 in SEQ ID NO: 1; the nucleotide sequence of positions 127 to 2079 in SEQ ID NO: 3; the nucleotide sequence of positions 668 to 2770 in SEQ ID NO: 33; the nucleotide sequence of positions 130 to 2232 in SEQ ID NO: 35; the nucleotide sequence of positions 199 to 2100 in SEQ ID NO: 37; the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39; the nucleotide sequence of positions 15 to 2325 in SEQ ID NO: 41; the nucleotide sequence of positions 15 to 1916 in SEQ ID NO: 43; the nucleotide sequence of positions 199 to 1950 in SEQ ID NO: 45; the nucleotide sequence of positions 15 to 1766 in SEQ ID NO: 47; or the nucleotide sequence of positions 196 to 2262 in SEQ ID NO: 49;

35 (ii) a polynucleotide encoding a protein that is selectively expressed in a GABA-producing neuron progenitor cell and which comprises an amino acid sequence encoded by a nucleotide

sequence comprising an insertion, substitution, deletion of one or more nucleotides, and/or addition of one or more nucleotides to either or both ends thereof, in the nucleotide sequence of positions 178 to 2280 in SEQ ID NO: 1; the nucleotide sequence of positions 127 to 2079 in SEQ ID NO: 3; the nucleotide sequence of positions 668 to 2770 in SEQ ID NO: 33; the
5 nucleotide sequence of positions 130 to 2232 in SEQ ID NO: 35; the nucleotide sequence of positions 199 to 2100 in SEQ ID NO: 37; the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39; the nucleotide sequence of positions 15 to 2325 in SEQ ID NO: 41; the nucleotide sequence of positions 15 to 1916 in SEQ ID NO: 43; the nucleotide sequence of positions 199 to 1950 in SEQ ID NO: 45; the nucleotide sequence of positions 15 to 1766 in
10 SEQ ID NO: 47; or the nucleotide sequence of positions 196 to 2262 in SEQ ID NO: 49;
(iii) a polynucleotide encoding a protein that is selectively expressed in a GABA-producing neuron progenitor cell, which hybridizes under a stringent condition to a polynucleotide comprising the nucleotide sequence of positions 178 to 2280 in SEQ ID NO: 1; the nucleotide sequence of positions 127 to 2079 in SEQ ID NO: 3; the nucleotide sequence of positions 668
15 to 2770 in SEQ ID NO: 33; the nucleotide sequence of positions 130 to 2232 in SEQ ID NO: 35; the nucleotide sequence of positions 199 to 2100 in SEQ ID NO: 37; the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39; the nucleotide sequence of positions 15 to 2325 in SEQ ID NO: 41; the nucleotide sequence of positions 15 to 1916 in SEQ ID NO: 43; the nucleotide sequence of positions 199 to 1950 in SEQ ID NO: 45; the nucleotide
20 sequence of positions 15 to 1766 in SEQ ID NO: 47; or the nucleotide sequence of positions 196 to 2262 in SEQ ID NO: 49; and
(iv) a polynucleotide encoding a protein that is selectively expressed in a GABA-producing neuron progenitor cell, which has 70% or higher sequence identity to a polynucleotide comprising the nucleotide sequence of positions 178 to 2280 in SEQ ID NO: 1; the nucleotide
25 sequence of positions 127 to 2079 in SEQ ID NO: 3; the nucleotide sequence of positions 668 to 2770 in SEQ ID NO: 33; the nucleotide sequence of positions 130 to 2232 in SEQ ID NO: 35; the nucleotide sequence of positions 199 to 2100 in SEQ ID NO: 37; the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39; the nucleotide sequence of positions 15 to 2325 in SEQ ID NO: 41; the nucleotide sequence of positions 15 to 1916 in SEQ ID NO:
30 43; the nucleotide sequence of positions 199 to 1950 in SEQ ID NO: 45; the nucleotide sequence of positions 15 to 1766 in SEQ ID NO: 47; or the nucleotide sequence of positions 196 to 2262 in SEQ ID NO: 49;
[31] the reagent of [30] for detecting or selecting a cerebellar cell or spinal cord cell, which further comprises in combination a polynucleotide that hybridizes to a transcript of one or
35 more genes selected from the group consisting of the Lbx1, Pax2, Lim1/2, Corl1, and Corl2 genes;

[32] the reagent of [30] or [31], which further comprises a cerebellar cell or spinal cord cell;

[33] a reagent for detecting or selecting a GABA-producing neuron progenitor cell, which comprises an antibody that binds to a protein selected from:

(v) a protein comprising the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44,

5 46, 48, or 50;

(vi) a protein that is selectively expressed in a GABA-producing neuron progenitor cell and comprises an amino acid sequence comprising an insertion, substitution, deletion of one or more amino acids in the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50, and/or addition of one or more amino acids to either or both ends thereof;

10 (vii) a protein that is selectively expressed in a GABA-producing neuron progenitor cell and encoded by a polynucleotide that hybridizes under stringent conditions to a polynucleotide encoding the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50; and

(viii) a protein that is selectively expressed in a GABA-producing neuron progenitor cell and
15 comprises an amino acid sequence having 70% or higher identity to the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50;

[34] a reagent for detecting or selecting a GABA-producing neuron progenitor cell, which comprises a polynucleotide comprising a polynucleotide encoding a marker protein linked to a promoter to express the mRNA translated into a protein selected from:

20 (v) a protein comprising the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50;

(vi) a protein that is selectively expressed in a GABA-producing neuron progenitor cell and comprises an amino acid sequence comprising an insertion, substitution, deletion of one or more amino acids in the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46,
25 48, or 50, and/or addition of one or more amino acids to either or both ends thereof;

(vii) a protein that is selectively expressed in a GABA-producing neuron progenitor cell and encoded by a polynucleotide that hybridizes under stringent conditions to a polynucleotide encoding the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50; and

30 (viii) a protein that is selectively expressed in a GABA-producing neuron progenitor cell and comprises an amino acid sequence having 70% or higher identity to the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50;

[35] the reagent of [33] or [34] for detecting or selecting a GABA-producing neuron progenitor cell, which further comprises in combination an antibody that binds to a protein
35 encoded by one or more genes selected from the group consisting of the Lbx1, Pax2, Lim1/2, Corl1, and Corl2 genes;

[36] the reagent of any one of [33] to [35], which further comprises a cerebellar cell or spinal cord cell;

[37] the reagent of any one of [33] to [36], wherein the target cerebellar cell for detection is a Purkinje cell;

5 [38] the reagent of any one of [33] to [36], wherein the target spinal cord cell for detection is dI4 or dILA;

[39] a polynucleotide for detecting or selecting a GABA-producing neuron progenitor cell for use in regeneration medicine to treat cerebellar degeneration or spinal cord injury, which can hybridize to a polynucleotide selected from (i), (ii), (iii), and (iv) below, or to a

10 complementary sequence thereof:

(i) a polynucleotide comprising the nucleotide sequence of positions 178 to 2280 in SEQ ID NO: 1; the nucleotide sequence of positions 127 to 2079 in SEQ ID NO: 3; the nucleotide sequence of positions 668 to 2770 in SEQ ID NO: 33; the nucleotide sequence of positions 130 to 2232 in SEQ ID NO: 35; the nucleotide sequence of positions 199 to 2100 in SEQ ID NO: 37; the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39; the nucleotide sequence of positions 15 to 2325 in SEQ ID NO: 41; the nucleotide sequence of positions 15 to 1916 in SEQ ID NO: 43; the nucleotide sequence of positions 199 to 1950 in SEQ ID NO: 45; the nucleotide sequence of positions 15 to 1766 in SEQ ID NO: 47; or the nucleotide sequence of positions 196 to 2262 in SEQ ID NO: 49;

20 (ii) a polynucleotide encoding a protein that is selectively expressed in a GABA-producing neuron progenitor cell and which comprises an amino acid sequence encoded by a nucleotide sequence comprising an insertion, substitution, deletion of one or more nucleotides, and/or addition of one or more nucleotides to either or both ends thereof, in the nucleotide sequence of positions 178 to 2280 in SEQ ID NO: 1; the nucleotide sequence of positions 127 to 2079 in SEQ ID NO: 3; the nucleotide sequence of positions 668 to 2770 in SEQ ID NO: 33; the nucleotide sequence of positions 130 to 2232 in SEQ ID NO: 35; the nucleotide sequence of positions 199 to 2100 in SEQ ID NO: 37; the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39; the nucleotide sequence of positions 15 to 2325 in SEQ ID NO: 41; the nucleotide sequence of positions 15 to 1916 in SEQ ID NO: 43; the nucleotide sequence of positions 199 to 1950 in SEQ ID NO: 45; the nucleotide sequence of positions 15 to 1766 in SEQ ID NO: 47; or the nucleotide sequence of positions 196 to 2262 in SEQ ID NO: 49;

30 (iii) a polynucleotide encoding a protein that is selectively expressed in a GABA-producing neuron progenitor cell, which hybridizes under a stringent condition to a polynucleotide comprising the nucleotide sequence of positions 178 to 2280 in SEQ ID NO: 1; the nucleotide sequence of positions 127 to 2079 in SEQ ID NO: 3; the nucleotide sequence of positions 668 to 2770 in SEQ ID NO: 33; the nucleotide sequence of positions 130 to 2232 in SEQ ID NO: 35; the nucleotide sequence of positions 199 to 2100 in SEQ ID NO: 37; the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39; the nucleotide sequence of positions 15 to 2325 in SEQ ID NO: 41; the nucleotide sequence of positions 15 to 1916 in SEQ ID NO: 43; the nucleotide sequence of positions 199 to 1950 in SEQ ID NO: 45; the nucleotide sequence of positions 15 to 1766 in SEQ ID NO: 47; or the nucleotide sequence of positions 196 to 2262 in SEQ ID NO: 49;

35 (iv) a polynucleotide encoding a protein that is selectively expressed in a GABA-producing neuron progenitor cell, which hybridizes under a stringent condition to a polynucleotide comprising the nucleotide sequence of positions 178 to 2280 in SEQ ID NO: 1; the nucleotide sequence of positions 127 to 2079 in SEQ ID NO: 3; the nucleotide sequence of positions 668 to 2770 in SEQ ID NO: 33; the nucleotide sequence of positions 130 to 2232 in SEQ ID NO: 35; the nucleotide sequence of positions 199 to 2100 in SEQ ID NO: 37; the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39; the nucleotide sequence of positions 15 to 2325 in SEQ ID NO: 41; the nucleotide sequence of positions 15 to 1916 in SEQ ID NO: 43; the nucleotide sequence of positions 199 to 1950 in SEQ ID NO: 45; the nucleotide sequence of positions 15 to 1766 in SEQ ID NO: 47; or the nucleotide sequence of positions 196 to 2262 in SEQ ID NO: 49;

35; the nucleotide sequence of positions 199 to 2100 in SEQ ID NO: 37; the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39; the nucleotide sequence of positions 15 to 2325 in SEQ ID NO: 41; the nucleotide sequence of positions 15 to 1916 in SEQ ID NO: 43; the nucleotide sequence of positions 199 to 1950 in SEQ ID NO: 45; the nucleotide sequence of positions 15 to 1766 in SEQ ID NO: 47; or the nucleotide sequence of positions 196 to 2262 in SEQ ID NO: 49; and

(iv) a polynucleotide encoding a protein that is selectively expressed in a GABA-producing neuron progenitor cell, which has 70% or higher sequence identity to a polynucleotide comprising the nucleotide sequence of positions 178 to 2280 in SEQ ID NO: 1; the nucleotide sequence of positions 127 to 2079 in SEQ ID NO: 3; the nucleotide sequence of positions 668 to 2770 in SEQ ID NO: 33; the nucleotide sequence of positions 130 to 2232 in SEQ ID NO: 35; the nucleotide sequence of positions 199 to 2100 in SEQ ID NO: 37; the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39; the nucleotide sequence of positions 15 to 2325 in SEQ ID NO: 41; the nucleotide sequence of positions 15 to 1916 in SEQ ID NO: 43; the nucleotide sequence of positions 199 to 1950 in SEQ ID NO: 45; the nucleotide sequence of positions 15 to 1766 in SEQ ID NO: 47; or the nucleotide sequence of positions 196 to 2262 in SEQ ID NO: 49;

[40] the polynucleotide of [39] for detecting or selecting a GABA-producing neuron progenitor cell, wherein the GABA-producing neuron progenitor cell is a spinal cord or cerebellar GABA-producing neuron progenitor cell;

[41] the polynucleotide of [39] or [40] for detection or selection, which comprises a polynucleotide comprising at least 10 consecutive nucleotides of the nucleotide sequence of [39] or [40] or a complementary sequence thereof;

[42] the polynucleotide of [39] or [40] for detection or selection, which comprises a polynucleotide comprising at least 15 consecutive nucleotides of the nucleotide sequence of [39] or [40] or a complementary sequence thereof;

[43] a probe comprising the detection polynucleotide of any one of [39] to [42];

[44] a primer comprising the detection polynucleotide of any one of [39] to [42];

[45] a primer set comprising two or more of the detection polynucleotides, each of which is shown in any one of [39] to [42];

[46] an antibody for detecting or selecting a GABA-producing neuron progenitor cell for use in regeneration medicine to treat cerebellar degeneration or spinal cord injury, which binds to a protein selected from:

(v) a protein comprising the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50;

(vi) a protein that is selectively expressed in a GABA-producing neuron progenitor cell and

comprises an amino acid sequence comprising an insertion, substitution, deletion of one or more amino acids in the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50, and/or addition of one or more amino acids to either or both ends thereof;

(vii) a protein that is selectively expressed in a GABA-producing neuron progenitor cell and encoded by a polynucleotide that hybridizes under stringent conditions to a polynucleotide encoding the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50; and

(viii) a protein that is selectively expressed in a GABA-producing neuron progenitor cell and comprises an amino acid sequence having 70% or higher identity to the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50;

[47] the antibody of [46], wherein the GABA-producing neuron progenitor cell is a cerebellar cell or spinal cord cell;

[48] the antibody of [46] or [47], which binds to a polypeptide comprising the entire or at least six consecutive amino acid residues of the amino acid sequence of positions 21 to 510 in the amino acid sequence of SEQ ID NO: 2, 4, 38, 40, 42, or 44; the amino acid sequence of positions 20 to 513 in the amino acid sequence of SEQ ID NO: 34 or 36; the amino acid sequence of positions 21 to 460 in the amino acid sequence of SEQ ID NO: 46 or 48; or the amino acid sequence of positions 21 to 490 in the amino acid sequence of SEQ ID NO: 50;

[49] the antibody of any one of [46] to [48], which binds to a polypeptide comprising at least six amino acid residues;

[50] a GABA-producing neuron progenitor cell for use in regeneration medicine to treat cerebellar degeneration or spinal cord injury, which is detected or selected by using a polynucleotide that can hybridize to a polynucleotide selected from (i), (ii), (iii), and (iv) below, or to a complementary sequence thereof:

(i) a polynucleotide comprising the nucleotide sequence of positions 178 to 2280 in SEQ ID NO: 1; the nucleotide sequence of positions 127 to 2079 in SEQ ID NO: 3; the nucleotide sequence of positions 668 to 2770 in SEQ ID NO: 33; the nucleotide sequence of positions 130 to 2232 in SEQ ID NO: 35; the nucleotide sequence of positions 199 to 2100 in SEQ ID NO: 37; the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39; the nucleotide sequence of positions 15 to 2325 in SEQ ID NO: 41; the nucleotide sequence of positions 15 to 1916 in SEQ ID NO: 43; the nucleotide sequence of positions 199 to 1950 in SEQ ID NO: 45; the nucleotide sequence of positions 15 to 1766 in SEQ ID NO: 47; or the nucleotide sequence of positions 196 to 2262 in SEQ ID NO: 49;

(ii) a polynucleotide encoding a protein that is selectively expressed in a GABA-producing neuron progenitor cell and which comprises an amino acid sequence encoded by a nucleotide sequence comprising an insertion, substitution, deletion of one or more nucleotides, and/or

addition of one or more nucleotides to either or both ends thereof, in the nucleotide sequence of positions 178 to 2280 in SEQ ID NO: 1; the nucleotide sequence of positions 127 to 2079 in SEQ ID NO: 3; the nucleotide sequence of positions 668 to 2770 in SEQ ID NO: 33; the nucleotide sequence of positions 130 to 2232 in SEQ ID NO: 35; the nucleotide sequence of positions 199 to 2100 in SEQ ID NO: 37; the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39; the nucleotide sequence of positions 15 to 2325 in SEQ ID NO: 41; the nucleotide sequence of positions 15 to 1916 in SEQ ID NO: 43; the nucleotide sequence of positions 199 to 1950 in SEQ ID NO: 45; the nucleotide sequence of positions 15 to 1766 in SEQ ID NO: 47; or the nucleotide sequence of positions 196 to 2262 in SEQ ID NO: 49;

(iii) a polynucleotide encoding a protein that is selectively expressed in a GABA-producing neuron progenitor cell, which hybridizes under a stringent condition to a polynucleotide comprising the nucleotide sequence of positions 178 to 2280 in SEQ ID NO: 1; the nucleotide sequence of positions 127 to 2079 in SEQ ID NO: 3; the nucleotide sequence of positions 668 to 2770 in SEQ ID NO: 33; the nucleotide sequence of positions 130 to 2232 in SEQ ID NO: 35; the nucleotide sequence of positions 199 to 2100 in SEQ ID NO: 37; the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39; the nucleotide sequence of positions 15 to 2325 in SEQ ID NO: 41; the nucleotide sequence of positions 15 to 1916 in SEQ ID NO: 43; the nucleotide sequence of positions 199 to 1950 in SEQ ID NO: 45; the nucleotide sequence of positions 15 to 1766 in SEQ ID NO: 47; or the nucleotide sequence of positions 196 to 2262 in SEQ ID NO: 49; and

(iv) a polynucleotide encoding a protein that is selectively expressed in a GABA-producing neuron progenitor cell, which has 70% or higher sequence identity to a polynucleotide comprising the nucleotide sequence of positions 178 to 2280 in SEQ ID NO: 1; the nucleotide sequence of positions 127 to 2079 in SEQ ID NO: 3; the nucleotide sequence of positions 668 to 2770 in SEQ ID NO: 33; the nucleotide sequence of positions 130 to 2232 in SEQ ID NO: 35; the nucleotide sequence of positions 199 to 2100 in SEQ ID NO: 37; the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39; the nucleotide sequence of positions 15 to 2325 in SEQ ID NO: 41; the nucleotide sequence of positions 15 to 1916 in SEQ ID NO: 43; the nucleotide sequence of positions 199 to 1950 in SEQ ID NO: 45; the nucleotide sequence of positions 15 to 1766 in SEQ ID NO: 47; or the nucleotide sequence of positions 196 to 2262 in SEQ ID NO: 49;

[51] the GABA-producing neuron progenitor cell of [50], wherein the GABA-producing neuron progenitor cell is a spinal cord or cerebellar GABA-producing neuron progenitor cell;

[52] the GABA-producing neuron progenitor cell of [50] or [51], wherein the polynucleotide comprises at least 10 consecutive nucleotides of a polynucleotide selected from (i), (ii), (iii), and (iv) below, or a complementary sequence thereof;

[53] the GABA-producing neuron progenitor cell of [50] or [51], wherein the polynucleotide comprises at least 15 consecutive nucleotides of a polynucleotide selected from (i), (ii), (iii), and (iv) below, or a complementary sequence thereof;

[54] a GABA-producing neuron progenitor cell for use in regeneration medicine to treat cerebellar degeneration or spinal cord injury, which has been detected or selected by using an antibody that binds to a protein selected from:

(v) a protein comprising the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50;

(vi) a protein that is selectively expressed in a GABA-producing neuron progenitor cell and comprises an amino acid sequence comprising an insertion, substitution, deletion of one or more amino acids in the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50, and/or addition of one or more amino acids to either or both ends thereof;

(vii) a protein that is selectively expressed in a GABA-producing neuron progenitor cell and encoded by a polynucleotide that hybridizes under stringent conditions to a polynucleotide encoding the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50; and

(viii) a protein that is selectively expressed in a GABA-producing neuron progenitor cell and comprises an amino acid sequence having 70% or higher identity to the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50;

[55] the GABA-producing neuron progenitor cell of [54], wherein the GABA-producing neuron progenitor cell is a cerebellar or spinal cord GABA-producing neuron progenitor cell;

[56] the GABA-producing neuron progenitor cell of [54] or [55], wherein the antibody binds to a polypeptide comprising the entire or at least six consecutive amino acid residues of the amino acid sequence of positions 21 to 510 in the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50;

[57] the GABA-producing neuron progenitor cell of any one of [54] to [56], wherein the antibody binds to a polypeptide comprising at least six amino acid residues;

[58] a method for treating cerebellar degeneration or spinal cord injury, which comprises transplanting a GABA-producing neuron progenitor cell detected or selected by using a polynucleotide that can hybridize to a polynucleotide selected from (i), (ii), (iii), and (iv) below, or to a complementary sequence thereof:

(i) a polynucleotide comprising the nucleotide sequence of positions 178 to 2280 in SEQ ID NO: 1; the nucleotide sequence of positions 127 to 2079 in SEQ ID NO: 3; the nucleotide sequence of positions 668 to 2770 in SEQ ID NO: 33; the nucleotide sequence of positions 130 to 2232 in SEQ ID NO: 35; the nucleotide sequence of positions 199 to 2100 in SEQ ID NO: 37; the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39; the nucleotide

sequence of positions 15 to 2325 in SEQ ID NO: 41; the nucleotide sequence of positions 15 to 1916 in SEQ ID NO: 43; the nucleotide sequence of positions 199 to 1950 in SEQ ID NO: 45; the nucleotide sequence of positions 15 to 1766 in SEQ ID NO: 47; or the nucleotide sequence of positions 196 to 2262 in SEQ ID NO: 49;

5 (ii) a polynucleotide encoding a protein that is selectively expressed in a GABA-producing neuron progenitor cell and which comprises an amino acid sequence encoded by a nucleotide sequence comprising an insertion, substitution, deletion of one or more nucleotides, and/or addition of one or more nucleotides to either or both ends thereof, in the nucleotide sequence of positions 178 to 2280 in SEQ ID NO: 1; the nucleotide sequence of positions 127 to 2079 in
10 SEQ ID NO: 3; the nucleotide sequence of positions 668 to 2770 in SEQ ID NO: 33; the nucleotide sequence of positions 130 to 2232 in SEQ ID NO: 35; the nucleotide sequence of positions 199 to 2100 in SEQ ID NO: 37; the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39; the nucleotide sequence of positions 15 to 2325 in SEQ ID NO: 41; the nucleotide sequence of positions 15 to 1916 in SEQ ID NO: 43; the nucleotide sequence of
15 positions 199 to 1950 in SEQ ID NO: 45; the nucleotide sequence of positions 15 to 1766 in SEQ ID NO: 47; or the nucleotide sequence of positions 196 to 2262 in SEQ ID NO: 49;

(iii) a polynucleotide encoding a protein that is selectively expressed in a GABA-producing neuron progenitor cell, which hybridizes under a stringent condition to a polynucleotide comprising the nucleotide sequence of positions 178 to 2280 in SEQ ID NO: 1; the nucleotide
20 sequence of positions 127 to 2079 in SEQ ID NO: 3; the nucleotide sequence of positions 668 to 2770 in SEQ ID NO: 33; the nucleotide sequence of positions 130 to 2232 in SEQ ID NO: 35; the nucleotide sequence of positions 199 to 2100 in SEQ ID NO: 37; the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39; the nucleotide sequence of positions 15 to 2325 in SEQ ID NO: 41; the nucleotide sequence of positions 15 to 1916 in SEQ ID NO:
25 43; the nucleotide sequence of positions 199 to 1950 in SEQ ID NO: 45; the nucleotide sequence of positions 15 to 1766 in SEQ ID NO: 47; or the nucleotide sequence of positions 196 to 2262 in SEQ ID NO: 49; and

(iv) a polynucleotide encoding a protein that is selectively expressed in a GABA-producing neuron progenitor cell, which has 70% or higher sequence identity to a polynucleotide
30 comprising the nucleotide sequence of positions 178 to 2280 in SEQ ID NO: 1; the nucleotide sequence of positions 127 to 2079 in SEQ ID NO: 3; the nucleotide sequence of positions 668 to 2770 in SEQ ID NO: 33; the nucleotide sequence of positions 130 to 2232 in SEQ ID NO: 35; the nucleotide sequence of positions 199 to 2100 in SEQ ID NO: 37; the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39; the nucleotide sequence of positions 15
35 to 2325 in SEQ ID NO: 41; the nucleotide sequence of positions 15 to 1916 in SEQ ID NO: 43; the nucleotide sequence of positions 199 to 1950 in SEQ ID NO: 45; the nucleotide

sequence of positions 15 to 1766 in SEQ ID NO: 47; or the nucleotide sequence of positions 196 to 2262 in SEQ ID NO: 49;

[59] the method of [58] for transplanting a GABA-producing neuron progenitor cell, wherein the GABA-producing neuron progenitor cell is a spinal cord or cerebellar GABA-producing neuron progenitor cell;

[60] the method of [58] or [59] for transplanting a GABA-producing neuron progenitor cell, wherein the polynucleotide comprises at least 10 consecutive nucleotides of a polynucleotide selected from (i), (ii), (iii), and (iv) mentioned above, or a complementary sequence thereof;

[61] the method of [58] or [59] for transplanting a GABA-producing neuron progenitor cell, wherein the polynucleotide comprises at least 15 consecutive nucleotides of a polynucleotide selected from (i), (ii), (iii), and (iv) mentioned above, or a complementary sequence thereof;

[62] a method for treating cerebellar degeneration or spinal cord injury, which comprises transplanting a GABA-producing neuron progenitor cell selected by using an antibody that binds to a protein selected from:

(v) a protein comprising the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50;

(vi) a protein that is selectively expressed in a GABA-producing neuron progenitor cell and comprises an amino acid sequence comprising an insertion, substitution, deletion of one or more amino acids in the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50, and/or addition of one or more amino acids to either or both ends thereof;

(vii) a protein that is selectively expressed in a GABA-producing neuron progenitor cell and encoded by a polynucleotide that hybridizes under stringent conditions to a polynucleotide encoding the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50; and

(viii) a protein that is selectively expressed in a GABA-producing neuron progenitor cell and comprises an amino acid sequence having 70% or higher identity to the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50;

[63] the method of [62] for transplanting a GABA-producing neuron progenitor cell, wherein the GABA-producing neuron progenitor cell is a cerebellar or spinal cord GABA-producing neuron progenitor cell;

[64] the method of [62] or [63] for transplanting a GABA-producing neuron progenitor cell, wherein the antibody binds to a polypeptide comprising the entire or at least six consecutive amino acid residues of the amino acid sequence of positions 21 to 510 in the amino acid sequence of SEQ ID NO: 2, 4, 38, 40, 42, or 44; the amino acid sequence of positions 20 to 513 in the amino acid sequence of SEQ ID NO: 34 or 36; the amino acid sequence of positions 21 to 460 in the amino acid sequence of SEQ ID NO: 46 or 48; or the amino acid sequence of

positions 21 to 490 in the amino acid sequence of SEQ ID NO: 50; and [65] the method of any one of [62] to [64] for transplanting a GABA-producing neuron progenitor cell, wherein the antibody binds to a polypeptide comprising at least six amino acid residues.

5

Brief Description of the Drawings

Fig. 1 shows the expression pattern of the 65B13 gene in the nervous system of fetal mouse. A, E12.5 sagittal section; B, E12.5 cerebellar primordium; C, E12.5 spinal cord; D, E10.5 spinal cord; E, E12.5 spinal cord; F, E14.5 spinal cord. CB, cerebellar primordium; SC, spinal cord.

10

Fig. 2A shows a developmental scheme for the dorsal spinal cord. Fig. 2B shows identification markers for various neurons.

Fig. 3 shows a comparison of the 65B13, Mash1, and Pax2 expressions in the spinal cord of E10.5 mouse.

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Fig. 4 shows a comparison of the 65B13, Corl1, and Mash1 expressions in the spinal cord of E12.5 mouse.

Fig. 5 shows differentiation of 65B13-positive cells isolated from the spinal cord of E12.5 mouse into GABA-producing dILA neurons. Fig. 5A shows a result of flow cytometry analysis using an anti-65B13 antibody; Fig. 5B shows marker staining of the isolated 65B13-positive cells after two days of culture.

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Fig. 6 shows a comparison of the 65B13, Corl2, and Pax2 expressions in the fetal cerebellar primordium. Fig. 6A shows the result of expression analysis at E12.5; Fig. 6B shows the result of expression analysis at E14.5. In Fig. 6B, the left and right photographs show the expression of 65B13 or Pax2, respectively.

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Fig. 7 shows that 65B13-positive cells could be isolated from the cerebellar primordia of fetal mouse. Fig. 7A shows results for the cerebella and 65B13-positive cells isolated from the E12.5 cerebella. Fig. 7B shows results of the cerebella and 65B13-positive cells isolated from the E14.5 cerebella.

Fig. 8 shows differentiation of 65B13-positive cells isolated from the cerebellar primordia of E12.5 (A) and E14.5 (B) mice into GABA-producing Purkinje cells and GABA-producing Golgi cells, respectively.

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Fig. 9 shows 65B13-positive cells could be isolated from a population of *in vitro* differentiated spinal cord neurons derived from ES cells and their differentiation into GABA-producing neurons.

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Fig. 10 shows the structures of DNA constructs that can be used to select GABA-producing neuron progenitor cells.

Fig. 11 shows that a foreign gene (Gsh1) could be expressed specifically in GABA-producing neuron progenitor cells by using the 65B13 promoter.

Best Mode for Carrying Out the Invention

5 The present inventors demonstrated that the 65B13 gene was selectively expressed in GABA neuron progenitor cells of spinal dorsal horn and cerebellum.

The present invention provides polynucleotides for detecting or selecting GABA-producing neuron progenitor cells.

10 The 65B13 gene of the present invention includes, for example, two types of genes named 65B13-a and 65B13-b, which are alternative isoforms of the 65B13 gene. The respective nucleotide sequences are shown in SEQ ID NOs: 1 and 3, and the amino acid sequences encoded by the genes are shown in SEQ ID NOs: 2 and 4. The coding region of 65B13-a starts with A at position 178 in SEQ ID NO: 1, and extends to the stop codon at positions 2278 to 2280, encoding a protein of 700 amino acids. The 17 amino acid residues
15 encoded by the sequence of positions 178 to 228 constitute a signal sequence, while the 17 amino acid residues encoded by the sequence of positions 1717 to 1767 constitute a transmembrane region. On the other hand, the coding region of 65B13-b starts with A at position 127 in SEQ ID NO: 2 and extends to the stop codon at positions 2277 to 2079, encoding a protein of 650 amino acids. The 17 amino acid residues encoded by the sequence
20 of positions 127 to 178 constitute a signal sequence, while the 17 amino acid residues encoded by the sequence of positions 1516 to 1566 constitute a transmembrane region. Furthermore, the 65B13 gene of the present invention also includes, for example, the genes of SEQ ID NOs: 33, 35, 37, 39, 41, 43, 45, 47, and 49. The amino acid sequences encoded by the genes are shown in SEQ ID NOs: 34, 36, 38, 40, 42, 44, 46, 48, and 50, respectively. The coding
25 region of SEQ ID NO: 33 starts with A at position 668 and extends to the stop codon at positions 2768 to 2770, encoding a protein of 700 amino acids. The 19 amino acid residues encoded by the sequence of positions 668 to 724 constitute a signal sequence, while the 494 amino acid residues encoded by the sequence of positions 725 to 2206 constitute an extracellular domain. The coding region of SEQ ID NO: 35 starts with A at position 130 and
30 extends to the stop codon at positions 2230 to 2232, encoding a protein of 700 amino acids. The 19 amino acid residues encoded by the sequence of positions 130 to 186 constitute a signal sequence, while the 494 amino acid residues encoded by the sequence of positions 187 to 1668 constitute an extracellular domain. The coding region of SEQ ID NO: 37 starts with A at position 199 and extends to the stop codon at positions 2098 to 2100, encoding a protein
35 of 633 amino acids. The 20 amino acid residues encoded by the sequence of positions 199 to 258 constitute a signal sequence, while the 490 amino acid residues encoded by the sequence

of positions 259 to 1728 constitute an extracellular domain. The coding region of SEQ ID NO: 39 starts with A at position 199 and extends to the stop codon at positions 2323 to 2325, encoding a protein of 708 amino acids. The 20 amino acid residues encoded by the sequence of positions 199 to 258 constitute a signal sequence, while the 490 amino acid residues encoded by the sequence of positions 259 to 1728 constitute an extracellular domain. The coding region of SEQ ID NO: 41 starts with A at position 199 and extends to the stop codon at positions 2323 to 2325, encoding a protein of 708 amino acids. The 20 amino acid residues encoded by the sequence of positions 199 to 258 constitute a signal sequence, while the 490 amino acid residues encoded by the sequence of positions 259 to 1728 constitute an extracellular domain. The coding region of SEQ ID NO: 43 starts with A at position 15 and extends to the stop codon at positions 1914 to 1916, encoding a protein of 633 amino acids. The 20 amino acid residues encoded by the sequence of positions 15 to 74 constitute a signal sequence, while the 490 amino acid residues encoded by the sequence of positions 75 to 1544 constitute an extracellular domain. The coding region of SEQ ID NO: 45 starts with A at position 199 and extends to the stop codon at positions 1948 to 1950, encoding a protein of 583 amino acids. The 20 amino acid residues encoded by the sequence of positions 199 to 258 constitute a signal sequence, while the 440 amino acid residues encoded by the sequence of positions 259 to 1578 constitute an extracellular domain. The coding region of SEQ ID NO: 47 starts with A at position 15 and extends to the stop codon at positions 1764 to 1766, encoding a protein of 583 amino acids. The 20 amino acid residues encoded by the sequence of positions 15 to 74 constitute a signal sequence, while the 440 amino acid residues encoded by the sequence of positions 75 to 1394 constitute an extracellular domain. The coding region of SEQ ID NO: 49 starts with A at position 196 and extends to the stop codon at positions 2260 to 2262, encoding a protein of 688 amino acids. The 20 amino acid residues encoded by the sequence of positions 196 to 255 constitute a signal sequence, while the 470 amino acid residues encoded by the sequence of positions 256 to 1665 constitute an extracellular domain. In addition, the 65B13 gene of the present invention also includes, for example, the sequences of accession numbers XM_994164, AL136654, XM_512603, XR_012248, XM_541684, and XM_583222.

In a preferred embodiment, the polynucleotides of the present invention include polynucleotides for detecting or selecting GABA-producing neuron progenitor cells, which can hybridize to a nucleotide selected from (i), (ii), (iii), and (iv) below, or to a complementary sequence thereof.

(i) a polynucleotide comprising the nucleotide sequence of positions 178 to 2280 in SEQ ID NO: 1; the nucleotide sequence of positions 127 to 2079 in SEQ ID NO: 3; the nucleotide sequence of positions 668 to 2770 in SEQ ID NO: 33; the nucleotide sequence of positions

130 to 2232 in SEQ ID NO: 35; the nucleotide sequence of positions 199 to 2100 in SEQ ID NO: 37; the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39; the nucleotide sequence of positions 15 to 2325 in SEQ ID NO: 41; the nucleotide sequence of positions 15 to 1916 in SEQ ID NO: 43; the nucleotide sequence of positions 199 to 1950 in SEQ ID NO: 45; the nucleotide sequence of positions 15 to 1766 in SEQ ID NO: 47; or the nucleotide sequence of positions 196 to 2262 in SEQ ID NO: 49;

(ii) a polynucleotide encoding a protein that is selectively expressed in a GABA-producing neuron progenitor cell and which comprises an amino acid sequence encoded by a nucleotide sequence comprising an insertion, substitution, deletion of one or more nucleotides, and/or

addition of one or more nucleotides to either or both ends thereof, in the nucleotide sequence of positions 178 to 2280 in SEQ ID NO: 1; the nucleotide sequence of positions 127 to 2079 in SEQ ID NO: 3; the nucleotide sequence of positions 668 to 2770 in SEQ ID NO: 33; the nucleotide sequence of positions 130 to 2232 in SEQ ID NO: 35; the nucleotide sequence of positions 199 to 2100 in SEQ ID NO: 37; the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39; the nucleotide sequence of positions 15 to 2325 in SEQ ID NO: 41; the nucleotide sequence of positions 15 to 1916 in SEQ ID NO: 43; the nucleotide sequence of positions 199 to 1950 in SEQ ID NO: 45; the nucleotide sequence of positions 15 to 1766 in SEQ ID NO: 47; or the nucleotide sequence of positions 196 to 2262 in SEQ ID NO: 49;

(iii) a polynucleotide encoding a protein that is selectively expressed in a GABA-producing neuron progenitor cell, which hybridizes under a stringent condition to a polynucleotide comprising the nucleotide sequence of positions 178 to 2280 in SEQ ID NO: 1; the nucleotide sequence of positions 127 to 2079 in SEQ ID NO: 3; the nucleotide sequence of positions 668 to 2770 in SEQ ID NO: 33; the nucleotide sequence of positions 130 to 2232 in SEQ ID NO: 35; the nucleotide sequence of positions 199 to 2100 in SEQ ID NO: 37; the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39; the nucleotide sequence of positions 15 to 2325 in SEQ ID NO: 41; the nucleotide sequence of positions 15 to 1916 in SEQ ID NO: 43; the nucleotide sequence of positions 199 to 1950 in SEQ ID NO: 45; the nucleotide sequence of positions 15 to 1766 in SEQ ID NO: 47; or the nucleotide sequence of positions 196 to 2262 in SEQ ID NO: 49; and

(iv) a polynucleotide encoding a protein that is selectively expressed in a GABA-producing neuron progenitor cell, which has 70% or higher sequence identity to a polynucleotide comprising the nucleotide sequence of positions 178 to 2280 in SEQ ID NO: 1; the nucleotide sequence of positions 127 to 2079 in SEQ ID NO: 3; the nucleotide sequence of positions 668 to 2770 in SEQ ID NO: 33; the nucleotide sequence of positions 130 to 2232 in SEQ ID NO: 35; the nucleotide sequence of positions 199 to 2100 in SEQ ID NO: 37; the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39; the nucleotide sequence of positions 15

to 2325 in SEQ ID NO: 41; the nucleotide sequence of positions 15 to 1916 in SEQ ID NO: 43; the nucleotide sequence of positions 199 to 1950 in SEQ ID NO: 45; the nucleotide sequence of positions 15 to 1766 in SEQ ID NO: 47; or the nucleotide sequence of positions 196 to 2262 in SEQ ID NO: 49.

5 In the present invention, a “polynucleotide” refers to a polymer comprising nucleotides or nucleotide pairs of multiple deoxyribonucleic acids (DNA) or ribonucleic acids (RNA), and includes DNA, cDNA, genomic DNA, chemically synthesized DNA, and RNA. If needed, polynucleotides can also contain non-naturally occurring nucleotides such as 4-acetylcytidine, 5-(carboxyhydroxymethyl)uridine, 2'-O-methylcytidine,
 10 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluridine, dihydrouridine, 2'-O-methylpseudouridine, β -D-galactosylqueuosine, 2'-O-methylguanosine, inosine, N6-isopentenyladenosine, 1-methyladenosine, 1-methylpseudouridine, 1-methylguanosine, 1-methylinosine, 2,2-dimethylguanosine, 2-methyladenosine, 2-methylguanosine, 3-methylcytidine, 5-methylcytidine, N6-methyladenosine,
 15 7-methylguanosine, 5-methylaminomethyluridine, 5-methoxyaminomethyl-2-thiouridine, β -D-mannosylqueuosine, 5-methoxycarbonylmethyl-2-thiouridine, 5-methoxycarbonylmethyluridine, 5-methoxyuridine, 2-methylthio-N6-isopentenyladenosine, N-((9- β -D-ribofuranosyl-2-methylthiopurin-6-yl)carbamoyl)threonine, N-((9- β -D-ribofuranosylpurin-6-yl)N-methylcarbamoyl)threonine, uridine-5-oxyacetic
 20 acid-methyl ester, uridine-5-oxyacetic acid, wybutoxosine, pseudouridine, queuosine, 2-thiocytidine, 5-methyl-2-thiouridine, 2-thiouridine, 4-thiouridine, 5-methyluridine, N-((9- β -D-ribofuranosylpurin-6-yl)carbamoyl)threonine, 2'-O-methyl-5-methyluridine, 2'-O-methyluridine, wybutosine, and 3-(3-amino-3-carboxy propyl)uridine.

The polynucleotides of the present invention can also be produced by chemical
 25 synthesis based on the known sequence of 65B13. Alternatively, such polynucleotides can be prepared from 65B13 gene-expressing cells using hybridization, PCR, etc.

Meanwhile, the phrase “functionally equivalent” means that a target protein has the same biological property as a 65B13 protein (for example, the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50). The biological property of a 65B13 protein
 30 (for example, the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50) includes, for example, the olfactory nerve network pattern. Furthermore, the selective expression in GABA neuron progenitor cells can also be regarded as a function (biological property).

Accordingly, whether a target protein has the equivalent biological property as the
 35 amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50 identified by the present inventors can be assessed by those skilled in the art, for example, by testing for the

olfactory nerve network pattern or selective expression in GABA neuron progenitor cells.

The polynucleotides of the present invention also include polynucleotides that hybridize under stringent conditions to a polynucleotide comprising the nucleotide sequence of positions 178 to 2280 in SEQ ID NO: 1, the nucleotide sequence of positions 127 to 2079 in
5 SEQ ID NO: 3, the nucleotide sequence of positions 668 to 2770 in SEQ ID NO: 33, the nucleotide sequence of positions 130 to 2232 in SEQ ID NO: 35, the nucleotide sequence of positions 199 to 2100 in SEQ ID NO: 37, the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39, the nucleotide sequence of positions 15 to 2325 in SEQ ID NO: 41, the nucleotide sequence of positions 15 to 1916 in SEQ ID NO: 43, the nucleotide sequence of
10 positions 199 to 1950 in SEQ ID NO: 45, the nucleotide sequence of positions 15 to 1766 in SEQ ID NO: 47, or the nucleotide sequence of positions 196 to 2262 in SEQ ID NO: 49, and each of which encodes a protein that is functionally equivalent (a preferred function is the selective expression in GABA-producing neuron progenitor cells) to a protein comprising the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50. The
15 polypeptides also include isoforms, alternative isoforms, and allelic mutants of 65B13.

Such polynucleotides can be obtained from cDNA libraries and genomic libraries of animals such as humans, mice, rats, rabbits, hamsters, chickens, pigs, cows, goats, sheep, monkeys, and dogs by known hybridization methods such as colony hybridization, plaque hybridization, and Southern blotting using as a probe a polynucleotide comprising the
20 nucleotide sequence of positions 1 to 2876 in SEQ ID NO: 1, the nucleotide sequence of positions 1 to 2243 in SEQ ID NO: 3, the nucleotide sequence of positions 1 to 3123 in SEQ ID NO: 33, the nucleotide sequence of positions 1 to 3247 in SEQ ID NO: 35, the nucleotide sequence of positions 1 to 2153 in SEQ ID NO: 37, the nucleotide sequence of positions 1 to 2979 in SEQ ID NO: 39, the nucleotide sequence of positions 1 to 2973 in SEQ ID NO: 41,
25 the nucleotide sequence of positions 1 to 1969 in SEQ ID NO: 43, the nucleotide sequence of positions 1 to 2003 in SEQ ID NO: 45, the nucleotide sequence of positions 1 to 1819 in SEQ ID NO: 47, or the nucleotide sequence of positions 1 to 2959 in SEQ ID NO: 49; preferably the nucleotide sequence of positions 178 to 2280 in SEQ ID NO: 1, the nucleotide sequence of positions 127 to 2079 in SEQ ID NO: 3, the nucleotide sequence of positions 668 to 2770 in
30 SEQ ID NO: 33, the nucleotide sequence of positions 130 to 2232 in SEQ ID NO: 35, the nucleotide sequence of positions 199 to 2100 in SEQ ID NO: 37, the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39, the nucleotide sequence of positions 15 to 2325 in SEQ ID NO: 41, the nucleotide sequence of positions 15 to 1916 in SEQ ID NO: 43, the nucleotide sequence of positions 199 to 1950 in SEQ ID NO: 45, the nucleotide sequence of
35 positions 15 to 1766 in SEQ ID NO: 47, or the nucleotide sequence of positions 196 to 2262 in SEQ ID NO: 49. Regarding methods for constructing cDNA libraries, one can refer to

"Molecular Cloning, A Laboratory Manual 2nd ed." (Cold Spring Harbor Press (1989)). It is also possible to use cDNA libraries and genomic libraries available on the market.

More specifically, in constructing a cDNA library, total RNA is first prepared from cells, organs, tissues, or such that express a polynucleotide of the present invention, by known techniques such as guanidine ultracentrifugation (Chirwin *et al.* Biochemistry 1979, 18:5294-5299) or AGPC (Chomczynski and Sacchi Anal. Biochem. 1987, 162:156-159), followed by mRNA purification using an mRNA purification kit (Pharmacia), etc. A kit for direct mRNA preparation, such as the QuickPrep mRNA Purification Kit (Pharmacia), may also be used. Next, cDNA is synthesized from the resulting mRNA using reverse transcriptase. cDNA synthesis kits such as the AMV Reverse Transcriptase First-strand cDNA Synthesis Kit (Seikagaku Corporation) are also available commercially. Other methods that use the 5'-RACE method to synthesize and amplify cDNA by PCR may also be used (Frohman *et al.* Proc. Natl. Acad. Sci. USA 1988, 85:8998-9002; Belyavsky *et al.* Nucleic Acids Res. 1989, 17:2919-32). In addition, in order to construct cDNA libraries containing a high percentage of full-length clones, known techniques such as the oligo-capping method (Maruyama and Sugano. Gene 1994, 138:171-4; Suzuki. Gene 1997, 200:149-56) can also be employed. The cDNA obtained in this manner is then incorporated into a suitable vector.

Examples of hybridization conditions in the present invention include "2x SSC, 0.1% SDS, 50°C", "2x SSC, 0.1% SDS, 42°C", and "1x SSC, 0.1% SDS, 37°C". Examples of conditions of higher stringency include "2x SSC, 0.1% SDS, 65°C", "0.5x SSC, 0.1% SDS, 42°C", and "0.2x SSC, 0.1% SDS, 65°C". More specifically, a method that uses the Rapid-hyb buffer (Amersham Life Science) can be carried out by performing pre-hybridization at 68°C for 30 minutes or more, adding a probe to allow hybrid formation at 68°C for one hour or more, washing three times in 2x SSC, 0.1% SDS at room temperature for 20 minutes each, washing three times in 1x SSC, 0.1% SDS at 37°C for 20 minutes each, and finally washing twice in 1x SSC, 0.1% SDS at 50°C for 20 minutes each. This can also be carried out using, for example, the Expresshyb Hybridization Solution (CLONTECH), by performing pre-hybridization at 55°C for 30 minutes or more, adding a labeled probe and incubating at 37°C to 55°C for one hour or more, washing three times in 2x SSC, 0.1% SDS at room temperature for 20 minutes each, and washing once at 37°C for 20 minutes with 1x SSC, 0.1% SDS. Here, conditions of higher stringency can be achieved by increasing the temperature for pre-hybridization, hybridization, or second wash. For example, the pre-hybridization and hybridization temperature can be raised to 60°C, and to 68°C for higher stringency. In addition to conditions such as salt concentration of the buffer and temperature, a person with ordinary skill in the art can also integrate other conditions such as probe

concentration, probe length, and reaction time, to obtain isoforms of 65B13 of the present invention, allelic mutants, and corresponding genes derived from other organisms.

References such as "Molecular Cloning, A Laboratory Manual 2nd ed." (Cold Spring Harbor Press (1989), Sections 9.47-9.58), "Current Protocols in Molecular Biology" (John Wiley & Sons (1987-1997), Sections 6.3-6.4), "DNA Cloning 1: Core Techniques, A Practical Approach 2nd ed." (Oxford University (1995), Section 2.10 for conditions in particular) can be referred to for detailed information on hybridization procedures. Examples of hybridizing polynucleotides include polynucleotides containing a nucleotide sequence that has at least 50% or more, preferably 70%, more preferably 80%, and even more preferably 90% (for example, 95% or more, or 99%) identity with a nucleotide sequence comprising the nucleotide sequence of positions 178 to 2280 in SEQ ID NO: 1, the nucleotide sequence of positions 127 to 2079 in SEQ ID NO: 3, the nucleotide sequence of positions 668 to 2770 in SEQ ID NO: 33, the nucleotide sequence of positions 130 to 2232 in SEQ ID NO: 35, the nucleotide sequence of positions 199 to 2100 in SEQ ID NO: 37, the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39, the nucleotide sequence of positions 15 to 2325 in SEQ ID NO: 41, the nucleotide sequence of positions 15 to 1916 in SEQ ID NO: 43, the nucleotide sequence of positions 199 to 1950 in SEQ ID NO: 45, the nucleotide sequence of positions 15 to 1766 in SEQ ID NO: 47, or the nucleotide sequence of positions 196 to 2262 in SEQ ID NO: 49. Such identities can be determined by the BLAST algorithm (Altschul. Proc. Natl. Acad. Sci. USA 1990, 87:2264-8; Karlin and Altschul. Proc. Natl. Acad. Sci. USA 1993, 90:5873-7). Examples of programs that have been developed based on this algorithm include the BLASTX program for determining the identity of amino acid sequences, and the BLASTN program for nucleotide sequences (Altschul *et al.* J. Mol. Biol. 1990, 215:403-10). These programs can be used for the sequences of the present invention.

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The 65B13 isoforms or allelic mutants, and other genes with a 65B13-like structure and function can be obtained from cDNA libraries and genome libraries of animals such as humans, mice, rats, rabbits, hamsters, chickens, pigs, cows, goats, sheep, monkeys, and dogs by designing primers based on the nucleotide sequence of positions 178 to 2280 in SEQ ID NO: 1, the nucleotide sequence of positions 127 to 2079 in SEQ ID NO: 3, the nucleotide sequence of positions 668 to 2770 in SEQ ID NO: 33, the nucleotide sequence of positions 130 to 2232 in SEQ ID NO: 35, the nucleotide sequence of positions 199 to 2100 in SEQ ID NO: 37, the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39, the nucleotide sequence of positions 15 to 2325 in SEQ ID NO: 41, the nucleotide sequence of positions 15 to 1916 in SEQ ID NO: 43, the nucleotide sequence of positions 199 to 1950 in SEQ ID NO: 45, the nucleotide sequence of positions 15 to 1766 in SEQ ID NO: 47, or the nucleotide

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sequence of positions 196 to 2262 in SEQ ID NO: 49, using gene amplification technology (PCR) (Current Protocols in Molecular Biology (John Wiley & Sons (1987), Sections 6.1-6.4)).

The polynucleotide sequences of the present invention can be confirmed by using conventional sequence determination methods. For example, the dideoxynucleotide chain termination method (Sanger *et al.* Proc. Natl. Acad. Sci. USA 1977, 74:5463) can be used. In addition, sequences can also be analyzed using a suitable DNA sequencer.

A "protein" in the present invention can also be referred to as a "polypeptide" in general. A "polypeptide" of the present invention refers to a peptide polymer encoded by a polynucleotide of the present invention. Preferred examples include proteins having the amino acid sequence described in SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50. The polypeptides of the present invention may comprise naturally occurring or modified amino acid residues. Examples of amino acid residue modifications include acylation, acetylation, amidation, arginylation, GPI anchor formation, crosslinking, γ -carboxylation, cyclization, covalent crosslink formation, glycosylation, oxidation, covalent bonding of a lipid or fat derivative, cystine formation, disulfide bond formation, selenoylation, demethylation, protein fragmentation treatment, covalent bonding of a nucleotide or nucleotide derivative, hydroxylation, pyroglutamate formation, covalent bonding of a flavin, prenylation, covalent bonding with a heme portion, covalent bonding of phosphatidyl inositol, formylation, myristoylation, methylation, ubiquitination, iodination, racemization, ADP-ribosylation, sulfation, and phosphorylation. Moreover, the polypeptides of the present invention include precursors containing a signal peptide portion, mature proteins lacking a signal peptide portion, and fusion proteins modified with other peptide sequences. Peptide sequences to be added to a polypeptide of the present invention can be selected from sequences that facilitate protein purification using, for example, pcDNA3.1/Myc-His vector (Invitrogen), or those that confer stability in recombinant protein production. Examples of such sequences are influenza agglutinin (HA), glutathione S transferase (GST), substance P, multiple histidine tag (such as 6x His and 10x His), protein C fragment, maltose-binding protein (MBP), immunoglobulin constant region, α -tubulin fragment, β -galactosidase, B-tag, c-myc fragment, E-tag (epitope on a monoclonal phage), FLAG (Hopp *et al.* Bio/Technol. 1988, 6:1204-10), Ick tag, p18 HIV fragment, HSV-tag (human simple Herpes virus glycoprotein), SV40T antigen fragment, T7-tag (T7 gene 10 protein), and VSV-GP fragment (vesicular stomatitis virus glycoprotein).

The GABA-producing neuron progenitor cells of the present invention preferably include, but are not limited to, GABA-producing neuron progenitor cells of the spinal cord and cerebellum.

The length of a polynucleotide of the present invention is not particularly limited, as

long as it allows for detection or selection of GABA-producing neuron progenitor cells. The polynucleotides of the present invention also include the so-called "oligonucleotides". In general, the polynucleotides of the present invention comprise at least ten consecutive nucleotides in the nucleotide sequence of the present invention or a complementary sequence thereof, and preferably comprise at least 15 consecutive nucleotides.

Furthermore, the present invention also provides nucleotide chains complementary to a polynucleotide for detecting or selecting GABA-producing neuron progenitor cells of the present invention, which comprise at least 15 consecutive nucleotides. Such polynucleotides comprising a nucleotide sequence that contains at least 15 consecutive nucleotides are useful as probes for detecting the generation of GABA-producing neuron progenitor cells or as primers for detecting GABA-producing neuron progenitor cells.

The nucleotide chain normally consists of 15 to 100, and preferably 15 to 35 nucleotides and the polynucleotide is appropriately labeled with a radioisotope, non-radioactive compound, or the like when used as a probe. The nucleotide chain preferably consists of at least 15 and preferably 30 nucleotides when used as a primer. A primer can be designed to have a restriction enzyme recognition sequence, a tag or such, added to the 5'-end side thereof, and at the 3' end, a sequence complementary to a target sequence. A nucleotide chain of the present invention can hybridize with a polynucleotide of the present invention. Moreover, mutations of a polynucleotide of the present invention within cells can be detected using these probes or primers. In some cases, such mutations may cause abnormalities in the activity or expression of the polypeptides of the present invention; therefore, nucleotide chains of the present invention are thought to be useful for disease diagnosis, etc.

Here, a "complementary sequence" refers to not only cases where at least 15 consecutive nucleotides of the nucleotide sequence completely pair with the template, but also includes those that have at least 70%, preferably 80%, more preferably 90%, and even more preferably 95% or more (for example, 97% or 99%) of the consecutive nucleotides paired with the template. Pair formation refers to the formation of a chain, in which T (U in the case of an RNA) corresponds to A, A corresponds to T or U, G corresponds to C, and C corresponds to G in the nucleotide sequence of the template polynucleotide. Identities can be determined by methods similar to that used in the aforementioned polynucleotide hybridization.

The present invention also provides primer sets comprising two or more polynucleotides for detecting or selecting GABA-producing neuron progenitor cells of the present invention.

The present invention also provides antibodies that bind to the translation products of the 65B13 gene, which are used in regeneration medicine to treat cerebellar degeneration or

spinal cord injury. In a preferred embodiment, the present invention provides antibodies for detecting or selecting GABA-producing neuron progenitor cells, which bind to, for example, a protein selected from:

(v) a protein comprising the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44,
5 46, 48, or 50;

(vi) a protein comprising an amino acid sequence that comprises an insertion, substitution, deletion of one or more amino acids in the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50, and/or addition of one or more amino acids to either or both ends thereof, which is functionally equivalent to a protein comprising the amino acid sequence of
10 SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50;

(vii) a protein encoded by a polynucleotide that hybridizes under stringent conditions to a polynucleotide encoding the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50, which is functionally equivalent to a protein comprising the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50; and

15 (viii) a protein comprising an amino acid sequence having 70% or higher identity to the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50, which comprises the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50.

Herein, "equivalent function to a protein comprising the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50" preferably refers to the selective expression
20 in GABA-producing neuron progenitor cells.

Furthermore, in a preferred embodiment, the antibodies bind to a polypeptide of the present invention which is an extracellular domain of GABA-producing neuron progenitor cells. The extracellular domains of polypeptides used in the present invention can be searched using the program, PSORT. Specifically,

25 extracellular domains obtained using the PSORT program are: the amino acid sequences of positions 21 to 510 in the amino acid sequence of SEQ ID NO: 2, 4, 38, 40, 42, or 44; of positions 20 to 513 in the amino acid sequence of SEQ ID NO: 34 or 36; of positions 21 to 460 in the amino acid sequence of SEQ ID NO: 46 or 48; and of positions 21 to 490 in the amino acid sequence of SEQ ID NO: 50.

30 It is well known that a mutant polypeptide comprising an amino acid sequence, in which one or more amino acids are deleted, inserted, substituted, or added, maintains the same biological activity as the original polypeptide (Mark *et al.* Proc. Natl. Acad. Sci. USA 1984, 81:5662-6; Zoller and Smith. Nucleic Acids Res. 1982, 10:6487-500; Wang *et al.* Science 1984, 224:1431-3; Dalbadie-McFarland *et al.* Proc. Natl. Acad. Sci. USA 1982, 79:6409-13).

35 Here, an amino acid substitution refers to a mutation in which one or more amino acid residues in a sequence are changed to a different type of amino acid residue. When the

amino acid sequence encoded by a polynucleotide of the present invention is altered by such a substitution, a conservative substitution is preferably carried out if the function of the protein is to be maintained. A conservative substitution means altering a sequence so that it encodes an amino acid that has properties similar to those of the amino acid before substitution.

5 Amino acids can be classified, based on their properties, into non-polar amino acids (Ala, Ile, Leu, Met, Phe, Pro, Trp, Val), non-charged amino acids (Asn, Cys, Gln, Gly, Ser, Thr, Tyr), acidic amino acids (Asp, Glu), basic amino acids (Arg, His, Lys), neutral amino acids (Ala, Asn, Cys, Gln, Gly, Ile, Leu, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val), aliphatic amino acids (Ala, Gly), branched amino acids (Ile, Leu, Val), hydroxyamino acids (Ser, Thr), amide-type amino
10 acids (Gln, Asn), sulfur-containing amino acids (Cys, Met), aromatic amino acids (His, Phe, Trp, Tyr), heterocyclic amino acids (His, Trp), imino acids (Pro, 4Hyp), etc. In particular, substitutions among Ala, Val, Leu, and Ile; Ser and Thr; Asp and Glu; Asn and Gln; Lys and Arg; and Phe and Tyr are preferable in order to maintain protein properties. There are no particular limitations on the number and sites of the mutated amino acids, as long as the amino
15 acid encoded by the polynucleotide has 65B13 antigenicity.

A polynucleotide encoding an amino acid sequence, in which one or more amino acids are deleted, inserted, substituted, or added to the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50, can be prepared according to methods such as site-directed mutagenesis described in "Molecular Cloning, A Laboratory Manual 2nd ed." (Cold Spring Harbor Press (1989)), "Current Protocols in Molecular Biology" (John Wiley & Sons (1987-1997), Sections 8.1-8.5), Hashimoto-Goto *et al.* (Gene 1995, 152:271-5), Kunkel (Proc. Natl. Acad. Sci. USA 1985, 82:488-92), Kramer and Fritz (Method. Enzymol. 1987, 154:350-67), Kunkel (Method. Enzymol. 1988, 85:2763-6), etc.
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The above-described proteins of the present invention also include proteins encoded
25 by polynucleotides that hybridize under stringent conditions to a polynucleotide encoding the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50, which are functionally equivalent to a protein comprising the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50.

"Equivalent function to a protein comprising the amino acid sequence of SEQ ID NO:
30 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50" preferably refers to the selective expression in GABA-producing neuron progenitor cells.

Examples of hybridization conditions in the present invention include "2x SSC, 0.1% SDS, 50°C", "2x SSC, 0.1% SDS, 42°C", and "1x SSC, 0.1% SDS, 37°C". Examples of conditions of higher stringency include "2x SSC, 0.1% SDS, 65°C", "0.5x SSC, 0.1% SDS,
35 42°C", and "0.2x SSC, 0.1% SDS, 65°C". More specifically, a method that uses the Rapid-hybTM buffer (Amersham Life Science) can be carried out by performing

pre-hybridization at 68°C for 30 minutes or more, adding a probe to allow hybrid formation at 68°C for one hour or more, washing three times in 2x SSC, 0.1% SDS at room temperature for 20 minutes each, washing three times in 1x SSC, 0.1% SDS at 37°C for 20 minutes each, and finally washing twice in 1x SSC, 0.1% SDS at 50°C for 20 minutes each. This can also be
5 carried out using, for example, the Expresshyb Hybridization Solution (CLONTECH), by performing pre-hybridization at 55°C for 30 minutes or more, adding a labeled probe and incubating at 37°C to 55°C for one hour or more, washing three times in 2x SSC, 0.1% SDS at room temperature for 20 minutes each, and washing once at 37°C for 20 minutes with 1x SSC, 0.1% SDS. Here, conditions of higher stringency can be achieved by increasing the
10 temperature for pre-hybridization, hybridization, or second wash. For example, the pre-hybridization and hybridization temperature can be raised to 60°C, and to 68°C for higher stringency. In addition to conditions such as salt concentration of the buffer and temperature, a person with ordinary skill in the art can also integrate other conditions such as probe concentration, probe length, and reaction time, to obtain 65B13 isoforms, allelic mutants, and
15 corresponding genes derived from other organisms.

References such as "Molecular Cloning, A Laboratory Manual 2nd ed." (Cold Spring Harbor Press (1989), Sections 9.47-9.58), "Current Protocols in Molecular Biology" (John Wiley & Sons (1987-1997), Sections 6.3-6.4), "DNA Cloning 1: Core Techniques, A Practical Approach 2nd ed." (Oxford University (1995), Section 2.10 for conditions) can be referred to
20 for detailed information on hybridization procedures. Examples of hybridizing polynucleotides include polynucleotides containing a nucleotide sequence that has at least 50% or more, preferably 70%, more preferably 80%, and even more preferably 90% (for example, 95% or more, or 99%) identity with a polynucleotide encoding the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50. Such identities can be
25 determined by the BLAST algorithm (Altschul. Proc. Natl. Acad. Sci. USA 1990, 87:2264-8; Karlin and Altschul. Proc. Natl. Acad. Sci. USA 1993, 90:5873-7). Examples of programs that have been developed based on this algorithm include the BLASTX program for determining the identity of amino acid sequences, and the BLASTN program for nucleotide sequences (Altschul *et al.* J. Mol. Biol. 1990, 215:403-10). These programs can be used for
30 the sequences of the present invention. One can refer to, for example, <http://www.ncbi.nlm.nih.gov> for a specific example of analysis methods.

The 65B13 isoforms or allelic mutants, and other genes with a 65B13-like structure or function can be obtained from cDNA libraries and genome libraries of animals such as humans, mice, rats, rabbits, hamsters, chickens, pigs, cows, goats, sheep, monkeys, and dogs by
35 designing primers based on the nucleotide sequence of positions 178 to 2280 in SEQ ID NO: 1, the nucleotide sequence of positions 127 to 2079 in SEQ ID NO: 3, the nucleotide sequence of

positions 668 to 2770 in SEQ ID NO: 33, the nucleotide sequence of positions 130 to 2232 in SEQ ID NO: 35, the nucleotide sequence of positions 199 to 2100 in SEQ ID NO: 37, the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39, the nucleotide sequence of positions 15 to 2325 in SEQ ID NO: 41, the nucleotide sequence of positions 15 to 1916 in SEQ ID NO: 43, the nucleotide sequence of positions 199 to 1950 in SEQ ID NO: 45, the nucleotide sequence of positions 15 to 1766 in SEQ ID NO: 47, or the nucleotide sequence of positions 196 to 2262 in SEQ ID NO: 49, using gene amplification technology (PCR) (Current Protocols in Molecular Biology (John Wiley & Sons (1987), Sections 6.1-6.4)).

Antibodies of the present invention also include polyclonal antibodies, monoclonal antibodies, chimeric antibodies, single-chain antibodies (scFV) (Huston *et al.* Proc. Natl. Acad. Sci. USA 1988, 85:5879-83; The Pharmacology of Monoclonal Antibody, vol.113, Rosenberg and Moore ed., Springer Verlag (1994) pp.269-315), humanized antibodies, multispecific antibodies (LeDoussal *et al.* Int. J. Cancer Suppl. 1992, 7:58-62; Paulus. Behring Inst. Mitt. 1985, 78:118-32; Millstein and Cuello. Nature 1983, 305:537-9; Zimmermann Rev. Physiol. Biochem. Pharmacol. 1986, 105:176-260; Van Dijk *et al.* Int. J. Cancer 1989, 43:944-9), and antibody fragments such as Fab, Fab', F(ab')₂, Fc, and Fv. Moreover, an antibody of the present invention may also be modified by PEG and such, as necessary. An antibody of the present invention may also be produced in the form of a fusion protein with β -galactosidase, maltose-binding protein, GST, green fluorescent protein (GFP), or such, to allow detection without the use of a secondary antibody. In addition, an antibody may be modified by labeling with biotin or such to allow recovery using avidin, streptoavidin, etc.

An antibody of the present invention can be produced using a polypeptide of the present invention, a fragment thereof, or a cell in which a polypeptide or polypeptide fragment of the present invention is expressed, as a sensitized antigen. In addition, a short polypeptide of the present invention or a fragment thereof may also be used as an immunogen by coupling to a carrier such as bovine serum albumin, Keyhole Limpet Hemocyanin, and ovalbumin. In addition, a polypeptide of the present invention or a fragment thereof may be used in combination with a known adjuvant such as aluminum adjuvant, Freund's complete (or incomplete) adjuvant, or pertussis adjuvant, to enhance the immune response to an antigen.

Polyclonal antibodies can be obtained from, for example, the serum of an immunized animal after immunizing a mammal with a polypeptide of the present invention or a fragment thereof, together with an adjuvant as necessary. Although there are no particular limitations on the mammals used, typical examples include rodents, lagomorphs, and primates. Specific examples include rodents such as mice, rats, and hamsters; lagomorphs such as rabbits; and primates such as monkeys including cynomolgus monkeys, rhesus monkeys, baboons, and chimpanzees. Animal immunization is carried out by suitably diluting and suspending a

sensitized antigen in phosphate-buffered saline (PBS) or physiological saline, mixing with an adjuvant as necessary until emulsified, and injecting into an animal intraperitoneally or subcutaneously. The sensitized antigen mixed with Freund's incomplete adjuvant is preferably administered several times, every 4 to 21 days. Antibody production can be confirmed by measuring the level of an antibody of interest in the serum using conventional methods. Finally, the serum itself may be used as a polyclonal antibody, or it may be further purified. See, for example, "Current Protocols in Molecular Biology" (John Wiley & Sons (1987), Sections 11.12-11.13), for specific methods.

A monoclonal antibody can be produced by removing the spleen from an animal immunized in the manner described above, separating immunocytes from the spleen, and fusing with a suitable myeloma cell using polyethylene glycol (PEG) or such to establish hybridomas. Cell fusion can be carried out according to the Milstein method (Galfre and Milstein. *Methods Enzymol.* 1981, 73:3-46). Here, suitable myeloma cells are exemplified particularly by cells that allow chemical selection of fused cells. When using such myeloma cells, fused hybridomas are selected by culturing in a culture medium (HAT culture medium) that contains hypoxanthine, aminopterin, and thymidine, which destroy cells other than the fused cells. Next, a clone that produces an antibody against a polypeptide of the present invention or a fragment thereof is selected from the established hybridomas. Subsequently, the selected clone is introduced into the abdominal cavity of a mouse or such, and ascite is collected to obtain a monoclonal antibody. See, in addition, "Current Protocols in Molecular Biology" (John Wiley & Sons (1987), Sections 11.4-11.11) for information on specific methods.

Hybridomas can also be obtained by first sensitizing human lymphocytes that have been infected by EB virus with an immunogen *in vitro*, and fusing the sensitized lymphocytes with human myeloma cells (such as U266) to obtain hybridomas that produce human antibodies (Japanese Patent Application Kokai Publication No. (JP-A) S63-17688 (unexamined, published Japanese patent application)). In addition, human antibodies can also be obtained by using antibody-producing cells generated by sensitizing a transgenic animal with a human antibody gene repertoire (WO92/03918; WO93/02227; WO94/02602; WO94/25585; WO96/33735; WO96/34096; Mendez *et al.* *Nat. Genet.* 1997, 15:146-156, etc.). Methods that do not use hybridomas can be exemplified by a method in which a cancer gene is introduced to immortalize immunocytes such as antibody-producing lymphocytes.

In addition, antibodies can also be produced by genetic recombination techniques (see Borrebaeck and Larrick (1990) *Therapeutic Monoclonal Antibodies*, MacMillan Publishers Ltd., UK). First, a gene that encodes an antibody is cloned from hybridomas or antibody-producing cells (such as sensitized lymphocytes). The resulting gene is then

inserted into a suitable vector, the vector is introduced into a host, and the host is then cultured to produce the antibody. This type of recombinant antibody is also included in the antibodies of the present invention. Typical examples of recombinant antibodies include chimeric antibodies comprising a non-human antibody-derived variable region and a human
 5 antibody-derived constant region, and humanized antibodies comprising a non-human-derived antibody complementarity determining region (CDR), human antibody-derived framework region (FR), and human antibody constant region (Jones *et al.* Nature 1986, 321:522-5; Reichmann *et al.* Nature 1988, 332: 323-9; Presta. Curr. Op. Struct. Biol. 1992, 2:593-6; Methods Enzymol. 1991, 203:99-121).

10 Antibody fragments of the present invention can be produced by treating the aforementioned polyclonal or monoclonal antibodies with enzymes such as papain or pepsin. Alternatively, an antibody fragment can be produced by genetic engineering techniques using a gene that encodes an antibody fragment (see Co *et al.* J. Immunol. 1994, 152:2968-76; Better and Horwitz. Methods Enzymol. 1989, 178:476-96; Pluckthun and Skerra. Methods
 15 Enzymol. 1989, 178:497-515; Lamoyi. Methods Enzymol. 1986, 121:652-63; Rousseaux *et al.* 1986, 121:663-9; Bird and Walker. Trends Biotechnol. 1991, 9:132-7).

The multispecific antibodies of the present invention include bispecific antibodies (BsAb), diabodies (Db), etc. Multispecific antibodies can be produced by methods such as (1) chemically coupling antibodies having different specificities with different types of
 20 bifunctional linkers (Paulus Behring Inst. Mill. 1985, 78:118-32), (2) fusing hybridomas that secrete different monoclonal antibodies (Millstein and Cuello. Nature 1983, 305:537-9), or (3) transfecting eukaryotic cell expression systems, such as mouse myeloma cells, with a light chain gene and a heavy chain gene of different polyclonal antibodies (four types of DNA), followed by the isolation of a bispecific monovalent portion (Zimmermann. Rev. Physio.
 25 Biochem. Pharmacol. 1986, 105:176-260; Van Dijk *et al.* Int. J. Cancer 1989, 43:944-9). On the other hand, diabodies are dimer antibody fragments comprising two bivalent polypeptide chains that can be constructed by gene fusion. They can be produced using known methods (see Holliger *et al.* Proc. Natl. Acad. Sci. USA 1993, 90:6444-8; EP404097; WO93/11161).

Recovery and purification of antibodies and antibody fragments can be carried out
 30 using Protein A and Protein G, or according to known protein purification techniques (Antibodies: A Laboratory Manual, Ed. Harlow and David Lane, Cold Spring Harbor Laboratory (1988)). For example, when using Protein A to purify an antibody of the present invention, known Protein A columns such as Hyper DTM, POROSTM, or Sepharose F.F. (Pharmacia) can be used. The concentration of the resulting antibody can be determined by measuring the
 35 absorbance or by enzyme linked immunoabsorbent assay (ELISA).

Antigen-binding activity of an antibody can be determined by absorbance

measurement, or by using fluorescent antibody methods, enzyme immunoassay (EIA) methods, radioimmunoassay (RIA) methods, or ELISA. When ELISA is used, an antibody of the present invention is first immobilized onto a support such as a plate. A polypeptide of the present invention is added, and then a sample containing the antibody of interest is added.

5 Here, samples containing an antibody of interest include, for example, culture supernatants of antibody-producing cells, purified antibodies, etc. Next, a secondary antibody that recognizes an antibody of the present invention is added, followed by the incubation of the plate. Subsequently, the plate is washed and the label attached to the secondary antibody is detected. Namely, if a secondary antibody is labeled with alkaline phosphatase, the antigen
10 binding activity can be determined by adding an enzyme substrate such as p-nitrophenyl phosphate, and measuring the absorbance. In addition, a commercially available system such as BIAcore (Pharmacia) can also be used to evaluate antibody activities.

The antibodies of the present invention can recognize or detect a polypeptide of the present invention or a fragment thereof. Furthermore, since the antibodies recognize a
15 polypeptide of the present invention or a fragment thereof, they can recognize or detect cells or the like expressing the polypeptide or a fragment thereof. In addition, the antibodies can be used to purify a polypeptide of the present invention or a fragment thereof. The antibodies can also be used to purify cells or the like expressing the polypeptide of the present invention or a fragment thereof.

20 The antibodies of the present invention preferably bind to a polypeptide comprising the entire or at least six consecutive amino acid residues of the amino acid sequence of positions 21 to 510 in the amino acid sequence of SEQ ID NO: 2, 4, 38, 40, 42, or 44; the amino acid sequence of positions 20 to 513 in the amino acid sequence of SEQ ID NO: 34 or 36; the amino acid sequence of positions 21 to 460 in the amino acid sequence of SEQ ID NO:
25 46 or 48; or the amino acid sequence of positions 21 to 490 in the amino acid sequence of SEQ ID NO: 50, and more preferably bind to a polypeptide comprising at least six consecutive amino acid residues of the above amino acid sequence.

Furthermore, the present invention relates to methods for detecting or selecting GABA-producing neuron progenitor cells, which comprise detecting the expression of the
30 above-described polynucleotides of the present invention.

In a preferred embodiment, the methods of the present invention comprise the step of detecting the expression of a polynucleotide that can hybridize to a polynucleotide of the present invention selected from (i), (ii), (iii), and (iv) described above, or to a complementary sequence thereof.

35 The “step of detecting the expression of a polynucleotide” in the above-described methods of the present invention preferably comprises the steps of:

(a) contacting a test cell sample with a polynucleotide that can hybridize to a polynucleotide selected by the above-described methods of the present invention or to a complementary sequence thereof, or with a probe comprising the polynucleotide; and
(b) detecting reactivity.

5 Alternatively, in a preferred embodiment of the methods of the present invention, first, the test cell sample is contacted with a polynucleotide of the present invention or with a probe comprising the polynucleotide. For example, it is possible to contact such a probe with mRNA prepared from the test cell sample or with a complementary DNA (cDNA) transcribed from the mRNA.

10 The absence and presence of reactivity is then determined in these methods. Herein, the presence of reactivity generally means that the contacted polynucleotide hybridizes (reacts) with the target sequence.

 The steps of the above-described methods of the present invention may include, for example, the steps of:

15 (a-1) conducting gene amplification using as a template a polynucleotide derived from the test cell sample, and polynucleotides of the present invention or primers comprising the polynucleotides of the present invention, or a set of primers comprising the polynucleotides of the present invention; and
(b-1) detecting the resulting amplification products.

20 The “gene amplification method” in the above step includes known methods, for example, PCR. Furthermore, the amplification products generated by the amplification method can also be detected by known methods.

 Meanwhile, for example, mRNA prepared from a test cell sample or complementary DNA (cDNA) transcribed from the mRNA may be used as a template in step (a-1) described
25 above.

 The detection step may be followed by the step of isolating GABA-producing neuron progenitor cells from the detected sample.

 Since the protein encoded by the 65B13 gene of the present invention is a membrane protein, viable GABA-producing neuron progenitor cells can be isolated (separate) by using
30 the protein as an indicator.

 In addition, the methods of the present invention may comprise, in addition to the above-described step, the step of detecting or selecting GABA-producing neuron progenitor cells using the expression of a gene selected from the group consisting of Corl1, Pax2, Lim1/2, Lbx1, and Corl2 genes as an indicator.

35 In a preferred embodiment of the present invention, the methods include methods comprising the step of detecting a protein selected from (v), (vi), (vii), and (viii) described

above.

Specifically, the protein detection step comprises the steps of:

- (d) contacting a test cell sample with an antibody that binds to a protein selected through the above-described step of detecting or selecting GABA-producing neuron progenitor cells; and
- 5 (e) detecting reactivity.

The proteins of the present invention can be detected by contacting an antibody of the present invention with cell samples that may contain GABA-producing neuron progenitor cells, and detecting reactivity. The antibody may be immobilized onto appropriate carriers for use before contact with the cells. Alternatively, cells bound to the antibody can be
10 selectively collected through affinity purification using the antibody after contacting and binding the cells with the antibody. For example, when an antibody of the present invention is linked to biotin, the cells can be purified by adding the cell sample to a plate or column immobilized with avidin or streptavidin.

The detection step may be followed by the step of isolating GABA-producing neuron
15 progenitor cells from the detected sample. In the present invention, GABA-producing neuron progenitor cells can be efficiently separated by flow cytometry using an anti-65B13 antibody.

Alternatively, GABA-producing neuron progenitor cells can also be selected using a 65B13 promoter (including modified promoters) (see, for example, JP-A No. 2002-51775).
20 For example, it is possible to transfect cells with a vector carrying a construct in which a gene encoding a detectable marker such as GFP is linked to a promoter portion obtained by analyzing the 65B13 expression region (analysis of the 65B13 expression region is described later). The construct may have a structure where the 65B13 gene is linked upstream or downstream of the marker gene under the control of the expression regulatory sequence
25 (including promoters, enhancers, etc.). Alternatively, the marker gene can be knocked-in at the 65B13 locus. In a preferred embodiment, the construct includes, for example, constructs having any one of Structures 2 to 4 schematically illustrated in Fig. 10. In any case, expression of the marker gene is detected specifically in GABA-producing neuron progenitor cells, and this enables specific cell detection.

30 The cell samples used in the methods are culture media containing *in vitro* differentiated GABA-producing neurons. GABA-producing neurons can be differentiated *in vitro* by known methods, using known ES cells or the like as a starting material. In general, GABA-producing neurons can be differentiated by co-culturing nerve tissue-derived supporting cell layer with brain tissues obtained from an area containing GABA-producing
35 neurons. The cell sample used for selection of GABA-producing neuron progenitor cells of the present invention may be a group of cells separated or cultured by any method.

In addition, it is necessary that a support used in immobilizing an antibody or a polypeptide of the present invention is safe to cells. Examples of such a support include synthetic or naturally occurring organic polymer compounds, inorganic materials such as glass beads, silica gel, alumina, and activated charcoal, and those that have their surfaces coated with a polysaccharide or synthetic polymer. There are no particular limitations on the form of the support, examples of which include films, fibers, granules, hollow fibers, non-woven fabric, porous supports, or honeycombed supports, and the contact surface area can be controlled by changing its thickness, surface area, width, length, shape, and size in various ways.

Marker proteins for GABA-producing neuron progenitor cells other than the proteins selected by the methods of the present invention include, for example, proteins encoded by genes selected from the group consisting of the Corl1, Pax2, Lim1/2, Lbx1, and Corl2 genes.

In the methods of the present invention, a transcript of the 65B13 gene can be detected by contacting a polynucleotide of the present invention with nucleic acid extract derived from a cell sample, and detecting for nucleic acid that hybridizes to the polynucleotide in the nucleic acid extract.

The polynucleotide probe is preferably labeled with radioisotope or non-radioactive compound to detect a transcript of the 65B13 gene. Such radioisotopes to be used as a label include, for example, ^{35}S and ^3H . When a radiolabeled polynucleotide probe is used, RNA that binds to a marker can be detected by detecting silver particles by emulsion autoradiography. Meanwhile, as for conventional non-radioisotopic compounds that are used to label polynucleotide probes include biotin and digoxigenin are known. The detection of biotin-labeled markers can be achieved, for example, using fluorescent labeled avidin or avidin labeled with an enzyme such as alkaline phosphatase or horseradish peroxidase. On the other hand, the detection of digoxigenin-labeled markers can be achieved by using fluorescent labeled anti-digoxigenin antibody or anti-digoxigenin antibody labeled with an enzyme such as alkaline phosphatase or horseradish peroxidase. When enzyme labeling is used, the detection can be made by allowing stable dye to deposit at marker positions by incubating with an enzyme substrate.

When polynucleotide primers are used for detection of a transcript of the 65B13 gene, 65B13 gene transcripts can be detected by amplifying nucleic acid that hybridizes to the polynucleotide primers, for example, using techniques such as RT-PCR.

The detection of translation products of the 65B13 gene with the methods of the present invention can be made by contacting the antibody described above with protein extract of cell samples and then detecting proteins bound to the antibody. As described above, assay methods for antigen binding activities of antibodies include absorbance measurement,

fluorescent antibody method, enzyme immunoassay (EIA), radioimmunoassay (RIA), ELISA, etc.

In the context of the present invention, highly accurate identification can be achieved by detecting, in addition to a transcript or translation product of the 65B13 gene, the
5 transcripts or translation products of one or more genes selected from the group consisting of Lbx1, Pax2, Lim1/2, Corl1, and Corl2. Such methods are also included in the present invention.

Furthermore, the present invention provides kits for detecting or selecting
10 GABA-producing neuron progenitor cells. The kits of the present invention may comprise, for example, probes, primers, or primer sets that enable detection of the expression of a polynucleotide that can hybridize to a polynucleotide selected from (i), (ii), (iii), and (iv) described above, or to a complementary sequence thereof. The kits may also comprise appropriate buffers, etc. Furthermore, the packages may contain instruction manuals containing a description of how to use the kits.

15 The kits of the present invention may further comprise polynucleotides that hybridize to transcripts of one or more genes selected from the group consisting of the Lbx1, Pax2, Lim1/2, Corl1, and Corl2 genes.

The kits of the present invention may further comprise cerebellar cells or spinal cord cells.

20 In an embodiment of the present invention, the kits for detecting or selecting GABA-producing neuron progenitor cells include kits containing antibodies that bind to a protein selected from (v), (vi), (vii), and (viii) described above.

The kits may further contain in combination antibodies that bind to proteins encoded by one or more genes selected from the group consisting of Lbx1, Pax2, Lim1/2, Corl1, and
25 Corl2 genes.

The kits may further contain cerebellar cells or spinal cord cells.

Meanwhile, the preferred target cerebellar cells in the methods or kits of the present invention are Purkinje cells, and the preferred target spinal cord cells are dI4 and dILA.

Furthermore, the expression of a polynucleotide of the present invention can be used
30 as an indicator to screen for substances that are effective in differentiating GABA-producing neuron progenitor cells.

The present invention provides methods of screening for substances that are effective for differentiating GABA-producing neuron progenitor cells. Since compounds obtained through screening by the methods of the present invention have the activity of differentiating
35 GABA-producing neuron progenitor cells, they are expected to become candidate compounds useful for treating diseases caused by defects in GABA-producing neurons. Target diseases

of treatment using a compound obtained by the screening methods include, for example, spinocerebellar ataxia.

In a preferred embodiment, the above-described screening methods of the present invention comprise the steps of:

- 5 (I) contacting a test compound with cells that can differentiate into GABA-producing neuron progenitor cells; and
(II) detecting in GABA-producing neuron progenitor cells the expression of a polynucleotide that can hybridize to a nucleotide sequence selected from (i), (ii), (iii), and (vi) described above or to a complementary sequence thereof.

10 Here, the “test substance” may be any type of compound, examples of which include the expression products of gene libraries, synthetic low molecular weight compound libraries, synthetic peptide libraries, antibodies, substances released by bacteria, cell (microbial, plant, or animal) extracts, cell (microbial, plant, or animal) culture supernatants, purified or partially purified polypeptides, marine organisms, plant or animal extracts, soil, random phage peptide
15 display libraries, etc.

Furthermore, since 65B13 is expressed specifically in differentiated GABA-producing neuron progenitor cells, it can be used in screening for reagents that differentiate GABA-producing neuron progenitor cells. Specifically, whether a test sample has the ability to differentiate GABA-producing neuron progenitor cells can be assessed by
20 inducing the differentiation into GABA-producing neuron progenitor cells from cells having the ability to differentiate into GABA-producing neuron progenitor cells in the presence of the test sample, and detecting the expression of 65B13 in the differentiated cells. Thus, the present invention provides methods of screening for candidate compounds as a reagent that differentiates GABA-producing neuron progenitor cells, which use as an indicator the
25 expression of 65B13 and comprise the steps of:

- (f) inducing the differentiation into GABA-producing neuron progenitor cells from cells having the ability to differentiate into GABA-producing neuron progenitor cells in the presence of a test sample;
(g) detecting the transcript or translation product of the 65B13 gene in the differentiated cells;
30 and
(h) selecting a compound that increases the level of the transcript or translation product as compared to that detected in the absence of the test sample.

In the above methods, the preferred “cells having the ability to differentiate into GABA-producing neuron progenitor cells” are cell samples containing cells that can be
35 differentiated into GABA-producing neuron progenitor cells, such as multipotent ES cells.

The transcript or translation product of the 65B13 gene of the present invention can

be detected using polynucleotides that hybridize to a transcript of the 65B13 gene or antibodies that bind to a translation product of the 65B13 gene, as described above.

In the present invention, cell growth and differentiation can be detected by comparing the cell condition with when the test substance is not contacted. Cell growth and
5 differentiation can be assessed through morphological observation under a microscope, or detecting or quantifying substances produced upon cell differentiation.

Cell differentiation can be assessed by comparing the expression level of the 65B13 gene with that in the absence of a test sample. Specifically, when a test sample increases the level of transcript or translation product of the 65B13 gene as compared to that in the absence
10 of the test sample, the test sample can be judged to have the ability to differentiate nerve cells. "Increase" means that, for example, the level becomes twice, preferably five times, and more preferably ten or more times.

In a preferred embodiment of the present invention, the screening methods of the present invention further comprise the step of selecting compounds with which the expression
15 of the polynucleotide is detected in step (II).

In another preferred embodiment of the present invention, the screening methods of the present invention also include, for example, methods comprising the steps of:
(IV) contacting a test compound with cells having the ability to differentiate into GABA-producing neuron progenitor cells; and
20 (V) detecting a protein selected from (v), (vi), (vii), and (viii) described above.

In a preferred embodiment, the above-described methods further comprise step (VI) of selecting compounds with which the protein is detected in step (V).

The above methods may comprise use of the 65B13 promoter (including modified promoters) (as a means) to detect proteins. For example, it is possible to transfect cells with
25 a vector carrying a construct in which a gene encoding a detectable marker such as green fluorescent protein (GFP) is linked to a promoter portion obtained by analyzing the expression region of 65B13. Alternatively, a marker gene can be knocked-in at the 65B13 locus. In both cases, the expression of the marker gene is detected in a manner specific to GABA-producing neuron progenitor cells, and thus enables detection of the protein. The
30 protein expression can be detected by methods (means) for detecting protein expression, and therefore the methods can also be used as methods (means) for detecting the protein-encoding gene.

In this case, "the gene encoding a marker is linked to a promoter portion" means that the gene encoding the marker is linked to the promoter portion in an expressible manner.
35 The gene may be directly linked to the promoter, or the gene may be linked distantly to but still under the control of the promoter. Furthermore, the promoter portion obtained by

analyzing the 65B13 expression region may be replaced with another promoter, as long as the promoter for 65B13 enables the expression of the 65B13 region.

The present invention also provides methods for producing a cell population of GABA-producing neurons. Such methods include, for example, methods comprising the

5 steps of:

(VII) obtaining a cell population potentially containing GABA-producing neuron progenitor cells;

(VIII) detecting GABA-producing neuron progenitor cells using a method of the present invention for detecting or selecting GABA-producing neuron progenitor cells; and

10 (IX) growing cells detected or selected in step (VIII).

The GABA-producing neuron progenitor cells obtained by the production methods described above are used, for example, in treating spinal cord injury or cerebellar degeneration.

The GABA-producing neuron progenitor cells obtained by the production methods

15 described above are also included in the present invention. It is preferable that cells produced by the above-described methods of the present invention are viable cells.

Since cells obtained in the present invention are GABA-producing neuron progenitor cells, they are preferable in transplant therapy for degenerative diseases and such in terms of their safety, survival rate, and network formation ability, compared to mixed cell populations or GABA-producing neurons carrying an exogenous gene. Moreover, since cells (or cell

20 populations) of the present invention obtained according to the methods are progenitor cells, they can be differentiated into a suitable stage by selecting *in vitro* conditions such as media, and are preferable materials for various types of neural transplant therapy. When neuron progenitor cells obtained using the methods of the present invention are used in transplants,

25 preferably 1×10^3 to 1×10^6 neurons, and more preferably 5×10^4 to 6×10^4 neurons are transplanted. The primary method is stereotaxic surgery in which a cell suspension is transplanted into the brain. In addition, cells may also be transplanted by microsurgery. See, Backlund *et al.* (J. Neurosurg. 1985, 62:169-73), Lindvall *et al.* (Ann. Neurol. 1987, 22:457-68), or Madrazo *et al.* (New Engl. J. Med. 1987, 316:831-4) for methods of

30 transplanting neuron tissues.

Moreover, the cells of the present invention can also be used to isolate genes specific to GABA-producing neuron progenitor cells, and genes specific to each stage of the maturation from progenitor cells into GABA-producing neurons. They can also be used for searching therapeutic targets for degenerative diseases, elucidating the maturation process of

35 GABA-producing neurons, and in screenings using maturation as an indicator.

The present invention also provides reagents for detecting or selecting

GABA-producing neuron progenitor cells. When identifying cell type by the reagents of the present invention, the representative target cerebellar cell is Purkinje cells and the representative target spinal cord cells are dI4 and dILA.

5 Herein, the “cell type identification” means not only when target cells are identified to be of a specific cell type, but also when target cells are judged not to be of a specific cell type. For example, when the 65B13 gene is substantially expressed in target cerebellar cells, the cells can be identified to be “possibly Purkinje cells” or the cells can be judged “not to be Purkinje cells”. When the 65B13 gene is not substantially expressed in target spinal cord cells, the cells can be identified to be “possibly dI4 or dILA” or the cells can be judged to be
10 “neither dI4 nor dILA”.

In an embodiment, the reagents of the present invention include, for example, reagents for detecting or selecting GABA-producing neuron progenitor cells, which comprise probes, primers, or primer sets that enable detection of the expression of a polynucleotide that can hybridize to a polynucleotide selected from (i), (ii), (iii), and (iv) described above, or to a
15 complementary sequence thereof.

The present invention also provides reagents for detecting or selecting cerebellar cells or spinal cord cells. For the reagents, the above-described reagents for detecting or selecting GABA-producing neuron progenitor cells may be appropriately combined with other known markers. Such reagents enable thorough cell type identification. Thus, in a preferred
20 embodiment, the present invention provides reagents for detecting or selecting cerebellar cells or spinal cord cells, which comprise a combination of the above-described reagents for detecting or selecting GABA-producing neuron progenitor cells and polynucleotides that hybridize to the transcripts of one or more genes selected from the group consisting of Lbx1, Pax2, Lim1/2, Corl1, and Corl2 genes.

25 The sequences of the above-described marker genes are known as listed below.

The nucleotide sequence of mouse Lbx1 is shown in SEQ ID NO: 5 and the amino acid sequence is shown in SEQ ID NO: 6; the nucleotide sequence of human Lbx1 is shown in SEQ ID NO: 7 and the amino acid sequence is shown in SEQ ID NO: 8.

30 The nucleotide sequence of mouse Pax2 is shown in SEQ ID NO: 9 and the amino acid sequence is shown in SEQ ID NO: 10; the nucleotide sequence of human Pax2 is shown in SEQ ID NO: 11 and the amino acid sequence is shown in SEQ ID NO: 12.

The nucleotide sequence of mouse Lim1 is shown in SEQ ID NO: 13 and the amino acid sequence is shown in SEQ ID NO: 14; the nucleotide sequence of human Lim1 is shown in SEQ ID NO: 15 and the amino acid sequence is shown in SEQ ID NO: 16.

35 The nucleotide sequence of mouse Lim2 is shown in SEQ ID NO: 17 and the amino acid sequence is shown in SEQ ID NO: 18; the nucleotide sequence of human Lim2 is shown

in SEQ ID NO: 19 and the amino acid sequence is shown in SEQ ID NO: 20; the nucleotide sequence of rat Lim2 is shown in SEQ ID NO: 21 and the amino acid sequence is shown in SEQ ID NO: 22.

5 The nucleotide sequence of mouse Corl1 is shown in SEQ ID NO: 23 and the amino acid sequence is shown in SEQ ID NO: 24; the nucleotide sequence of human Corl1 is shown in SEQ ID NO: 25 and the amino acid sequence is shown in SEQ ID NO: 26; the nucleotide sequence of rat Corl1 is shown in SEQ ID NO: 27 and the amino acid sequence is shown in SEQ ID NO: 28.

10 The nucleotide sequence of mouse Corl2 is shown in SEQ ID NO: 29 and the amino acid sequence is shown in SEQ ID NO: 30; the nucleotide sequence of human Corl2 is shown in SEQ ID NO: 31 and the amino acid sequence is shown in SEQ ID NO: 32.

The above-described reagents may further comprise cerebellar cells or spinal cord cells.

15 In another embodiment, the reagents of the present invention for detecting or selecting GABA-producing neuron progenitor cells include, for example, reagents for detecting or selecting GABA-producing neuron progenitor cells, which comprise antibodies that bind to a protein selected from (v), (vi), (vii), and (viii) described above.

20 The above-described reagents for detecting or selecting GABA-producing neuron progenitor cells may further comprise in combination antibodies that bind to proteins encoded by one or more genes selected from the group consisting of Lbx1, Pax2, Lim1/2, Corl1, and Corl2 genes.

The above-described reagents may further comprise cerebellar cells or spinal cord cells.

25 Cerebellar cells that can be identified using the above-described reagents include Purkinje cells, while spinal cord cells that can be identified using the above-described reagents include dI4 and dILA.

30 Examples

Hereinbelow, the present invention is specifically described with reference to the Examples; however, it should not be construed as being limited thereto.

35 [Example 1] 65B13 expression analysis in fetal spinal cord and isolation of 65B13-positive cells

65B13 (Neph3) is selectively expressed in several areas of fetal brain (Fig. 1;

WO2004/038018; Minaki Y, Mizuhara E, Morimoto K, Nakatani T, Sakamoto Y, Inoue Y, Satoh K, Imai T, Takai Y, Ono Y. Migrating postmitotic neural precursor cells in the ventricular zone extend apical processes and form adherens junctions near the ventricle in the developing spinal cord. *Neurosci Res.* 2005, 52(3):250-62). In the midbrain, dopaminergic neuron progenitor cells have been demonstrated to express 65B13 (WO2004/038018); however, the types of cells that express 65B13 remain unidentified in other areas. Thus, the present inventors attempted to identify 65B13-expressing cells in the spinal cord and cerebellar primordia.

First, detailed analysis was carried out to clarify the expression of 65B13 in the spinal cord. The analysis was performed by the method described in WO2004/038018. In E10.5 mouse spinal cord, expression of the 65B13 mRNA was restricted to a very narrow dorsal area (Fig. 1D). At E12.5, 65B13 was observed to be expressed in a broader dorsal area (Fig. 1E), and the pattern remained unaltered until E14.5 (Fig. 1F) (Minaki Y, Mizuhara E, Morimoto K, Nakatani T, Sakamoto Y, Inoue Y, Satoh K, Imai T, Takai Y, Ono Y. Migrating postmitotic neural precursor cells in the ventricular zone extend apical processes and form adherens junctions near the ventricle in the developing spinal cord. *Neurosci Res.* 2005, 52(3):250-62).

It is known that in the spinal cord, six types of neurons are generated at an early stage (E10.5 to E11.5) of development and two types of neurons are generated at a late stage (E12 to E13.5) (Fig. 2; Caspary T, Anderson KV. Patterning cell types in the dorsal spinal cord: what the mouse mutants say. *Nat Rev Neurosci.* 2003, 4(4):289-97; Helms AW, Johnson JE. Specification of dorsal spinal cord interneurons. *Curr Opin Neurobiol.* 2003, 13(1):42-9; Matisse M. A dorsal elaboration in the spinal cord. *Neuron.* 2002, 34(4):491-3). Of these, four types (dI4, dI5, dILA, and dILB) form the dorsal horn and transmit sensory, pain, and other signals from the periphery to center. Of these dorsal horn neurons, two types (dI4 and dILA) are GABA-producing inhibitory neurons, and the remaining two (dI5 and dILB) are glutamic acid-producing excitatory neurons (Fig. 2).

At an early stage of development, spinal dorsal neurons are generated from each of the six domains arranged in the dorsoventral direction. To determine the types of 65B13-positive cells, the expression of 65B13 (at the protein level) was compared to those of various neuron markers according to the protocol described below.

Mouse E10.5 embryos were collected and fixed in 4% PFA/PBS(-) at 4°C for two hours. After replacing with 20% sucrose/PBS(-) at 4°C overnight, the embryos were embedded in OCT. Sections of 12-μm thickness were prepared and placed onto slide glasses. Then, the sections were dried at room temperature for 30 minutes, and again wetted with PBS(-). Next, after 30 minutes of blocking (BlockAceTM) at room temperature, a primary antibody was reacted at room temperature for one hour. The reaction was followed by

incubation at 4°C overnight. The sections were washed three times with 0.1% Tween-20/PBS(-) at room temperature for 15 minutes each, and then incubated with a fluorescently labeled secondary antibody at room temperature for one hour. After washing in the same way, the sections were washed with PBS(-) at room temperature for ten minutes, and then mounted. The primary antibodies used were: 65B13 (see WO2004/038018; Minaki Y, Mizuhara E, Morimoto K, Nakatani T, Sakamoto Y, Inoue Y, Satoh K, Imai T, Takai Y, Ono Y. Migrating postmitotic neural precursor cells in the ventricular zone extend apical processes and form adherens junctions near the ventricle in the developing spinal cord. *Neurosci Res.* 2005, 52(3):250-62), Pax2 (purchased from Zymed), and Mash1 (purchased from BD PharMingen).

The result showed that 65B13 was selectively expressed in the dI4 region (Fig. 3). Furthermore, the finding that 65B13 is expressed only within the ventricular zone (VZ) containing undifferentiated progenitor cells (Fig. 1) demonstrates that 65B13-positive cells in E10.5 spinal cord were progenitor cells of dI4.

Next, 65B13-positive cells at a late stage (E12.5) of development were identified by the same method as described above. The Corl1 antibody used was described in WO2006/022243 and Mizuhara E, Nakatani T, Minaki Y, Sakamoto Y, Ono Y. Corl1, a novel neuronal lineage-specific transcriptional corepressor for the homeodomain transcription factor Lbx1. *J Biol Chem.* 2005, 280(5):3645-55.

At E12.5, 65B13 was found to be expressed broadly in the VZ of dorsal spinal cord (Fig. 1). The spatial expression pattern along the dorsoventral axis (Fig. 4A) was identical to the pattern of progenitor cell-selective Mash1 expression in dILA and dILB, and the pattern of Corl1 expression in these neurons (Mizuhara E, Nakatani T, Minaki Y, Sakamoto Y, Ono Y. Corl1, a novel neuronal lineage-specific transcriptional corepressor for the homeodomain transcription factor Lbx1. *J Biol Chem.* 2005, 280(5):3645-55). This finding demonstrates that 65B13 is selectively expressed in progenitor cells of either or both of dILA and dILB. Unlike at the early stage, two types of neurons develop at this stage in the same area of dorsal spinal cord; therefore, the cell types cannot be identified according to their expression sites. In this context, to identify the cell types, 65B13-positive cells were isolated and cultured using the protocol described below.

First, spinal cord mass was excised from E12.5 mice and dispersed using Cell Suspension Buffer (Invitrogen). Then, without fixation and permeability treatment, the cells were stained at 4°C for 20 minutes using an anti-65B13 monoclonal antibody (100 times diluted purified antibody, 1% bovine fetal serum, 1 mM EDTA/SDIA differentiation medium (Kawasaki *et al.* *Neuron* 2000, 28(1):31-40)). After washing three times with 1 mM EDTA/PBS containing 1% bovine fetal serum at 4°C for three minutes, the cells were stained

with a PE-labeled anti-hamster IgG antibody (Jackson; 10 µg/ml, 1% bovine fetal serum, 1 mM EDTA/SDIA differentiation medium) at 4°C for 30 minutes and washed in the same way as described above. After staining, 65B13-expressing cells were separated with a cell sorter. The isolated cells were placed onto slide glasses coated with poly-L-ornithine (Sigma, 0.002% in PBS), laminin (Invitrogen, 5 µg/ml in PBS), and fibronectin (Sigma, 5 µg/ml in PBS) and cultured at 37°C for two days in SDIA differentiation medium supplemented with Knockout Serum Replacement (Gibco, 5%), N2 (Invitrogen, 1x), B27 (Invitrogen, 1x), ascorbic acid (Sigma, 200 µM), and BDNF (Invitrogen, 20 ng/ml). The cultured cells were fixed with 2% PFA/PBS at 4°C for 20 minutes, and then washed twice with PBS at 4°C for 10 minutes. Then, cell permeability treatment was performed using 0.3% Triton X-100/PBS at room temperature for 30 minutes, and the cells were blocked with 10% normal donkey serum/BlockAce at room temperature for 20 minutes. Next, the cells were incubated with a primary antibody (10% normal donkey serum, 2.5% BlockAce, 0.1% Triton X-100/PBS) at room temperature for one hour and then at 4°C overnight. On the next day, after washing three times with 0.1% Triton X-100/PBS at room temperature for ten minutes, the cells were incubated with a fluorescently labeled secondary antibodies (all from Jackson, 10 µg/ml, 10% normal donkey serum, 2.5% BlockAce, 0.1% Triton X-100/PBS) at room temperature for 30 minutes. After washing in the same way as described above, the cells were washed with PBS at room temperature for five minutes, mounted, and observed. The primary antibodies used were: Lim1/2 (purchased from Developmental Studies Hybridoma Bank), HuC/D (purchased from Molecular Probe), and Gad65 (purchased from BD PharMingen). The antibody Lmx1b was prepared by the method described below. First, an expression vector was constructed for a GST fusion protein with amino acids of 271 to 306 of Lmx1b as an immunization antigen. After the resulting vector was introduced into *E. coli* (JM109 strain), the expression was induced with IPTG. The fusion protein was collected using glutathione beads. After rabbits were immunized several times with the fusion proteins, blood was collected from the rabbits. Anti-Lmx1b polyclonal antibody was obtained by affinity purification of the serum using the same GST-Lmx1b antigen used in the immunization. Nuclear staining was performed using SYTOX Green (Molecular Probe).

The first finding was that 65B13-expressing cells in the dorsal spinal cord can be isolated alive by using an anti-65B13 antibody (Fig. 5A). Another finding was that almost the entire population of 65B13-expressing cells in the dorsal spinal cord differentiated into HuC/D-positive neurons after two days of culturing, and nearly all of the neurons were Lim1/2-positive and Gad1-positive GABA-producing dILA neurons (Fig. 5B). Thus, in E12.5 spinal cord, 65B13 was revealed to be selectively expressed in progenitor cells of dILA. Specifically, it was demonstrated that in the fetal spinal cord, 65B13 was specifically

expressed in GABA neuron progenitor cells (dI4 and dILA) of the dorsal horn, and these progenitor cells can be separated by using an anti-65B13 antibody.

[Example 2] 65B13 expression analysis in cerebellar primordium and isolation of
5 65B13-positive cells

The cerebellum is constituted of glutamic acid-producing granule cells and GABA-producing neurons such as Purkinje cells, Golgi cells, stellate cells, and basket cells (Wang VY, Zoghbi HY. Genetic regulation of cerebellar development. *Nat Rev Neurosci.* 2001, 2(7):484-91). The granule cells are known to develop in the rhombic lip region at E12.5 to
10 E14.5 (Wang VY, Zoghbi HY. Genetic regulation of cerebellar development. *Nat Rev Neurosci.* 2001, 2(7):484-91). By contrast, the development of GABA-producing neurons is still poorly understood. However, recent findings suggest that Purkinje cells are generated at E11.5 to E13.5 in the dorsal rhombomere 1 (cerebellar primordium area) (Chizhikov VV, Lindgren AG, Currle DS, Rose MF, Monuki ES, Millen KJ. The roof plate regulates cerebellar
15 cell-type specification and proliferation. *Development.* 2006, 133(15):2793-804). Although there is no detailed report on Golgi cells, they are thought to be generated at a late stage (E13.5 to E15.5) of development in the same region. The stellate cells and basket cells are thought to be generated from progenitor cells in the white matter after birth (Zhang L, Goldman JE. Generation of cerebellar interneurons from dividing progenitors in white matter.
20 *Neuron.* 1996, 16(1):47-54).

To identify 65B13-expressing cells in the cerebellar primordium, the spatial expression pattern of 65B13 was compared to those of various markers by the same methods described in Example 1. The anti-Corl2 antibody used was the same as described in WO2006/082826.

25 The result showed that in the cerebellar primordium of E12.5, 65B13 was selectively expressed in VZ where Corl2-positive Purkinje cells develop (Fig. 6A). In addition, neurons that are thought to be Pax2-positive Golgi cells revealed to start to emerge at E14.5 in the same 65B13-positive area (Maricich SM, Herrup K. Pax-2 expression defines a subset of GABAergic interneurons and their precursors in the developing murine cerebellum. *J*
30 *Neurobiol.* 1999, 41(2):281-94) (Fig. 6B).

Thus, experiments were carried out to isolate and culture 65B13-positive cells for the purpose of confirming that the 65B13-positive cells are progenitor cells of Purkinje and Golgi cells.

The cerebellar primordium areas at E12.5 and E14.5 were excised, and
35 65B13-positive cells were detected and isolated by the same method as described in Example 1 using a cell sorter. The result showed that 65B13-positive cells could be isolated alive

from the cerebellar primordium areas at both of the developmental stages (Fig. 7).

Furthermore, it was demonstrated that almost the entire population of the cells differentiated into neurons after two days of culturing, and nearly all of the E12.5 65B13-positive cells differentiated into Corl2-positive Purkinje cells (Fig. 8A) while nearly all of the E14.5 65B13-positive cells differentiated into Pax2-positive Golgi cell-like neurons (Fig. 8B).

Thus, it was revealed that in the fetal cerebellum, 65B13 was selectively expressed in progenitor cells of Purkinje and Golgi cells, and these progenitor cells could be separated by using an anti-65B13 antibody. Specifically, 65B13 was demonstrated to be useful as a marker for separating GABA-producing neuron progenitor cells in the spinal dorsal horn and cerebellum.

[Example 3] Isolation of ES cell-derived spinal dorsal horn GABA neuron progenitor cells

Next, to assess whether *in vitro* differentiated GABA neuron progenitor cells can be separated by using 65B13 as a marker, spinal cord nerve cells differentiated from ES cells (Wichterle H, Lieberam I, Porter JA, Jessell TM. Directed differentiation of embryonic stem cells into motor neurons. Cell 2002, 110(3):385-97) were separated according to the protocol described below.

10 ml of Glasgow Minimum Essential Medium (Invitrogen) supplemented with 5% fetal calf blood, 2 mM L-glutamine (Invitrogen), 0.1 mM non-essential amino acid (Invitrogen), 1 mM sodium pyruvate (Sigma), 0.1 mM 2-mercaptoethanol (Sigma), 100 U/ml penicillin (Invitrogen), and 100 µg/ml streptomycin (Invitrogen) was added to a 10-cm dish. 1.5×10^6 cells were cultured in the dish at 37°C under 5% carbon dioxide and 95% humidity for two days. The resulting cell mass (EB) was harvested and placed in the medium described above. After adding 2 µM retinoic acid (Sigma), the cell mass was further cultured for four days. Then, the mass was dispersed into cells by the same method as described in Example 1. After staining with the anti-65B13 antibody, the cells were isolated with a cell sorter.

As a result, 65B13-positive cells were detected in the population of spinal cord cells differentiated from ES cells. This demonstrates that, like fetal spinal cord cells, dorsal spinal cord neuron progenitor cells derived from ES cells express 65B13 (Fig. 9A). It was also observed that about 50% of the isolated cells differentiated into HuC/D-positive neurons and about 70% of the neurons differentiated into Pax2-positive GABA neurons (Fig. 9B). Thus, 65B13 was demonstrated to be a useful marker for separating not only fetal but also ES cell-derived GABA neuron progenitor cells.

[Example 4] Expression of foreign genes using the 65B13 promoter specifically in

GABA-producing neuron progenitor cells

Next, whether foreign genes can be expressed in a GABA-producing neuron progenitor cell-specific manner using the 65B13 promoter was assessed by creating transgenic mice and analyzing the expression of foreign genes according to the protocol described below.

5 First, the poly A addition sequence of bovine growth hormone (SEQ ID NO: 55; derived from Invitrogen pcDNA3.1+ vector) was amplified and inserted into the *HindIII/XhoI* site of pSP73 (Promega) to construct pSP73-polyA. Then, the synthetic DNAs of SEQ ID NOs: 56 and 57 were annealed to each other and inserted into the *Asp718I/BamHI* site of pSP73-polyA to construct pSP73-polyA II. A mouse genomic fragment (SEQ ID NO: 58) located about 3.2 kb upstream of the translation initiation codon of 65B13 was inserted into the *Clal/Asp718I* site of pSP73-polyA II to construct pN3. Finally, mouse Gsh1 cDNA (SEQ ID NO: 59) was inserted as a foreign gene into the *Asp718I/SalI* site of pN3 to construct pN3-Gsh1. After linearized with *Clal*, pN3-Gsh1 was injected into the pronuclei of mouse fertilized eggs according to the method of Gordon *et al.* (Gordon JW, Scangos GA, Plotkin DJ, 10 Barbosa JA, Ruddle FH. Genetic transformation of mouse embryos by microinjection of purified DNA. Proc Natl Acad Sci USA. 1980 Dec;77(12):7380-4), and the eggs were transplanted into foster mothers. The fetuses were recovered at embryonic day 12.5, and the expression of Neph3 and Gsh1 in the cerebellar primordia was analyzed by the methods described in Example 1. An anti-Gsh1 antibody was prepared by the method described 20 below. First, an expression vector was constructed for a GST fusion protein with amino acids of 1 to 72 of Gsh1 as an immunization antigen. After the resulting vector was introduced into *E. coli* (JM109 strain), the expression was induced with IPTG. The fusion protein was collected using glutathione beads. After the rats were immunized with the fusion protein collected, lymphocytes were collected and fused with myeloma cell P3U1. Thus, anti-Gsh1 25 antibody-producing hybridomas were obtained (hybridoma preparation was outsourced to Kohjin Bio Co.).

The result showed that the wild-type cerebellum expressed Gsh1 only in a very small ventral fraction of 65B13-positive GABA-producing neuron progenitor cells, while the transgenic mice expressed Gsh1 specifically in the entire 65B13-positive area (Fig. 11). This 30 finding demonstrates that foreign genes can be expressed in a GABA-producing neuron progenitor cell-specific manner by using the 65B13 promoter.

Industrial Applicability

35 The present invention identified a selective marker 65B13 for spinal dorsal horn and cerebellar GABA neuron progenitor cells, and successfully isolated GABA neuron progenitor cells by using an antibody against 65B13. This technique can provide viable GABA neuron

progenitor cells, and is expected to be useful in preparing materials for transplantation therapy for degenerative diseases, search of specific genes, discovery of drugs targeting GABA neurons, etc.

5 Since the marker gene identified by the present inventors encodes a membrane protein, the marker can be used as an indicator to detect and select GABA neuron progenitor cells and to isolate (separate) viable GABA neuron progenitor cells.

Highly pure GABA neurons can be obtained by methods for preparing GABA neuron progenitor cells using the marker of the present invention. Thus, the methods are applicable to drug discovery, for example, targeting pain associated with spinal cord GABA neurons, or
10 cerebellar degeneration associated with cerebellar GABA neurons.

Sequence Listing Free Texts

SEQ ID NO: 33 Mouse 65B13 NM_172898 extracellular: 20-513 a.a.

SEQ ID NO: 35 Mouse 65B13 BC052773 extracellular: 20-513 a.a.

15 SEQ ID NO: 37 Human 65B13 NM_032123 extracellular: 21-510 a.a.

SEQ ID NO: 39 Human 65B13 NM_199180 extracellular: 21-510 a.a.

SEQ ID NO: 41 Human 65B13 AY358742 extracellular: 21-510 a.a.

SEQ ID NO: 43 Human 65B13 AY305301 extracellular: 21-510 a.a.

SEQ ID NO: 45 Human 65B13 NM_199179 extracellular: 21-460 a.a.

20 SEQ ID NO: 47 Human 65B13 AY305302 extracellular: 21-460 a.a.

SEQ ID NO: 49 Human 65B13 BC064925 extracellular: 21-490 a.a.

SEQ ID NO: 51 Chimpanzee 65B13 (predicted) XM_512603 extracellular: 21-445 a.a.

SEQ ID NO: 53 Cattle 65B13 (predicted) XM_583222 extracellular: 44-607 a.a.

CLAIMS

1. A method for detecting a GABA-producing neuron progenitor cell from a test cell sample comprising in vitro differentiated GABA-producing neuron progenitor cells or GABA-producing neuron progenitor cells obtained from the spinal dorsal horn or the cerebellum, which comprises the step of detecting the expression of a polynucleotide of (i) below, or a complementary sequence thereof:
 - (i) a polynucleotide comprising the nucleotide sequence of positions 178 to 2280 in SEQ ID NO: 1; the nucleotide sequence of positions 127 to 2079 in SEQ ID NO: 3; the nucleotide sequence of positions 668 to 2770 in SEQ ID NO: 33; the nucleotide sequence of positions 130 to 2232 in SEQ ID NO: 35; the nucleotide sequence of positions 199 to 2100 in SEQ ID NO: 37; the nucleotide sequence of positions 199 to 2325 in SEQ ID NO: 39; the nucleotide sequence of positions 15 to 2325 in SEQ ID NO: 41; the nucleotide sequence of positions 15 to 1916 in SEQ ID NO: 43; the nucleotide sequence of positions 199 to 1950 in SEQ ID NO: 45; the nucleotide sequence of positions 15 to 1766 in SEQ ID NO: 47; or the nucleotide sequence of positions 196 to 2262 in SEQ ID NO: 49.
2. The method of claim 1, wherein the step of detecting the expression of a polynucleotide comprises the steps of:
 - (a) contacting the test cell sample with a polynucleotide that can hybridize to the polynucleotide selected in claim 1 or to a complementary sequence thereof, or with a probe comprising the polynucleotide; and
 - (b) detecting reactivity.
3. The method of claim 2, wherein the probe is contacted with mRNA prepared from the test cell sample or a complementary DNA (cDNA) transcribed from the mRNA in step (a).
4. The method of claim 1, wherein the step of detecting the expression of a polynucleotide selected in claim 1 or a complementary sequence thereof comprises the steps of:

- (a-1) conducting gene amplification using a polynucleotide derived from the test cell sample as a template, and a primer comprising a polynucleotide that can hybridize to the polynucleotide selected in claim 1 or to a complementary sequence thereof, or a set of primers comprising a polynucleotide that can hybridize to the polynucleotide selected in claim 1 or to a complementary sequence thereof; and
- (b-1) detecting the resulting amplification product.

5. The method of claim 4, wherein mRNA prepared from the test cell sample or a complementary DNA (cDNA) transcribed from the mRNA is used as a template in step (a-1).
6. The method of any one of claims 1 to 5, wherein the detection step is followed by the step of separating a GABA-producing neuron progenitor cell from the detected sample.
7. The method of any one of claims 1 to 6, which further comprises the step of detecting or selecting a GABA-producing neuron progenitor cell, using as an indicator the expression of a gene selected from the group consisting of the Corl1, Pax2, Lim1/2, Lbx1, and Corl2 genes.
8. A method for detecting or selecting a GABA-producing neuron progenitor cell from a test cell sample comprising in vitro differentiated GABA-producing neuron progenitor cells or GABA-producing neuron progenitor cells obtained from the spinal dorsal horn or the cerebellum, which comprises the step of detecting a protein of (i) below:
 - (i) a protein comprising the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50.
9. A method for detecting or selecting a GABA-producing neuron progenitor cell from a test cell sample comprising in vitro differentiated GABA-producing neuron progenitor cells or GABA-producing neuron progenitor cells obtained from the spinal dorsal horn or the cerebellum, which comprises the step of detecting a marker protein translated from a marker protein mRNA transcribed under the control of a promoter linked to a polynucleotide encoding the marker protein to express the mRNA, wherein the protein to be translated from the mRNA is a protein of (i) below:

- (i) a protein comprising the amino acid sequence of SEQ ID NO: 2, 4, 34, 36, 38, 40, 42, 44, 46, 48, or 50.
10. The method of claim 8 or 9, wherein the step of detecting the protein comprises the steps of:
- (a) contacting the test cell sample with an antibody that specifically binds to the protein selected in claim 8 or 9; and
 - (b) detecting reactivity.
11. The method of any one of claims 8 to 10, wherein the detection step is followed by the step of separating a GABA-producing neuron progenitor cell from the detected sample.
12. The method of any one of claims 8 to 11, further comprising detecting the GABA-producing neuron progenitor cell by detecting a marker protein encoded by a gene selected from the group consisting of the *Corl1*, *Pax2*, *Lim1/2*, *Lbx1*, and *Corl2* genes.
13. A method for producing a GABA-producing neuron progenitor cell, which comprises the steps of:
- (I) detecting a GABA-producing neuron progenitor cell, from a cell population containing in vitro differentiated GABA-producing neuron progenitor cells using the method of any one of claims 1 to 12; and
 - (II) growing the cell detected in step (I).
14. A method for isolating, separating, or selecting GABA-producing neuron progenitor cells, comprising:
- contacting a test cell sample comprising in vitro differentiated GABA-producing neuron progenitor cells or GABA-producing neuron progenitor cells obtained from the spinal dorsal horn or the cerebellum with an anti-65B13 antibody that binds to the 65B13 protein of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID

NO:48, or SEQ ID NO:50, wherein the cells bound by the anti-65B13 antibody comprise GABA-producing neuron progenitor cells; and

isolating, separating, or selecting cells bound by the anti-65B13 antibody from the test cell sample.

15. The method of claim 14, wherein the cells bound by the anti-65B13 antibody are isolated using flow cytometry.

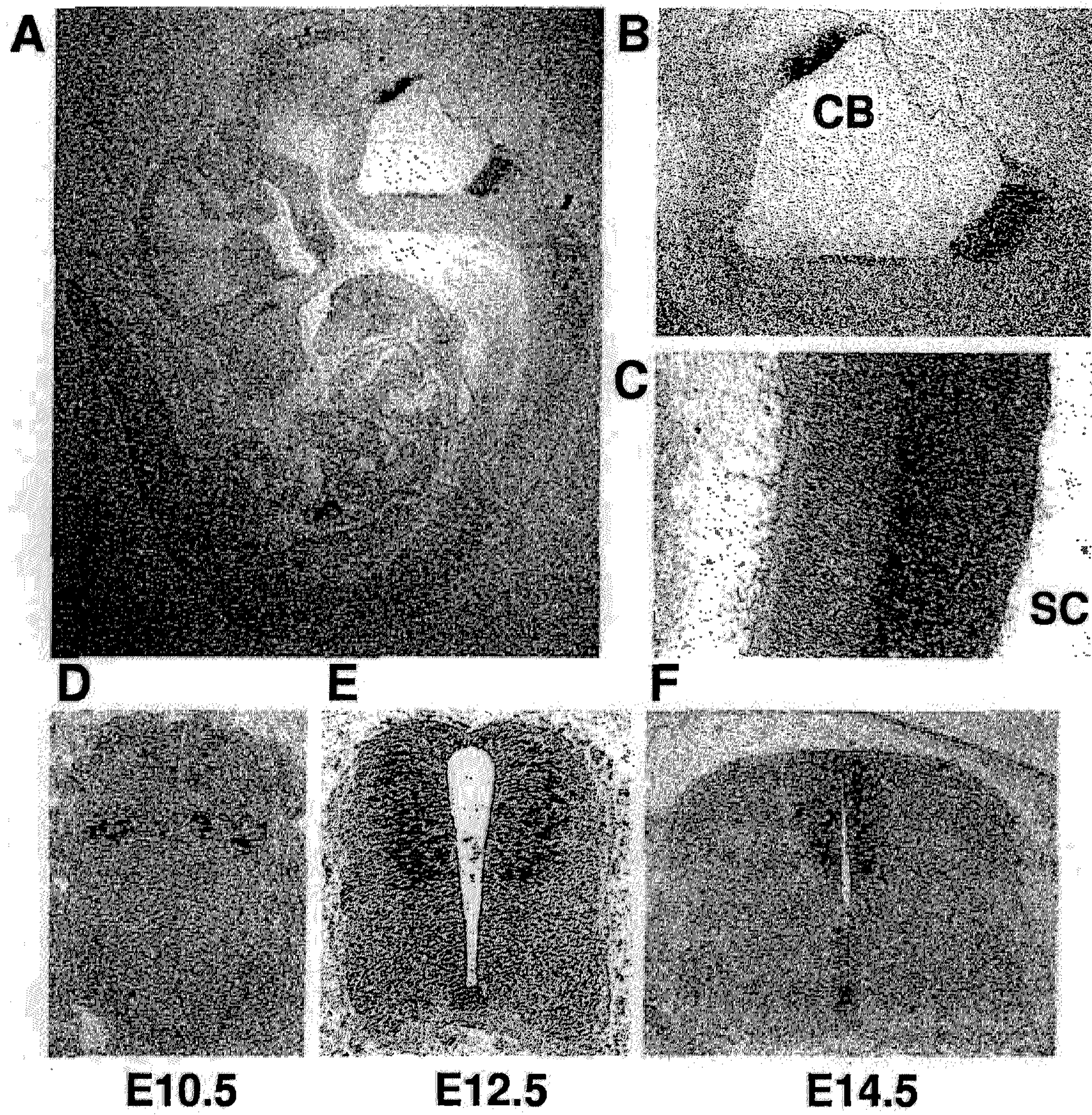
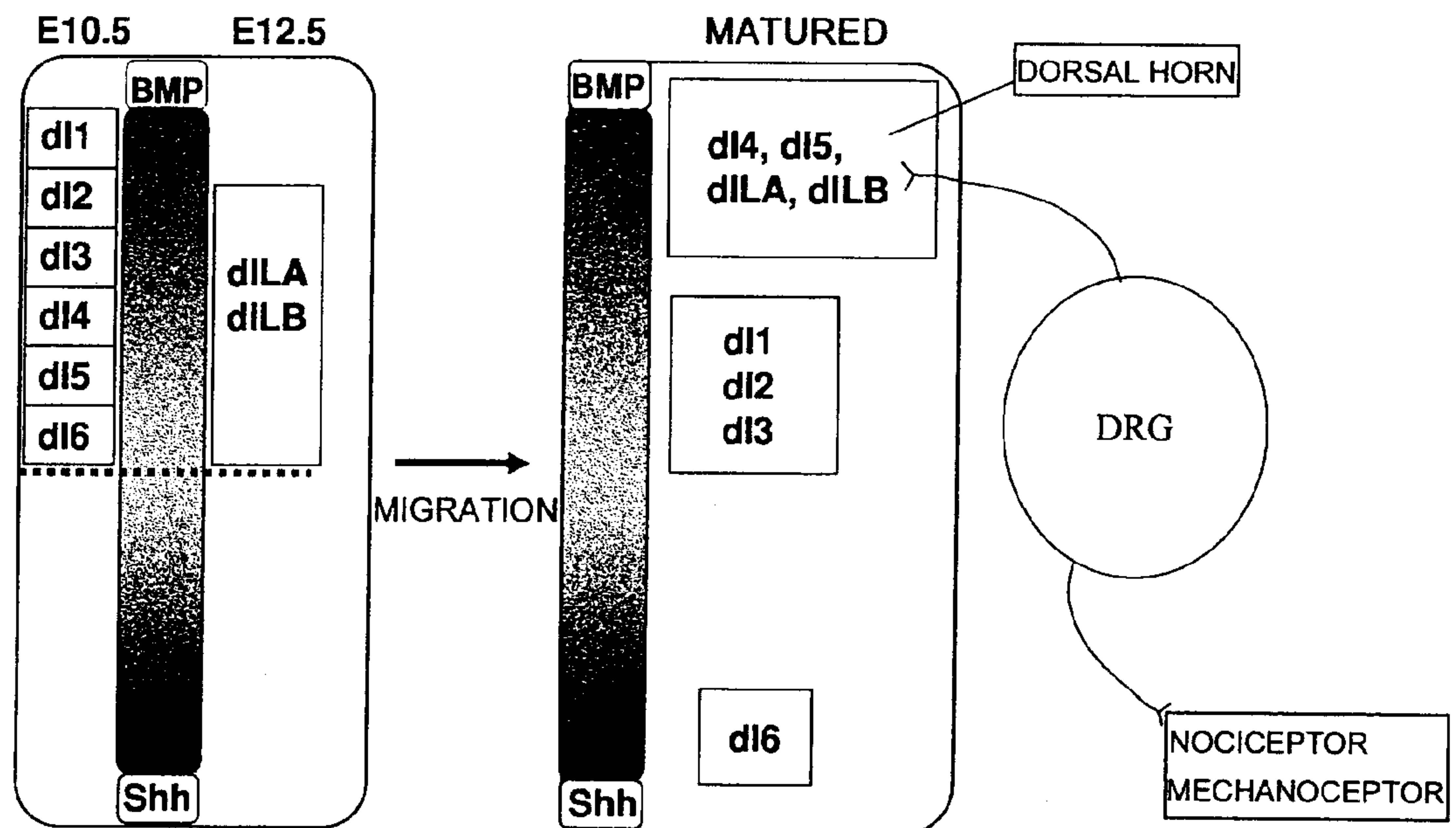


FIG. 1

A**B**

dI1—LH2A/B, Brn3a
 dI2—Lim1/2, Brn3a
 EARLY dI3—Isl1, Brn3a
 STAGE dI4—Lbx1, Pax2, Lim1/2, Corl1 —GABAergic
 dI5—Lbx1, Lmx1b, Brn3a, Corl1 —Glutamatergic
 dI6—Lbx1, Pax2, Lim1/2
 LATE dILA—Lbx1, Pax2, Lim1/2, Corl1 —GABAergic
 STAGE dILB—Lbx1, Lmx1b, Brn3a, Corl1—Glutamatergic

FIG. 2

Pax2 65B13 Mash1

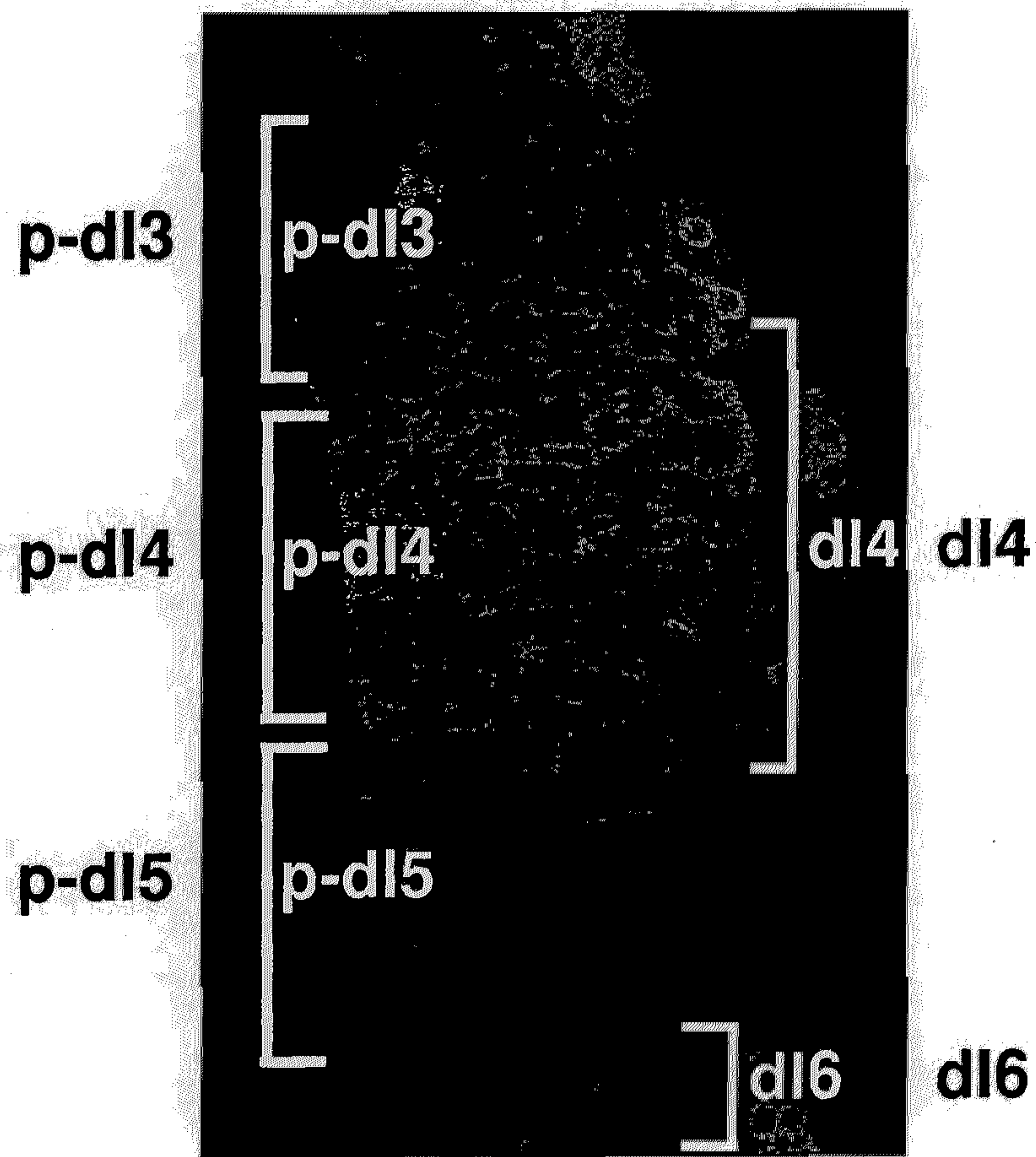


FIG. 3

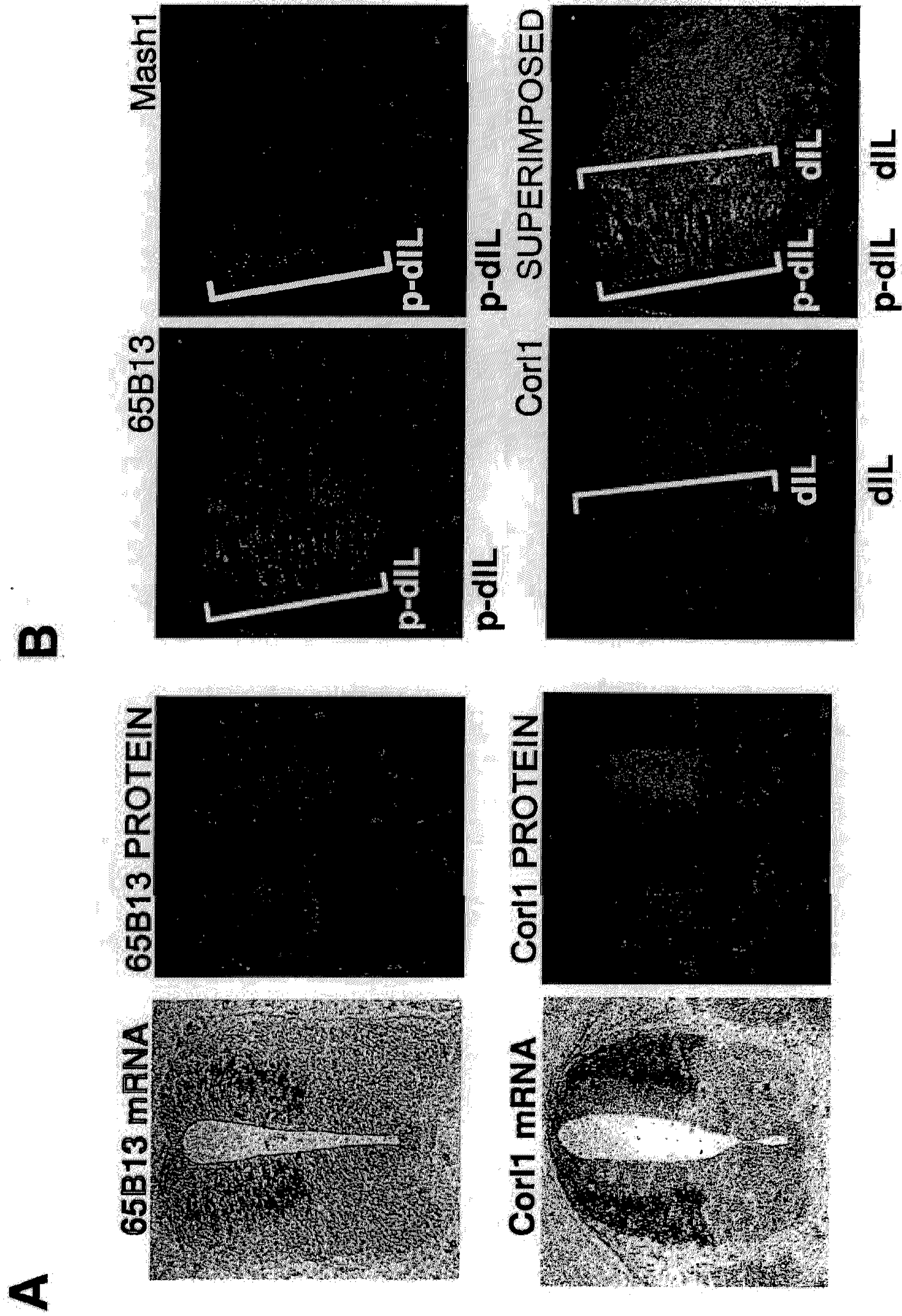


FIG. 4

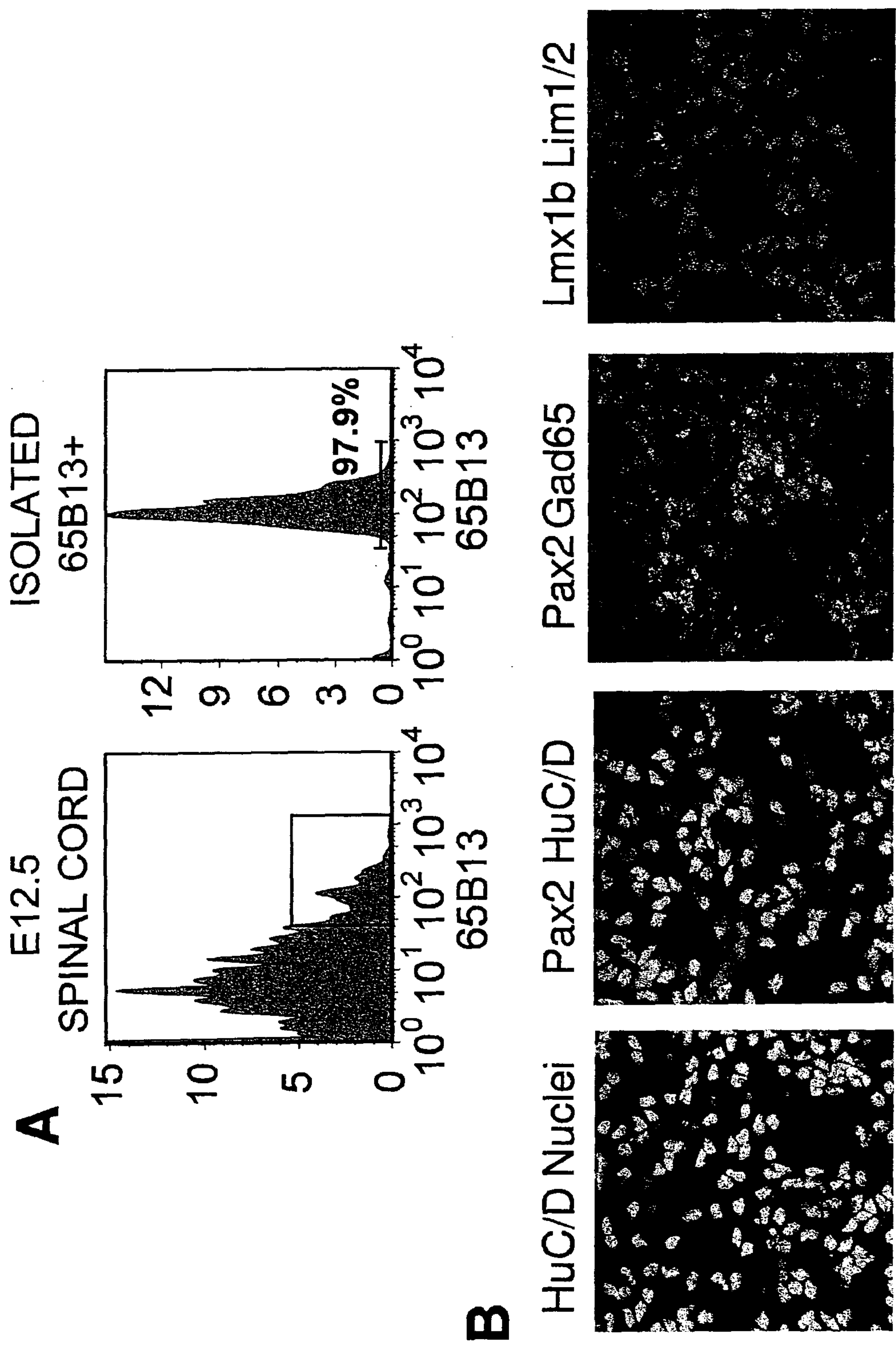
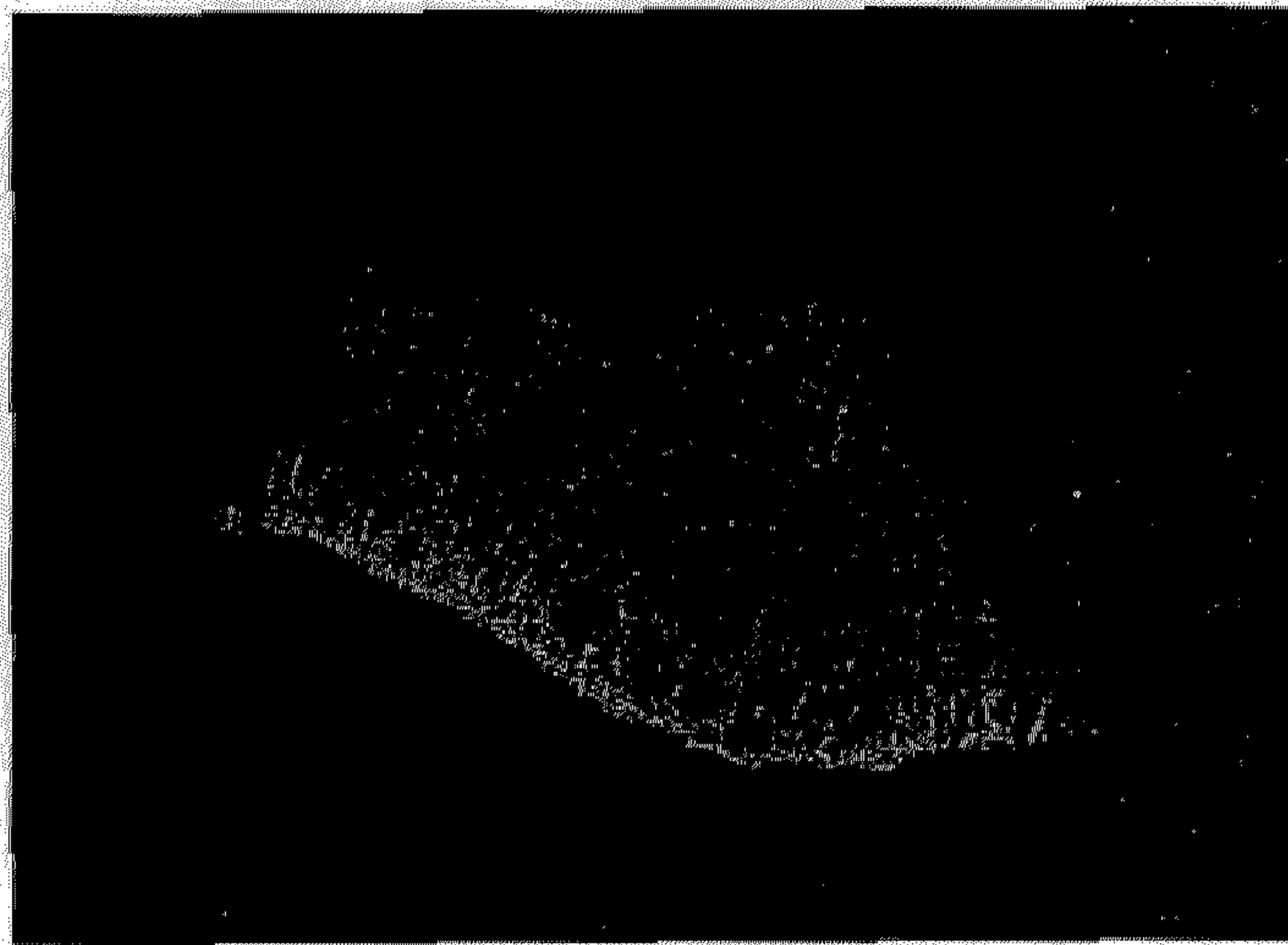


FIG. 5

A

E12.5

65B13 Corl2

**B**

E14.5

65B13



Pax2

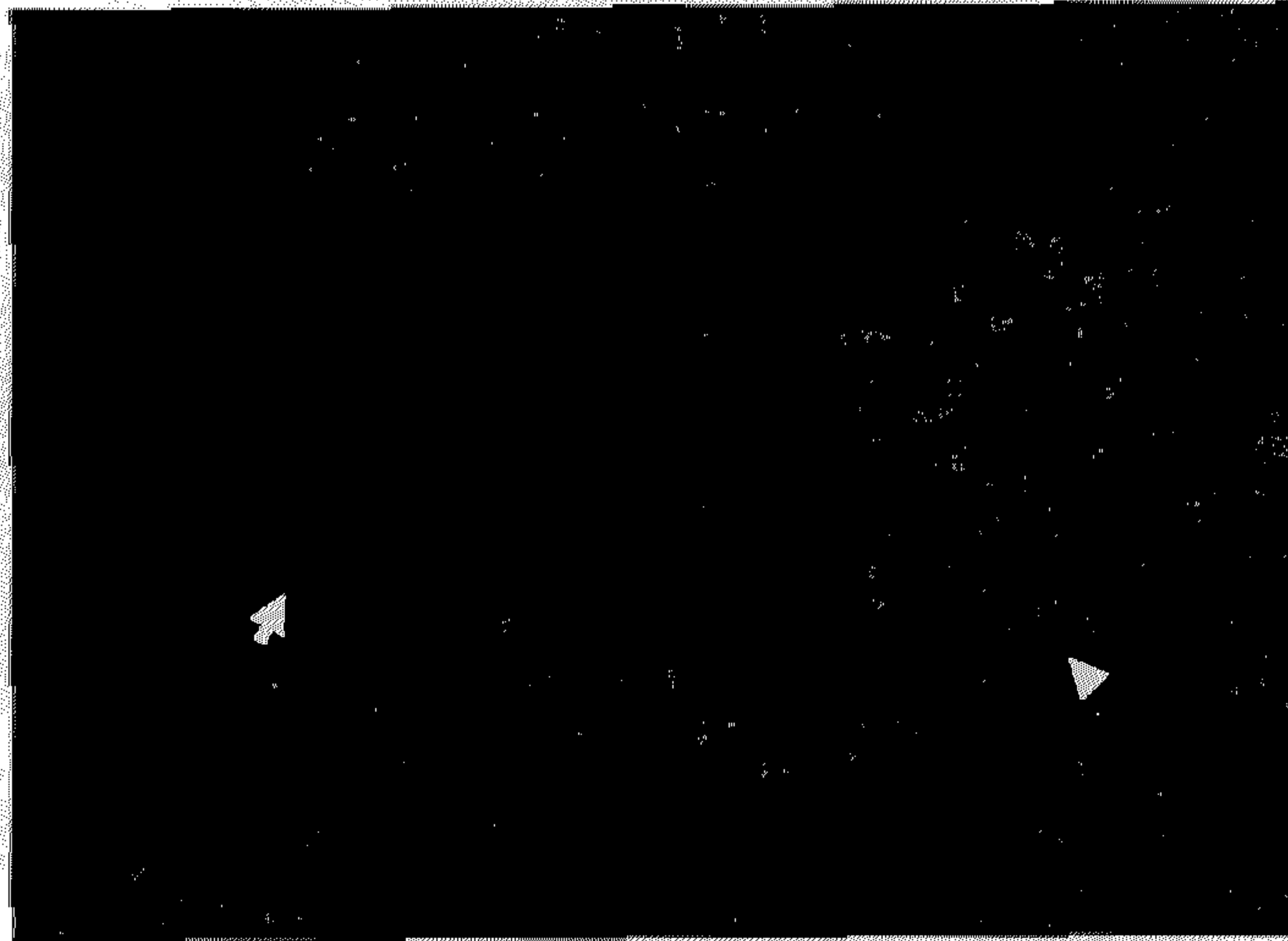
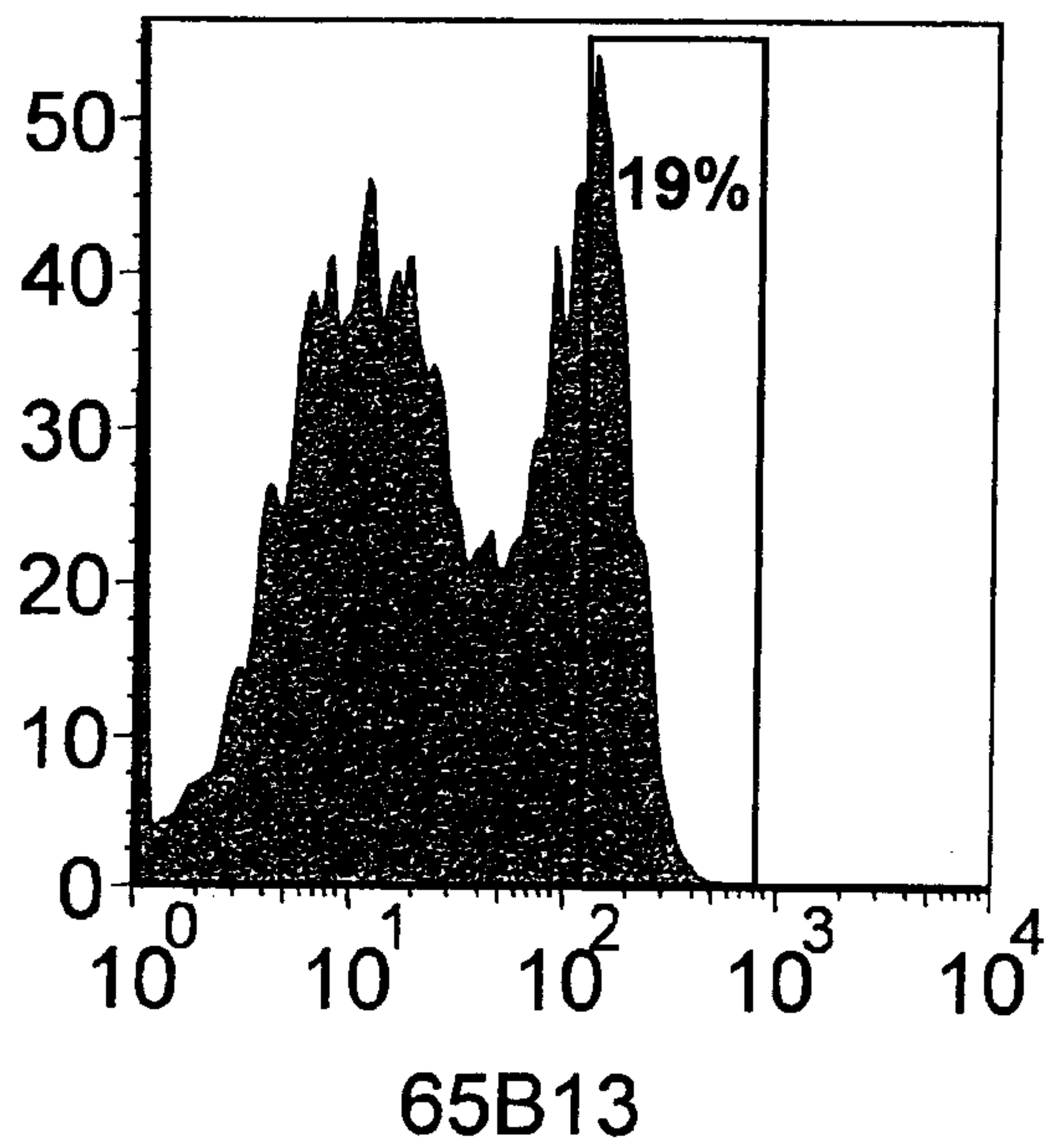


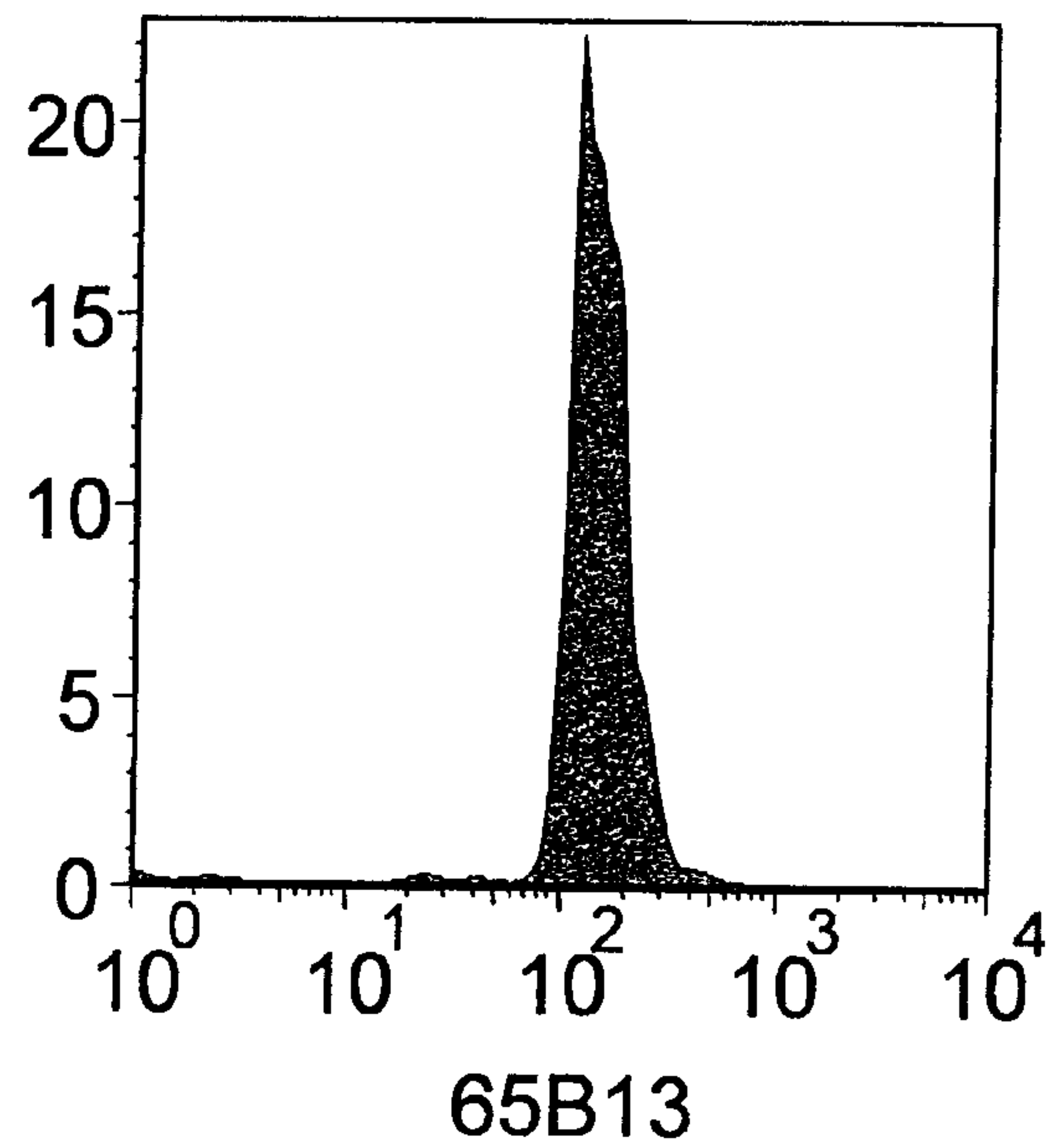
FIG. 6

A

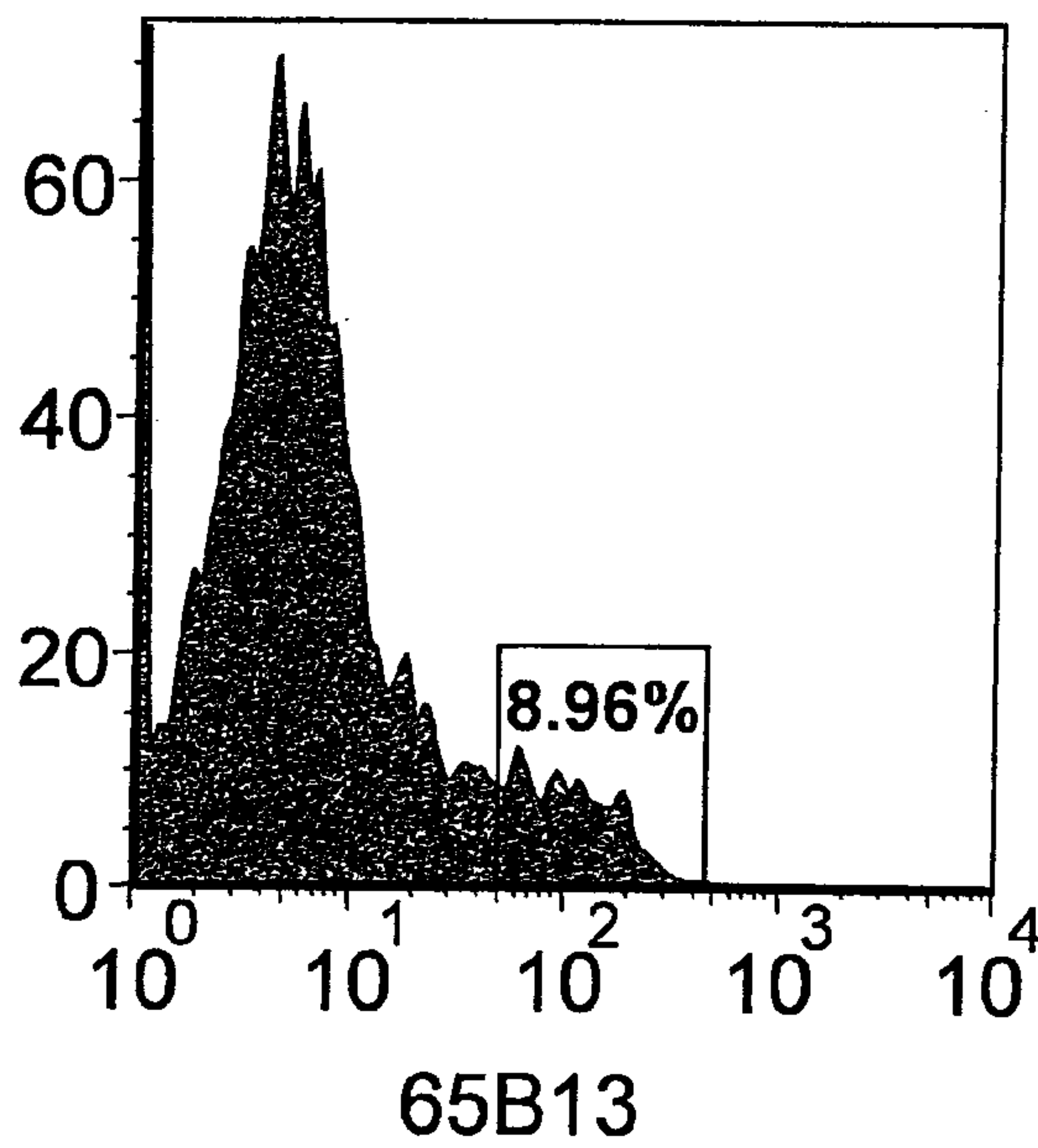
E12.5 CEREBELLUM



ISOLATED 65B13+

**B**

E14.5 CEREBELLUM



ISOLATED 65B13+

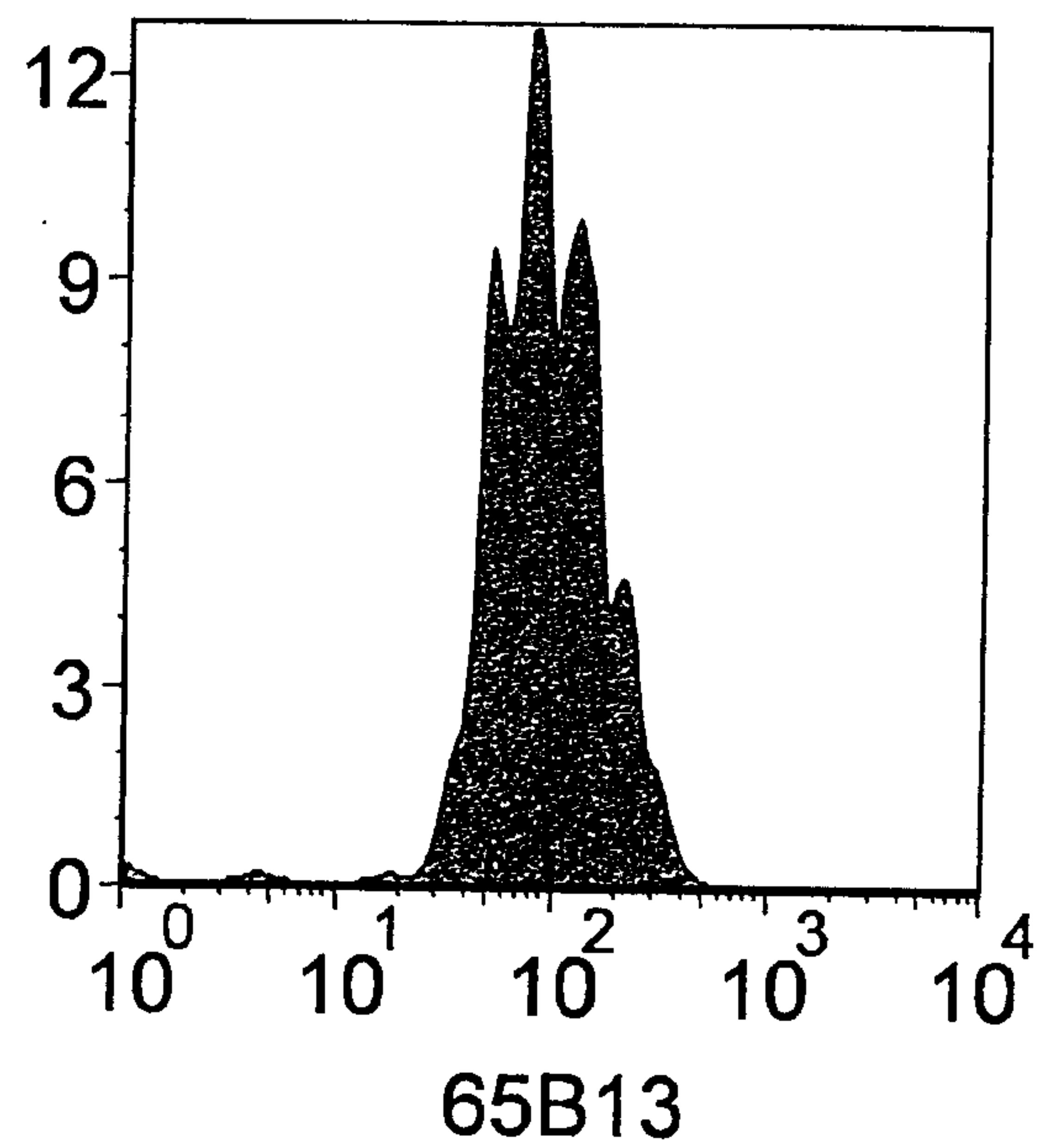
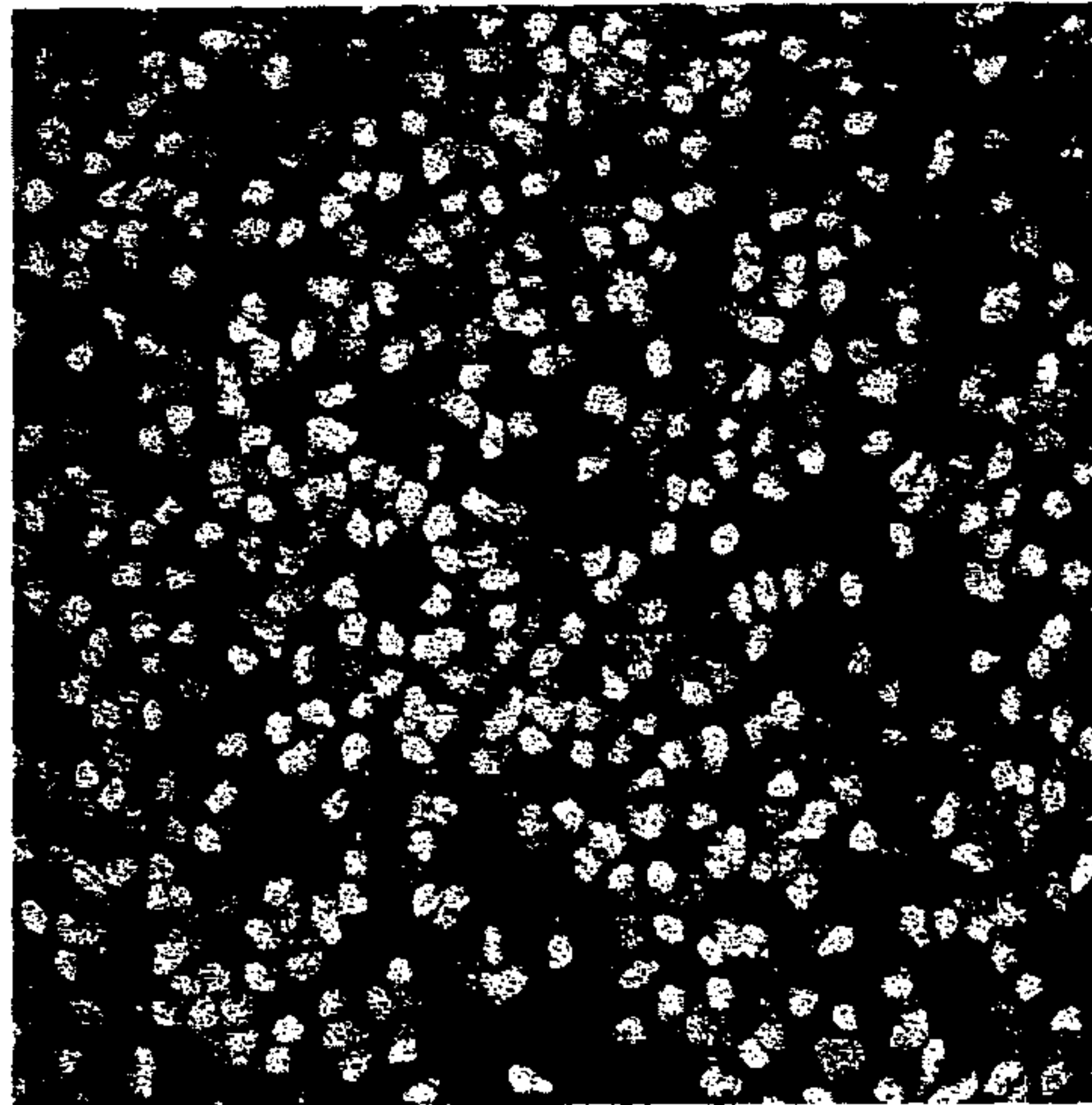


FIG. 7

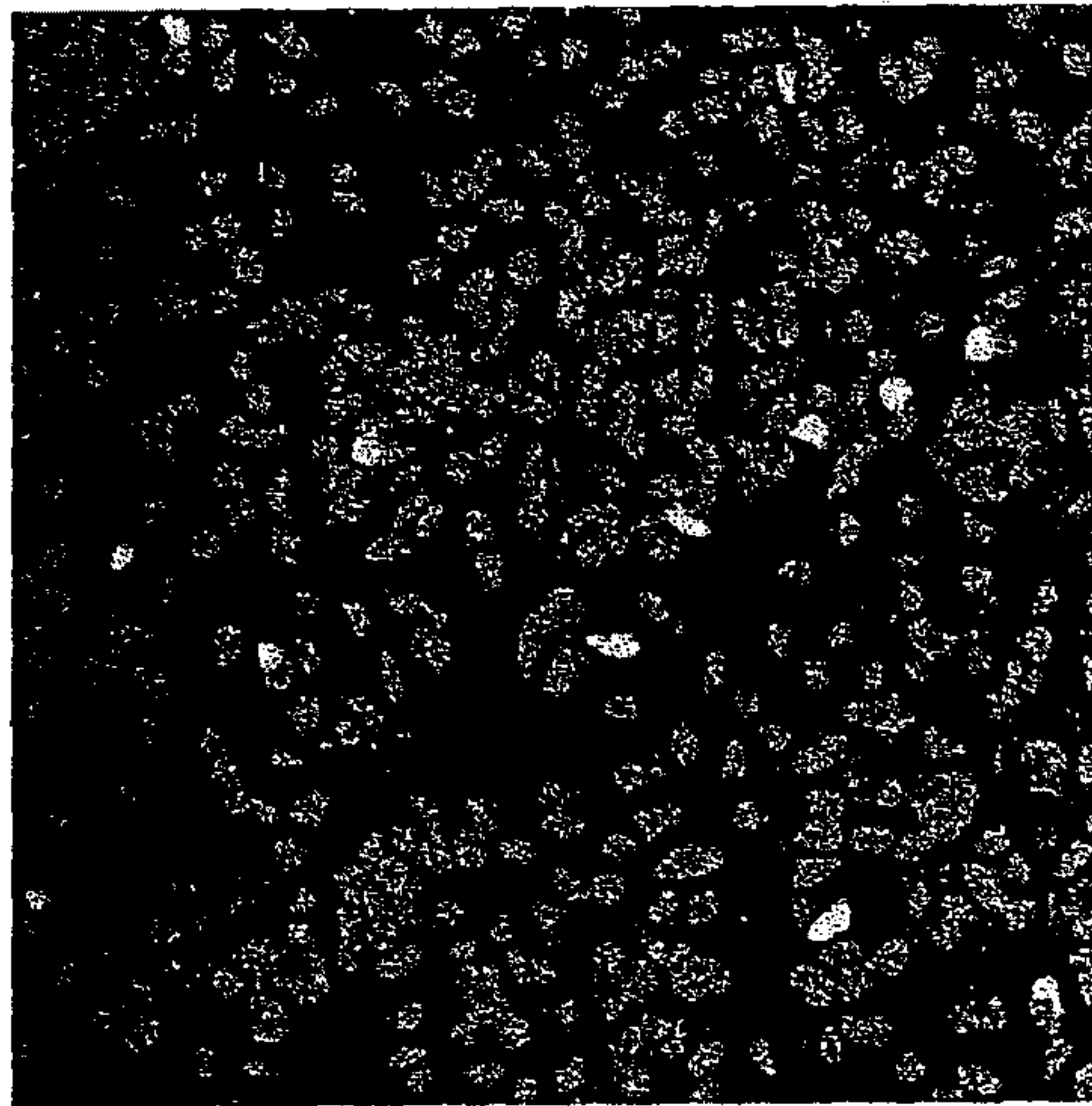
A

E12.5 65B13+ CELLS

Corl2 HuC/D



Pax2 HuC/D



B

E14.5 65B13+ CELLS

Pax2 HuC/D

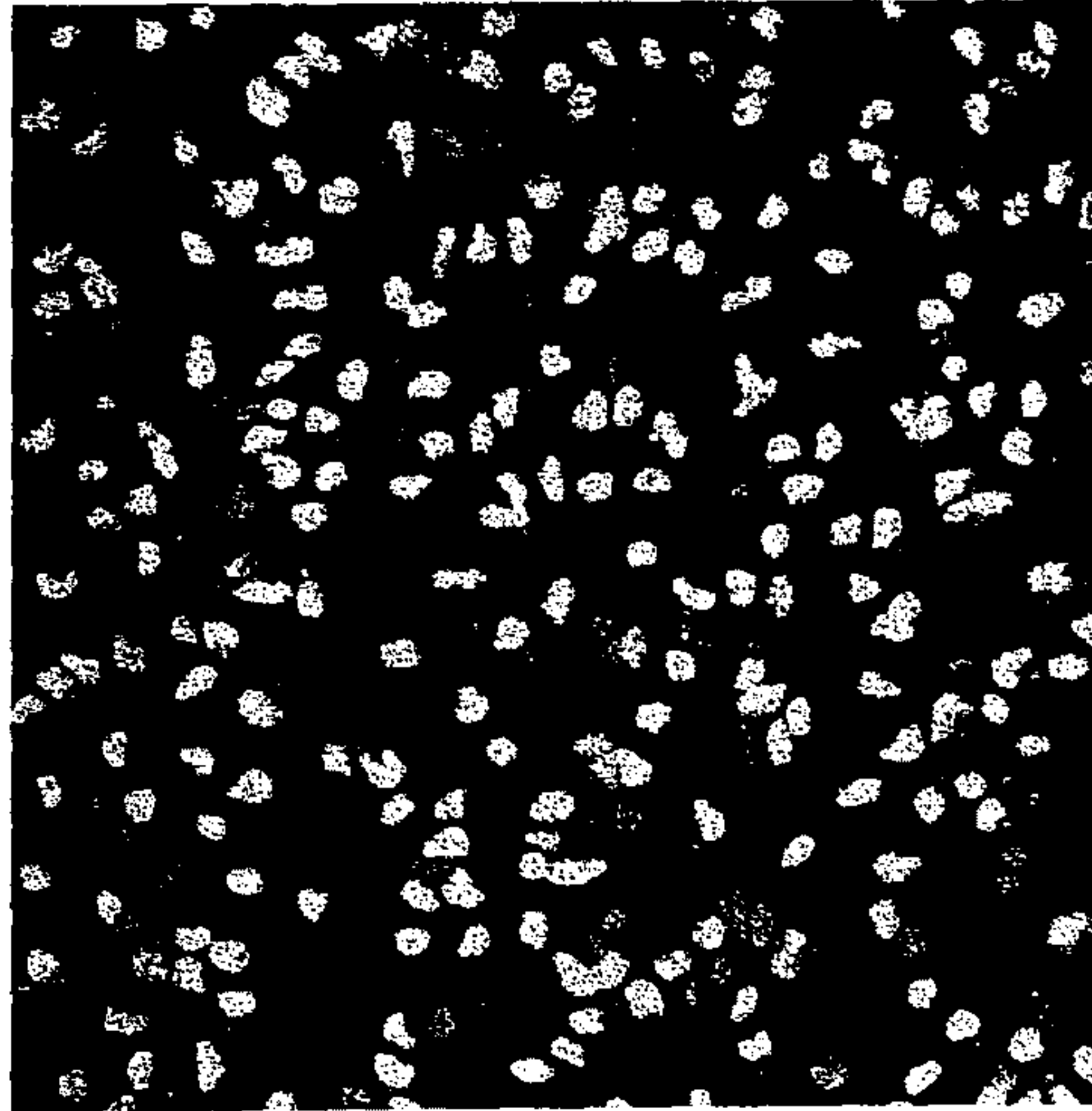


FIG. 8

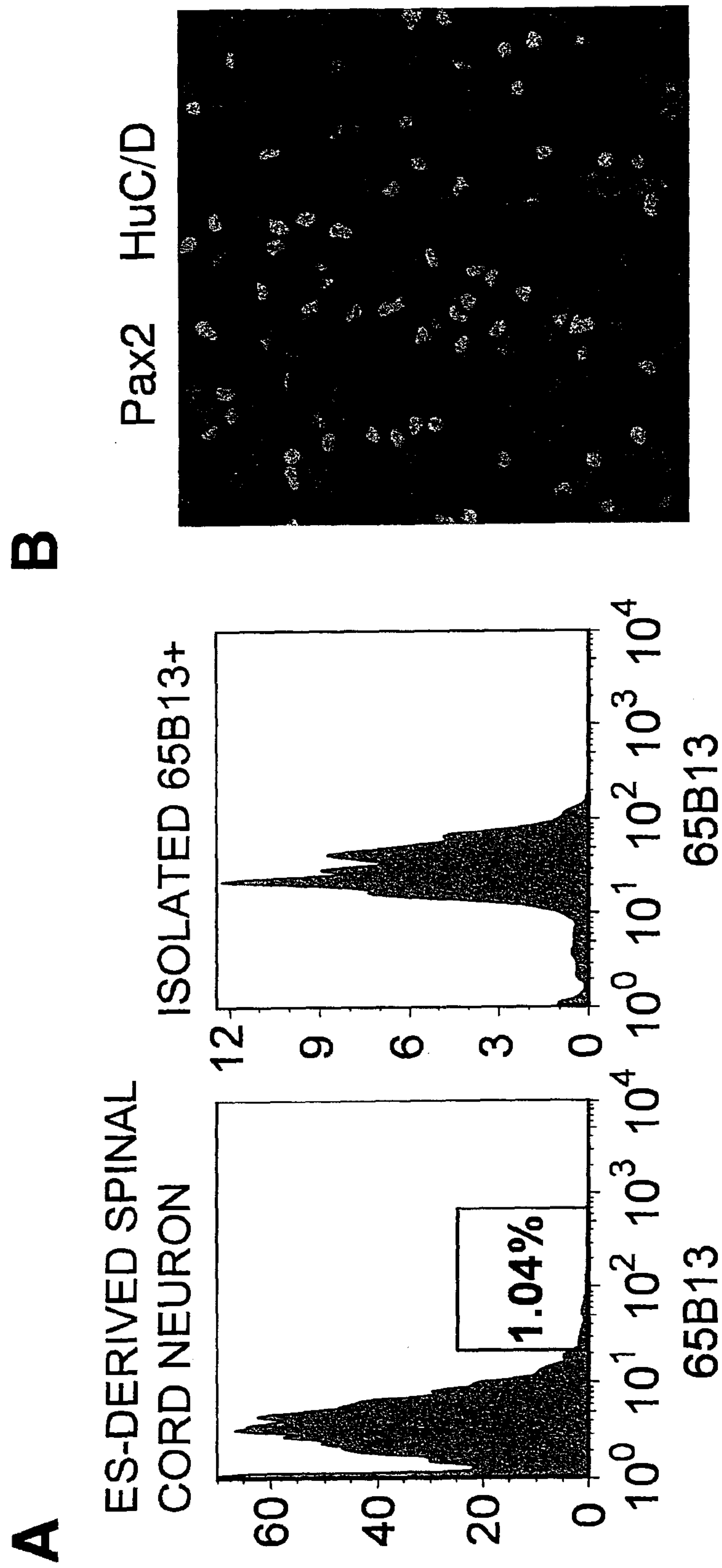


FIG. 9

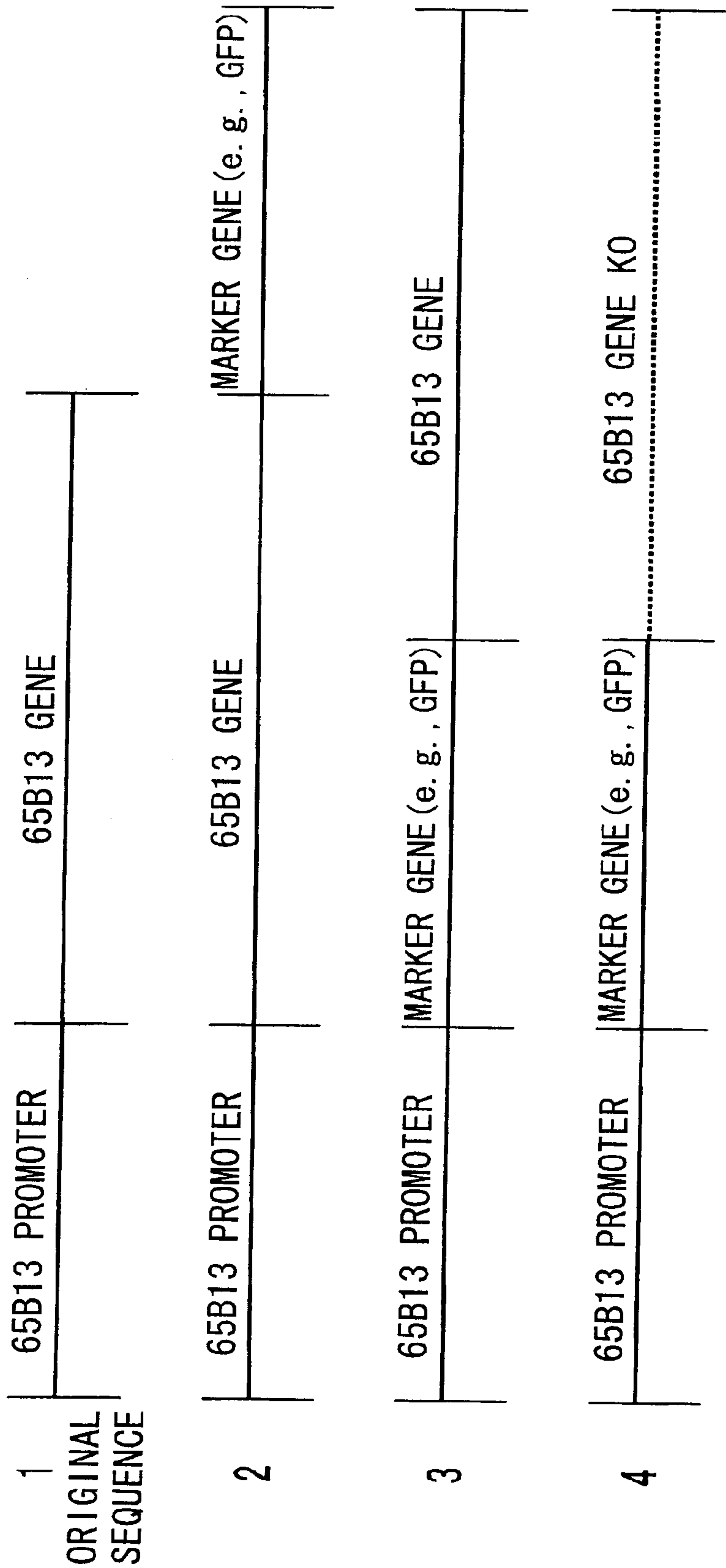


FIG. 10

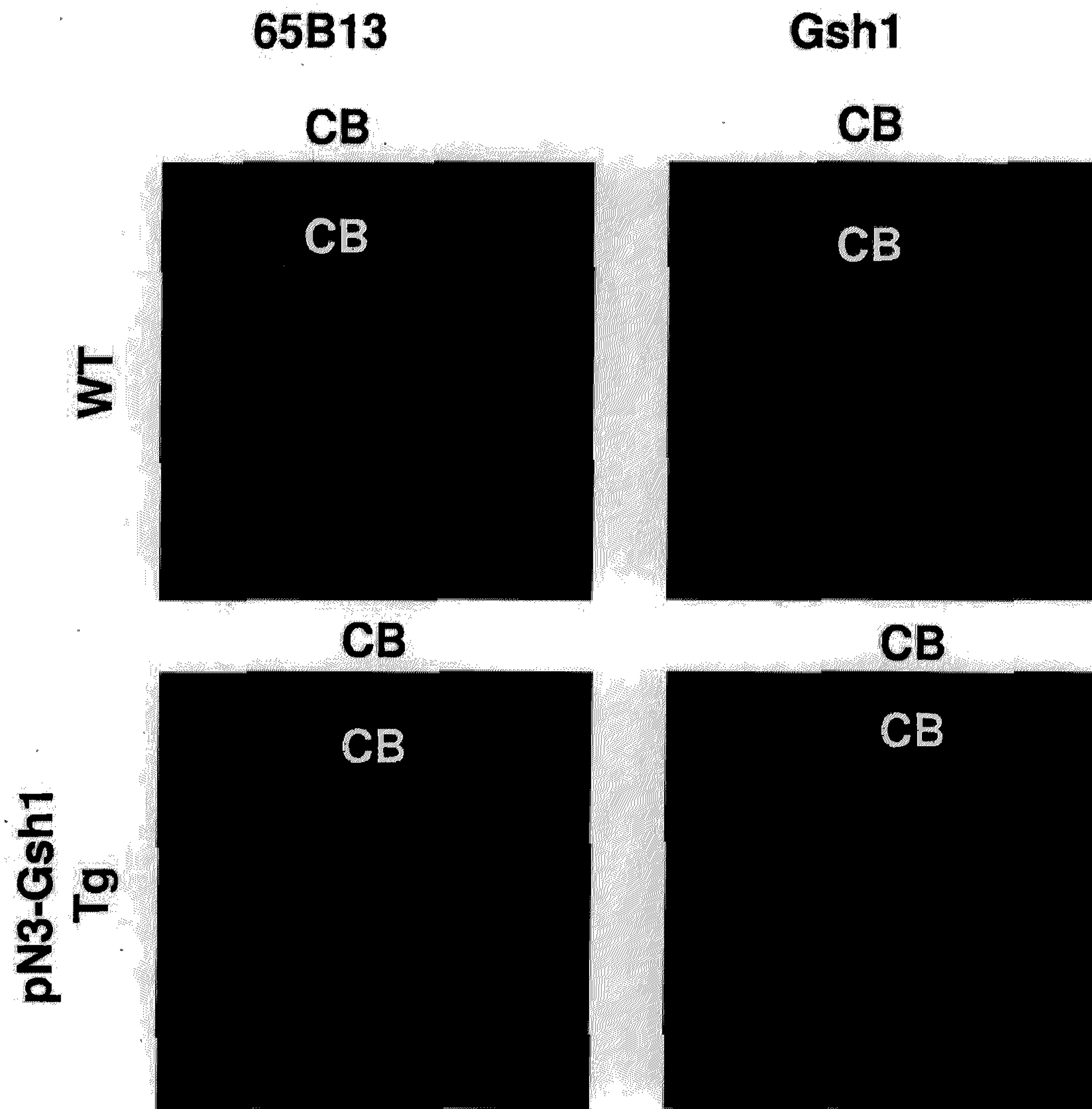


FIG. 11