Glycans are structurally diverse molecules found on the surface of living cells. The protocol details a system developed for combined analysis of glycan and RNA in single cells (scGR-seq) using human induced pluripotent stem cells (hiPSCs) and hiPSC-derived neural progenitor cells (NPCs). scGR-seq consists of DNA-barcoded lectin-based glycan profiling by sequencing (scGlycan-seq) and single-cell transcriptome profiling (scRNA-seq). scGR-seq will be an essential technique to delineate the cellular heterogeneity of glycans across multicellular systems.
Protocol

scGR-seq: Integrated analysis of glycan and RNA in single cells

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SUMMARY

Glycans are structurally diverse molecules found on the surface of living cells. The protocol details a system developed for combined analysis of glycan and RNA in single cells (scGR-seq) using human induced pluripotent stem cells (hiPSCs) and hiPSC-derived neural progenitor cells (NPCs). scGR-seq consists of DNA-barcoded lectin-based glycan profiling by sequencing (scGlycan-seq) and single-cell transcriptome profiling (scRNA-seq). scGR-seq will be an essential technique to delineate the cellular heterogeneity of glycans across multicellular systems.

For complete details on the use and execution of this profile, please refer to Minoshima et al. (2021).

BEFORE YOU BEGIN

The protocol below describes the specific steps for using human induced pluripotent stem cells (hiPSCs) and hiPSC-derived neural progenitor cells (NPCs). However, we have also used this protocol in other cells, such as human dermal fibroblasts, hiPSC-derived neurons, and several cell lines.

This protocol consists of three major steps (Figure 1)-

(i) Single cell Glycan-seq (scGlycan-seq)
(ii) Single cell RNA-seq (scRNA-seq)
(iii) Integrated data analysis (scGR-seq)

Standard cell culture procedures and humidified incubators are required for the maintenance of cell culture.

For scRNA-seq, we use RamDA-seq, a plate-based single-cell total RNA sequencing method (Hayashi et al., 2018).

We use the next-generation sequencer, MiSeq (Illumina) and the barcode DNA counting system (Minoshima et al., 2021) to count DNA-barcodes derived from each lectin.
Preparation of sugar-immobilized column

갑 Timing: 2 days

1. Wash 20 g of Sepharose CL-4B gel using a glass filter in 500 mL of Milli-Q water.
2. Suspend the Sepharose CL-4B gel in 30 mL of Milli-Q water and add 3 mL of epichlorohydrin and 13 mL of 2 N NaOH followed by incubation at 40°C for 2 h with shaking.
3. Wash the Sepharose CL-4B with 500 mL of Milli-Q water.
4. Dissolve 10 mmol of sugar ligands for lectins (see Table 1) in 80 mL of 0.1 M NaOH (pH 13.0) and mix all of it with epoxy-activated Sepharose CL-4B (prepared in step 3). Incubate at 40°C for 24 h followed by washing with 500 mL of Milli-Q water.
5. Resuspend the sugar-immobilized Sepharose CL-4B in 50 mL of 1 M monoethanolamine (pH 8.0) and incubate at 37°C overnight to block the excess epoxy groups.
6. Wash the sugar-immobilized Sepharose CL-4B with 0.1 M acetate buffer (pH 4.0)/0.5 M NaCl and 0.1 M Tris-HCl (pH 8.0)/0.5 M NaCl.
7. Resuspend the sugar-immobilized Sepharose CL-4B in Milli-Q water, and store it at 4°C until further use.
8. Pack 1 mL of the sugar-immobilized Sepharose CL-4B into Micro Bio-Spin Chromatography Columns and store it at 4°C until further use.

Note: 1 g of Sepharose gel contains 0.5 mmol of epoxy groups. The amount of gel to prepare can be reduced depending on the purification scale.

△ CRITICAL: Epichlorohydrin is a toxic substance.

Conjugation of lectin with DNA-barcode

갑 Timing: 1 day
| Name       | Species (origin)       | Rough specificity | Oligo DNA sequence                                                                 | Sugar-immobilized column used for purification | Eluents used for purification |
|------------|------------------------|-------------------|------------------------------------------------------------------------------------|-----------------------------------------------|-------------------------------|
| 1 rPVL     | Psathyrella velutina    | Sia, GlcNAc       | CGACGCTCTCCGATCTCTGCGCCCTGAAAACGGCAAGCGAGTCAGCTGCGAGGACGACAC                     | GlcNAc                                        | 0.2 M GlcNAc                 |
| 2 SNA      | Sambucus nigra         | s2-6Sia           | CGACGCTCTCCGATCTCTGCGCTAGCCAGTTGGTAGCTGAGGACGAGACAC                                 | Lac                                           | 0.2 M Lac                    |
| 3 SSA      | Sambucus sieboldiana   | s2-6Sia           | CGACGCTTCATCGCGCCGCGCGCGGAAAGGCAGTCAGCTGCGAGGACGACAC                              | Lac                                           | 0.2 M Lac                    |
| 4 TJAI     | Trichosanthes japonica|                  | CGACGCTCTCCGATCTCTGCGCCGAGAGGTAGCGAGTCAGCTGCGAGGACGACAC                           | Lac                                           | 0.2 M Lac                    |
| 5 rPSL1a   | Polyporus squamosus    | s2-6Sia           | CGACGCTCTCCGATCTCTGCGCCGAGAGGTAGCGAGTCAGCTGCGAGGACGACAC                           | Lac                                           | 0.2 M Lac                    |
| 6 rDiscoidinII | Dictyostelium dicio - | LacNAc, GaI1-3GaI1Anal (I), sGalNAc (Tn) | CGACGCTCTCCGATCTCTGCGCCGAGAGGTAGCGAGTCAGCTGCGAGGACGACAC | Lac                                           | 0.2 M Lac                    |
| 7 rCGL2    | Coprinopsis cinerea    | GalI NAce1-3GalI1A, PolyIacNAc | CGACGCTCTCCGATCTCTGCGCCGAGAGGTAGCGAGTCAGCTGCGAGGACGACAC | Lac                                           | 0.2 M Lac                    |
| 8 rC14     | Gallus gallus domesticus |                  | CGACGCTCTCCGATCTCTGCGCCGAGAGGTAGCGAGTCAGCTGCGAGGACGACAC                           | Lac                                           | 0.2 M Lac                    |
| 9 GSLII    | Griffonia simplicifolia|                  | CGACGCTCTCCGATCTCTGCGCCGAGAGGTAGCGAGTCAGCTGCGAGGACGACAC                           | GlcNAc                                        | 0.2 M GlcNAc                 |
| 10 rSRL    | Sclerotium rolfsii     |                  | CGACGCTCTCCGATCTCTGCGCCGAGAGGTAGCGAGTCAGCTGCGAGGACGACAC                           | GlcNAc                                        | 0.2 M GlcNAc                 |
| 11 rF17AG  | Escherichia coli       |                  | CGACGCTCTCCGATCTCTGCGCCGAGAGGTAGCGAGTCAGCTGCGAGGACGACAC                           | GlcNAc                                        | 0.2 M GlcNAc                 |
| 12 rGRFT   | Griffithsia sp.        |                  | CGACGCTCTCCGATCTCTGCGCCGAGAGGTAGCGAGTCAGCTGCGAGGACGACAC                           | Man                                           | 0.2 M Man                    |
| 13 ConA    | Canavalia ensiformis   |                  | CGACGCTCTCCGATCTCTGCGCCGAGAGGTAGCGAGTCAGCTGCGAGGACGACAC                           | Man                                           | 0.2 M Me-α-Man               |
| 14 rOrysata | Oryza sativa          |                  | CGACGCTCTCCGATCTCTGCGCCGAGAGGTAGCGAGTCAGCTGCGAGGACGACAC                           | Man                                           | 0.2 M Man                    |
| 15 rPALa   | Phlebodium aureum      |                  | CGACGCTCTCCGATCTCTGCGCCGAGAGGTAGCGAGTCAGCTGCGAGGACGACAC                           | Man                                           | 0.2 M Man                    |
| 16 rBanana | Musa acuminata        |                  | CGACGCTCTCCGATCTCTGCGCCGAGAGGTAGCGAGTCAGCTGCGAGGACGACAC                           | Man                                           | 0.2 M Man                    |
| 17 rCalsepa | Calystegia sepium     |                  | CGACGCTCTCCGATCTCTGCGCCGAGAGGTAGCGAGTCAGCTGCGAGGACGACAC                           | Man                                           | 0.2 M Man                    |
| 18 rRLS    |Ralstonia solanacearum |                  | CGACGCTCTCCGATCTCTGCGCCGAGAGGTAGCGAGTCAGCTGCGAGGACGACAC                           | Man                                           | 0.2 M Man                    |
| 19 rBC2LA  | Burkholderia cenocepacia |                  | CGACGCTCTCCGATCTCTGCGCCGAGAGGTAGCGAGTCAGCTGCGAGGACGACAC                           | Man                                           | 0.2 M Man                    |
| 20 rAAL    | Aleuria aurantia      | Fucose            | CGACGCTCTCCGATCTCTGCGCCGAGAGGTAGCGAGTCAGCTGCGAGGACGACAC                           | Fuc                                           | 0.2 M Fuc                    |

(Continued on next page)
| Name       | Species (origin) | Rough specificity | Oligo DNA sequence | Sugar-immobilized column used for purification | Eluents used for purification |
|------------|------------------|-------------------|--------------------|-----------------------------------------------|-------------------------------|
| 21 rRSIL   | Ralstonia solanacearum | α-Man, α-1-2Fuc (H), α-1-3Fuc (Lex), α-1-4Fuc (Lea) | CGACGCTTCTCGATCTCTGTCGTCGATCGGAGAGGAAGCAAACAC | Fuc | 0.2 M Fuc |
| 22 rPhoSL  | Pholiota squarrosa  | α-1-6Fuc | CGACGCTTCTCGATCTCTGAGCTCGGATCCTGGTTGGAACAGGACAC | Fuc | 0.2 M Fuc |
| 23 rAOL    | Aspergillus oryzae | α-Man, α-1-2Fuc (H), α-1-3Fuc (Lex), α-1-4Fuc (Lea) | CGACGCTTCTCGATCTCTGATGTCGATCGGAGAGGAAGCAAACAC | Fuc | 0.2 M Fuc |
| 24 rBC2LCN | Burkholderia cenocepacia | Fuc | CGACGCTTCTCGATCTCTGAGCTCGGATCCTGGTTGGAACAGGACAC | Fuc | 0.2 M Fuc |
| 25 UEAI    | Ulex europaeus     | Fuc | CGACGCTTCTCGATCTCTGAGCTCGGATCCTGGTTGGAACAGGACAC | Fuc | 0.2 M Fuc |
| 26 TJAlII  | Trichosanthes japonica | α-1-2Gal (B) | CGACGCTTCTCGATCTCTGAGCTCGGATCCTGGTTGGAACAGGACAC | Fuc | 0.2 M Fuc |
| 27 rGC2    | Geodia cydonium     | α-1-2Fuc (H), α-GalNAc (A), α-Gal (B) | CGACGCTTCTCGATCTCTGAGCTCGGATCCTGGTTGGAACAGGACAC | Lac | 0.1 M Lac |
| 28 rMOA    | Marasmius oreades   | α-Gal (B) | CGACGCTTCTCGATCTCTGAGCTCGGATCCTGGTTGGAACAGGACAC | Melibiose | 0.1 M Lac |
| 29 rPAIL   | Pseudomonas aeruginosa | α,β-Gal, α-GalNAc (Tn) | CGACGCTTCTCGATCTCTGAGCTCGGATCCTGGTTGGAACAGGACAC | Lac | 0.1 M Lac |
| 30 rGal3C  | Homo sapiens       | LacNAc, polylactosamine | CGACGCTTCTCGATCTCTGAGCTCGGATCCTGGTTGGAACAGGACAC | Lac | 0.1 M Lac |
| 31 rLSLN   | Latitoporus sulphureus | LacNAc, polylactosamine | CGACGCTTCTCGATCTCTGAGCTCGGATCCTGGTTGGAACAGGACAC | Lac | 0.1 M Lac |
| 32 HPA     | Helix pomatia      | α-GalNAc (A, Tn) | CGACGCTTCTCGATCTCTGAGCTCGGATCCTGGTTGGAACAGGACAC | GalNAc | 0.2 M GalNAc |
| 33 rPPL    | Pleurocybella porrigens | α,β-GalNAc (A, Tn, LacDiNAc) | CGACGCTTCTCGATCTCTGAGCTCGGATCCTGGTTGGAACAGGACAC | Lac | 0.1 M Lac |
| 34 rCNL    | Clitocybe nebularis | α,β-GalNAc (A, Tn, LacDiNAc) | CGACGCTTCTCGATCTCTGAGCTCGGATCCTGGTTGGAACAGGACAC | Lac | 0.1 M Lac |
| 35 WFA     | Wisteria floribunda | Terminal GalNAc, LacDiNAc | CGACGCTTCTCGATCTCTGAGCTCGGATCCTGGTTGGAACAGGACAC | GalNAc | 0.2 M GalNAc |
| 36 rABA    | Agaricus bisporus  | Gal(1-3)GalNAc (T), GlcNAc | CGACGCTTCTCGATCTCTGAGCTCGGATCCTGGTTGGAACAGGACAC | GlcNAc | 0.2 M GlcNAc |
| 37 rDiscoindl | Dictyostelium Discoideum | Gal | CGACGCTTCTCGATCTCTGAGCTCGGATCCTGGTTGGAACAGGACAC | Lac | 0.2 M Lac |
| 38 rMalectin | Homo sapiens | Glcα1-2Glc | CGACGCTTCTCGATCTCTGAGCTCGGATCCTGGTTGGAACAGGACAC | Maltose | 0.2 M Maltose |
| 39 CSA     | Oncohynchus keta   | Rhamnose, Galα1-4Gal | CGACGCTTCTCGATCTCTGAGCTCGGATCCTGGTTGGAACAGGACAC | Rhamnose | 0.2 M Rhamnose |
| 40 mIgG    | Mus musculus      | ND | CGACGCTTCTCGATCTCTGAGCTCGGATCCTGGTTGGAACAGGACAC | Protein G | 0.1 HCl |
| 41 gIgG    | Ovis aries       | ND | CGACGCTTCTCGATCTCTGAGCTCGGATCCTGGTTGGAACAGGACAC | Protein G | 0.1 M Glycine-HCl |
9. Mix 100 µL of lectin (1 mg/mL in PBS) with 10× concentration of photocleavable-dibenzyloclclooctyne-N-hydroxysuccinimide ester (PC-DBCO-NHS) and incubate at 20°C for 1 h in the dark.

10. Add 10 µL of 1 M Tris-HCl (pH 8.0), and incubate at 20°C for 15 min in the dark to block excess PC-DBCO-NHS.

11. After incubation, remove the free PC-DBCO-NHS using the G-25 desalting miniature column (see materials and equipment).

12. Add 200 µM of 5'-azide-DNA (10× concentration, see Table 1) to the PC-DBCO-lectin to generate DNA-barcode lectins.

### Purification of DNA-barcode lectins

© Timing: 2 days

13. The sugar-immobilized Sepharose CL-4B column (1 mL in miniature column) is washed with 1 mL PBSE at 4°C.

14. Add 100 µL of PBSE into the DNA-barcode lectin solutions and apply onto the sugar-immobilized Sepharose CL-4B column. Recover the flow-through fraction (100 µL).

15. Wash the sugar-immobilized Sepharose CL-4B column with 400 µL of PBSE three times. Recover each of the wash fractions (400 µL each).

16. Add 400 µL of the elution solution comprising PBSE containing an appropriate sugar for each lectin (see Table 1). Repeat this step three times. Recover each of the elution fractions (400 µL each).

17. Analyze the DNA-barcode lectins by SDS-PAGE (Figure 2). Mix 4 µL of each fraction of the purification steps (lectin only, flow-through, wash, elution) with 4 µL of SDS sample buffer.

18. Load 8 µL of the samples as well as 5 µL of Prestained Protein Size Marker onto 17% SDS-PAGE gel. Run the SDS-PAGE using SDS running buffer at 100 V for 20 min.

19. Stain the SDS-PAGE gel with GelRed followed by the manufacturer’s protocol. This step can stain free as well as lectin-conjugated DNA-barcode.

20. Stain the SDS-PAGE gel with silver staining reagents followed by the manufacturer’s protocol. The gel used for the GelRed staining can be used for silver staining.

**Figure 2. Preparation of DNA-barcode lectins**

(A) Illustration of reaction process to conjugate DNA oligonucleotides to lectin.

(B) rBC2LCN shows a single band at 16 kDa (lane 1). DNA-barcode rBC2LCN exhibited a high-molecular weight smear band at >140 kDa (lane 2). Cleavage of DNA barcode from rBC2LCN by UV exposure collapses the smear to the MW of rBC2LCN (16 kDa) (lane 3). Figure reprinted with permission from Minoshima et al. (2021).
21. Recover the elution fractions and dialyze the purified DNA-barcoded lectins against 0.1 x PBS (for dialysis) using Tube-O-Dialyzer, Medi 8kD.
22. Concentrate the DNA-barcoded lectins using a centrifugal filter (Amicon ultra 0.5 mL 10K) having a 10 kDa molecular weight cut off.
23. Quantify the protein and DNA concentration using the Bradford and Quant-iT OliGreen ssDNA Reagent Kit, respectively, and determine the DNA-to-lectin ratio.
24. Mix 41 DNA-barcoded probes (5 μg/mL, final concentration, for each lectin) (Table 1) into a 1.5 mL tube and fill up to 100 μL with PBS/BSA.
25. Store it at –30°C.

**Note:** Any lectins can be used for scGR-seq, but we recommend to check whether the lectins show no reaction each other using assays such as lectin blotting. We labeled 41 probes with DNA barcodes (Table 1), which cover a wide range of glycans such as sialylated, galactosylated, mannosylated, GlcNAcylated, and fucosylated glycans.

**Caution:** Some lectins are eluted in washing fractions. In this case, recover the wash fractions and use for the experiments.

**KEY RESOURCES TABLE**

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**      |        |            |
| PE Mouse anti-Human PAX6 | BD Biosciences | clone No. O18-1330 |
| PE Mouse anti-NESTIN | BD Biosciences | clone 25/NESTIN (RUO) |
| Mouse anti-Oct3/4   | Santa Cruz Biotechnology, Inc. | Cat#sc-5279 |
| Mouse anti-SSEA-4   | Merck | Cat#MAB4304 |
| Donkey anti-mouse IgG Alexa Fluor 488 polyclonal antibody (pAb) | Thermo Fisher Scientific | Cat#A21202 |
| **Chemicals, peptides, and recombinant proteins** | | |
| PBS                 | Fujifilm Wako Pure Chemical Co. | Cat#045-29795 |
| EDTA                | Sigma-Aldrich | Cat#09-1420-5 |
| BSA                 | Merck KGaA | Cat#A3059 |
| Tris-HCl            | Nacalai Tesque, Inc. | Cat#35434-21 |
| Na2HPO4 12H2O       | Fujifilm Wako Pure Chemical Co. | Cat#196-02835 |
| NaCl                | Fujifilm Wako Pure Chemical Co. | Cat#191-01665 |
| KCl                 | Fujifilm Wako Pure Chemical Co. | Cat#163-03545 |
| KH2PO4              | Fujifilm Wako Pure Chemical Co. | Cat#166-04255 |
| Nuclease-free water | QIAGEN | Cat#129114 |
| Epichlorohydrin     | NacalaiTesque, Inc. | Cat#14415-05 |
| Monoethanolamine    | Cytiva | Cat#BR-1000-50 |
| PC-DBCO-NHS ester   | Click Chemistry Tools | Cat#1160-10 |
| WIDE-VIEW Prestained Protein Size Marker | Fujifilm Wako Pure Chemical Co. | Cat#230-02221 |
| GelRed              | Biotium | Cat#41002 |
| mTeSR Plus          | VERITAS | Cat#ST-100-0276 |
| Matrigel            | CORNING | Cat#REF 354230 |
| Gentle Cell Dissociation Reagent | VERITAS | Cat#ST-100-0485 |
| mFreSR Cryopreservation Medium | VERITAS | Cat#ST-05855 |
| MesenPRO RS medium  | Thermo Fisher Scientific KK | Cat#12746012 |
| Dibenzocylooctyne-N-hydroxy succinimidyl ester | Funakoshi Co., Ltd | Cat#A133-25 |
| Y-27632             | Fujifilm Wako Pure Chemical Co. | Cat#3924591 |
| Accutase            | Innovative Cell Technologies, Inc. | Cat#AT104 |
| DMEM/F12 media      | Thermo Fisher Scientific KK | Cat#11330032 |

(Continued on next page)
### Critical commercial assays

- **Silver Stain MS Kit**
  - Fujifilm Wako Pure Chemical Co.
  - Cat#299-58901
- **Bradford protein assay**
  - Bio-Rad Laboratories
  - Cat#500001JA
- **Quanti-T OliGreen ssDNA Reagent**
  - Thermo Fisher Scientific KK
  - Cat#Q7582
- **PowerUp SYBR Green Master Mix**
  - Thermo Fisher Scientific KK
  - Cat#A25741
- **STEMdiff SMADi Neural Induction Kit (STEMdiff™ Neural Induction Medium/SMADi Neural Induction Supplement)**
  - VERITAS
  - Cat#08581
- **NEBNext Ultra II Q5 Master Mix**
  - New England BioLabs Japan Inc
  - Cat#MS05445
- **Agencourt AMPure XP kit**
  - Beckman Coulter, Inc.
  - Cat#BC-A63880
- **MiSeq Reagent Kit v2 50 Cycles**
  - Illumina KK
  - Cat#MS-102-2001
- **GenNext RamDA-seq Single Cell Kit**
  - TOYOBO
  - Cat#RMD-101

(Continued on next page)
Note: Dissolve sodium acetate trihydrate and NaCl with 500 mL MilliQ, adjust the pH to 4.0 by acetic acid, and fill up to 1 L with MilliQ.

### MATERIALS AND EQUIPMENT

#### 0.1 M acetate buffer (pH 4.0)/0.5 M NaCl

| Reagent                        | Final concentration | Amount   |
|--------------------------------|---------------------|----------|
| Sodium acetate trihydrate      | 0.1 M               | 13.6 g   |
| NaCl                           | 0.5 M               | 29.22 g  |

#### Deposited data

- All raw data of scRNA-seq: Minoshima et al. (2021) Cat#GSE151642
- All raw data of scGlycan-seq: Minoshima et al. (2021) N/A

#### Experimental models: Cell lines

- Human: iPS cell line 201B7: RIKEN BioResource Center Cat#HPS0063

#### Oligonucleotides

- Primer: i7 index primer: Table S1
- Primer: i5 index primer: Table S1
- 5'-azide-DNA oligonucleotide: Table 1

#### Software and algorithms

- Barcode DNA counting system: Minoshima et al. (2021) [https://github.com/bioinfo-tsukuba/barcode-dna-counting-system](https://github.com/bioinfo-tsukuba/barcode-dna-counting-system)
- PoolQ-3.3.2: The Broad Institute Genetic Perturbation Platform [https://portals.broadinstitute.org/gpp/public/software/poolq](https://portals.broadinstitute.org/gpp/public/software/poolq)
- Subio Platform version 1.24.5849: Subio Inc. [https://www.subioplatform.com/](https://www.subioplatform.com/)
- R version 3.6.1: The R Foundation [https://www.r-project.org/](https://www.r-project.org/)
- Seurat version 4.02: Satija Lab [https://satijalab.org/seurat/index.html](https://satijalab.org/seurat/index.html)

#### Other

- Sepharose CL-4B: Cytiva Cat#17015001
- Protein low binding microtubule: WATSON Co., Ltd. Cat#PK-15C-500
- Protein low binding microtubule: WATSON Co., Ltd. Cat#PK-15C-500
- Amicon ultra 0.5 mL 10K: Merck KGaA Cat#UFCS01096
- TOPick I Live Cell Pick system: Yodaka Giken N/A
- Glass needle: Yodaka Giken N/A
- Optical Flat 8-Cap Strips for 0.2 mL tube strips: Bio-Rad Laboratories Cat#TCS0803
- DynaMag-2 Magnet (magnetic stand): Thermo Fisher Scientific KK Cat#12321D
- MiSeq: Illumina, Inc. N/A
- MultiNA: Shimadzu Co. N/A
- UVP Blak-Ray XX-15L UV Bench Lamp: Analytik Jena US, An Endress+Hauser Company Cat#95-0042-12
- Sephades G-25 fine: GE Healthcare Cat#17-0032-01
- Tubu-O-Dialyzer, Medi 8kD: BIOSCIENCES Cat#786617
- PANETRA Gel 17% 20w: DRC Cat#NSV-3X6P20
- Micro Bio-Spin Chromatography Columns: Bio-Rad Laboratories Cat#7326204
### 0.1 M Tris-HCl (pH 8.0)/0.5 M NaCl

| Reagent          | Final concentration | Amount  |
|------------------|---------------------|---------|
| Tris-HCl         | 0.1 M               | 12.1 g  |
| NaCl             | 0.5 M               | 29.22 g |

**Note:** Dissolve Tris-HCl and NaCl with 500 mL MilliQ, adjust the pH to 4.0 by acetic acid, and fill up to 1 L with Milli Q.

### SDS sample buffer

| Reagent                  | Final concentration | Amount  |
|--------------------------|---------------------|---------|
| 1 M Tris-HCl (pH 6.8)    | 0.125 M             | 1.25 mL |
| SDS                      | 4%                  | 4 g     |
| Sucrose                  | 10%                 | 1 g     |
| Bromophenol Blue         | 0.004%              | 0.4 mg  |

**Note:** Fill up to 10 mL with MilliQ.

### SDS running buffer

| Reagent | Final concentration | Amount  |
|---------|---------------------|---------|
| Tris-HCl| 25 mM               | 3.03 g  |
| Glycine | 192 mM              | 14.4 g  |
| SDS     | 0.1%                | 1 g     |

**Note:** Fill up to 10 mL with MilliQ.

### PBS (for dialysis)

| Reagent                  | Final concentration | Amount  |
|--------------------------|---------------------|---------|
| Na₂HPO₄·12H₂O            | 5.86 mM             | 2.1 g   |
| NaCl                     | 130 mM              | 8 g     |
| KCl                      | 2.7 mM              | 0.2 g   |
| KH₂PO₄                  | 1.48 mM             | 0.2 g   |
| EDTA                     | 1 mM                | 3.72 g  |

**Note:** Fill up to 1 L with MilliQ.

### PBSE

| Reagent                  | Final concentration | Amount  |
|--------------------------|---------------------|---------|
| Na₂HPO₄·12H₂O            | 5.86 mM             | 2.1 g   |
| NaCl                     | 130 mM              | 8 g     |
| KCl                      | 2.7 mM              | 0.2 g   |
| KH₂PO₄                  | 1.48 mM             | 0.2 g   |
| EDTA                     | 1 mM                | 3.72 g  |

**Note:** Fill up to 1 L with MilliQ.

### G-25 desalting miniature column

| Reagent                  | Final concentration | Amount  |
|--------------------------|---------------------|---------|
| Sephadex G-25 fine       | n/a                 | 0.8 mL  |
| Micro Bio-Spin Chromatography Columns | n/a             | n/a  |

**Note:** Wash Sephadex G-25 fine with TBS, and dispense 0.8 mL of Sephadex G-25 fine into the column and store it at 4°C.
Note: Filter the reagent using 0.22 μm PVDF membrane and store it at 4°C.

### PBS/BSA

| Reagent       | Final concentration | Amount |
|---------------|---------------------|--------|
| PBS           | n/a                 | n/a    |
| BSA           | 1%                  | 10 g   |
| Total         | n/a                 | 1 L    |

Note: Aliquot and store at –20°C; however, it can be stored at 2°C–8°C for up to 2 weeks if not used immediately.

### PBS/BSA/CaCl₂

| Reagent       | Final concentration | Amount |
|---------------|---------------------|--------|
| PBS/BSA       | n/a                 | 1 L    |
| CaCl₂         | 1 mM                | N/A    |
| Total         | n/a                 | 1 L    |

### STEMdiff™ Neural Induction Medium/SMADi Neural Induction Supplement

| Reagent                        | Final concentration | Amount |
|--------------------------------|---------------------|--------|
| STEMdiff™ Neural Induction Medium | n/a              | 250 mL |
| STEMdiff™ SMADi Neural Induction Supplement | n/a              | 0.5 mL |
| Total                          | n/a               | 250.5 mL |

Note: Cell culture of hiPSCs

- **Timing:** 3–4 days
- **CRITICAL:** Perform all cell culture experiments inside a biosafety cabinet, and wear personal protective equipment, including gloves and goggles.

1. Coat each well of 6-well plate with 1 mL of Matrigel and let it sit at room temperature (RT) for 1 h.
2. Thaw the mTeSR Plus media at 37°C for 5–15 min. Plate the appropriate number of 201B7 hiPSCs in a 6-well plate containing 2 mL of the mTeSR Plus media.
3. Culture the cells for 2–3 days in a CO₂ incubator (with CO₂ level set to 5%).
4. Recover the cells with gentle cell dissociation reagent and resuspend them in the mTeSR Plus media supplemented with 10 μM Y-27632.

Pause point: hiPSC are suspended in mFreSR Cryopreservation Medium and stored in liquid nitrogen.

### Generation of hiPSC-derived neural progenitor cells (NPCs)

- **Timing:** 11 days

5. Thaw STEMdiff™ Neural Induction Medium and STEMdiff™ SMADi Neural Induction Supplement at room temperature (15°C–25°C) or overnight (2°C–8°C). Swirl both media thoroughly.
6. Add 0.5 mL of STEMdiff™ SMADi Neural Induction Supplement to 250 mL of STEMdiff™ Neural Induction Medium (NIM). Mix them thoroughly and warm them to room temperature before use.
7. Coat each well of 6-well plate with 1 mL of Matrigel and let it sit at room temperature for 1 h.
8. Wash the hiPSC cultured well with 2 mL of phosphate-buffered saline (PBS).
9. Add 1 mL of gentle cell dissociation reagent and incubate at 37°C for 8–10 min.
10. Pipet up and down 3–5 times to dissociate the cell aggregates. Collect the cells in a 15 mL conical tube.
11. Wash the plate with 2 mL PBS and collect the remaining cells into a 15 mL conical tube.
12. Count the viable cells with a hemocytometer using Trypan blue dye method.
13. Centrifuge the 15 mL conical tube at 300×g for 4 min. Aspirate and discard the supernatant without disturbing the cell pellet.
14. Resuspend the cell pellets with NIM supplemented with 10 μM Y-27632 to achieve a final concentration of 1 × 10⁶ cells/mL.
15. Aspirate the Matrigel from a 6-well plate and add 2 mL cell suspension (2 × 10⁶ cells/well) into a single well of Matrigel-coated plate.
16. Incubate the cells at 37°C, 5% CO₂ for 11 days. Fresh NIM without Y-27632 is used for medium changes every other day.
17. Aspirate the media from the plates, add 1 mL of Accutase per well, and incubate at 37°C, 5% CO₂ for 5 min.
18. Collect the cell suspension in a 15 mL conical tube. Wash the plates with 2 mL pre-warmed DMEM/F12 media, and collect the residual in the same tube. Centrifuge the cell suspension at 300×g for 4 min, and the resulting cell pellet is resuspended in NIM and used for the evaluation of the differentiation state by qRT-PCR and fluorescence staining of hiPSC markers (POUSF1) and NPC markers (SOX1, NESTIN, PAX6, FOXG1).

Pause point: hiPSC-derived NPCs are suspended in mFreSR Cryopreservation Medium and stored in liquid nitrogen.

Single cell glycan-seq

© Timing: 2 days

△ CRITICAL: Contamination with DNA and DNase will significantly affect the experiments. Perform all experiments inside a biosafety cabinet in the dark, and wear personal protective equipment, including gloves and goggles. Take extreme care while handling all the reagents to prevent contamination with DNA and DNase. Prepare and dispense all reagents on ice unless otherwise stated.

19. Incubate the cells with DNA-barcoded lectins.
   a. Take 1 × 10⁵ cells in a 1.5 mL microtube and centrifuge at 600×g for 4 min at 4°C.
   b. Discard the supernatant and resuspend the pellet in 1 mL of PBS/BSA. Repeat the washing step twice.
   c. Discard the supernatant and resuspend in 90 μL of PBS/BSA/CaCl₂ containing 10 μL of DNA-barcoded lectin mix (5 μg/mL of each lectin, 10 μL).
   d. Incubate the cells on ice for 1 h in the dark followed by centrifuge at 600×g for 4 min at 4°C.
   e. Discard the supernatant and resuspend the pellet in 1 mL of PBS/BSA/CaCl₂. Repeat the washing step three times.
   f. Add 200 μL of PBS/BSA/CaCl₂ and count the cell number.

△ CRITICAL: CaCl₂ should be added in the solution when you use lectins, which require Calcium for glycan-binding activity.

20. Dispense single-cells into tubes.
   a. Dispense single cells into the cap of Optical Flat 8-Cap Strips (for 0.2 mL tubes) by manually picking them using TOPick I Live Cell Pick system or other methods.
   b. Cover the tubes with the cap containing single cells, spin down, and keep them on ice.
21. Expose the cells to UV light for 15 min using UVP Blak-Ray XX-15L UV Bench Lamp to liberate DNA-barcodes from lectins.
22. Centrifuge at 3,549 \times g for 30 s at 4°C and transfer the supernatant into a new 0.2 mL 8-tube strips, kept on ice.

\[\Delta \text{CRITICAL: Be careful not to touch the cells with the pipette tip. You can leave 0.5 } \mu\text{L of supernatant in the tube.}\]

\[\text{Pause point: The supernatant can be stored at } -80^\circ\text{C.}\]

23. Add 2.5 \mu\text{L of cell lysis buffer into the tube and cover the tube with a cap.}

24. Spin down and store at \(-80^\circ\text{C}\) for single-cell RNA-seq (see the next section "single cell RNA-seq.").

25. Perform PCR to amplify the DNA-barcodes for sequencing.
   a. Prepare the PCR mix as follows: each sample contains 25 \mu\text{L of total reaction volume- 9.75 } \mu\text{L of supernatant (template), 12.5 } \mu\text{L of NEBNext UltraII Q5 Master Mix, 0.25 } \mu\text{L of i5 index primer (100 mM), and 2.5 } \mu\text{L of i7 index primer (10 mM).}
   b. Perform the PCR reactions as follows.

| PCR cycling conditions |
|------------------------|
| Steps                  | Temperature | Time | Cycles |
| Initial Denaturation   | 98°C        | 45 s | 1      |
| Denaturation           | 98°C        | 10 s | 25–35 cycles |
| Annealing and extension| 65°C        | 50 s |        |
| Final extension        | 65°C        | 5 min| 1      |
| Hold                   | 4°C         | forever|

26. Purify the PCR products.
   a. Combine 16 samples into a 1.5 mL microtube.
   b. Add 320 \mu\text{L of AMPure into it and gently pipette the contents 10 times.}
   c. After incubation at RT for 5 min, expose the tubes to the magnetic stand for 2 min.
   d. Discard the supernatants without disturbing the magnetic beads on the magnetic stand.
   e. Add 1 mL of 80% ethanol into the 1.5 mL microtube followed by incubation at RT for 30 s on the magnetic stand. Discard the supernatant and repeat the washing step three times.
   f. Air-dry the magnetic beads at RT for 10 min.

\[\Delta \text{CRITICAL: When beads are dried, the color of the beads becomes lighter. If it dried too much, it would be difficult to elute.}\]

g. Remove 1.5 mL microtube from the magnetic stand and add 160 \mu\text{L of 10 mM Tris (pH 8.5).}
   h. Gently pipette the contents 10 times and incubate at RT for 2 min.
   i. Expose the tube to the magnetic stand and carefully collect the supernatant to a 15 mL tube.

27. Concentrate the PCR products.
   a. Add 5 volumes of DNA Binding Buffer to the 15 mL tube and mix briefly by vortexing.
   b. Transfer the mixture into a Collection Tube containing Zymo-Spin Column.
   c. Centrifuge for 30 s and discard the flow-through.
   d. Add 200 \mu\text{L of DNA Wash Buffer to the column and centrifuge it for 30 s. Repeat the washing step.}
   e. Add 20 \mu\text{L DNA Elution Buffer directly to the column matrix and incubate at RT for 1 min. Transfer the column to a 1.5 mL microcentrifuge tube and centrifuge at 20,400 \times g for 30 s at 4°C to elute the DNA.}

28. Use 6.5 \mu\text{L of the elution fraction to analyze the size and quantity of the PCR products, using the microchip electrophoresis system–MultiNA with DNA-500 kit–according to the manufacturer’s}
instructions. A single band between 150 and 175 bp will appear if the DNA library is constructed successfully.

Pause point: DNA library can be stored at -20°C.

**Alternatives:** For the analysis of the size and quantity of the PCR products, Agilent Bioanalyzer or Agilent TapeStation could be considered.

29. Denaturing library DNA
   (for > 4 nM of library DNA)
   a. Dilute the concentration of library DNA to 4 nM with nuclease-free water and mix it in equal amounts.
   b. Mix 4 μL of the library DNA mixture of all the samples with 4 μL of 0.1 N NaOH, briefly by vortexing and spin down.
   c. Incubate at RT for 5 min and keep them on ice.
   d. Add 4 μL of 2 nM library DNA to 796 μL of pre-chilled HT1 for a total of 800 μL (10 pM) library DNA. Mix briefly by vortexing and spin down.
   (for < 4 nM of library DNA)
   e. Mix each library DNA in equal amounts
   f. Mix 2 μL of the library DNA mixture of all the samples with 2 μL of 0.1 N NaOH, briefly by vortexing and spin down.
   g. Incubate at RT for 5 min and keep them on ice.
   h. Add 2 μL of 200 mM Tris-HCl (pH 7.0) and mix briefly by vortexing and spin down.
   i. Add 6 μL of library DNA to 534 μL of pre-chilled HT1 for a total of 540 μL, library DNA. Mix briefly by vortexing and spin down.

30. Denaturing PhiX
   a. Mix 1 μL of 10 nM PhiX, 4 μL of nuclease-free water, and 5 μL of 0.1 N NaOH for a total of 10 μL, 1 nM of PhiX. Mix briefly by vortexing and spin down.
   b. Incubate at RT for 5 min and chill on ice.
   c. Mix 2 μL of 1 nM denatured PhiX and 248 μL of pre-chilled HT1 for a total of 250 μL (8 pM PhiX). Mix briefly by vortexing and spin down.

31. Mix 540 μL of library DNA (step 29) with 130 μL of 8 pM PhiX (step 30).
32. Heat at 96°C for 2 min and chill on ice immediately followed by incubation for 5 min.
33. Load 600 μL of the library mix into the reagent cartridge of MiSeq Reagent kit and run the setup according to the manufacturer’s instructions.

**Single cell RNA-seq**

Timing: 1–2 days

CRITICAL: Contamination with RNase and DNA will significantly affect the experiments. Perform all experiments inside a biosafety cabinet in the dark, and wear personal protective equipment, including gloves, masks, and goggles. Wipe all instruments used for the experiment, i.e., pipette, centrifuge, mixer, thermal cycler, laboratory bench, with RNase remover –RnaseAway. Take extreme care while handling all the reagents to prevent contamination with RNase. In order to inactivate RNase and maintain enzyme activity, prepare and dispense all reagents on ice, unless otherwise stated.

34. Prepare cDNA library using a full-length total RNA-sequencing method –Random displacement amplification sequencing (RamDA-seq)–from single cells, according to manufacturer’s instructions.
35. Quantify the library DNA from individual samples derived from single cells, using the microchip electrophoresis system–MultiNA with DNA-12000 kit–according to the manufacturer’s instructions. A band of 150–600 bp will appear if the DNA library is constructed successfully.

36. Pool and mix each library DNA and transfer 50–100 fmol into a 1.5 mL tube.

37. Sequence the mixed library DNA using a next-generation sequencer such as Novaseq6000 according to the sequencer guidelines.

EXPECTED OUTCOMES

A successful scGR-seq output amounts to approximately 5,000 of the total DNA barcode counts. In the case of scRNA-seq, approximately 10,000 genes should be detected. In UMAP, hiPSCs and NPCs are separated into two clusters based on Glycan-seq and RNA-seq data (Figure 3). hiPSC-specific lectin, rBC2LCN, shows higher binding to hiPSCs than NPCs (Figure 4A). In contrast, rBanana shows higher binding to NPCs than hiPSCs (Figure 4A). hiPSCs show higher expression of hiPSC-specific genes such as NANOG and POU5F1 (Figure 4B); in comparison, NPCs show higher expression of NPC marker genes such as NES (NESTIN), PAX6, and SOX1 (Figure 4B).

QUANTIFICATION AND STATISTICAL ANALYSIS

Preprocessing of data

Our in-house developed software, Barcode DNA counting system (Mizuho Information & Research Institute, Inc., Tokyo, Japan), processed the Glycan-seq readout in the FASTQ format, which is accessible from the github (https://github.com/bioinfo-tsukuba/barcode-dna-counting-system). Each read sequence is aligned with the DNA-barcode reference that corresponds to each lectin in this system. Two mismatches in the flanking region and one mismatch in the middle region were accommodated to the maximum extent. As a result, the DNA barcode count data in each cell is a readout. Each lectin count is normalized with the total count of DNA barcode and expressed as % of total count.

Alternatives: Command line interface such as PoolQ might be considered.

Processing of the scRNA-seq readout in the FASTQ format was performed with Subio Platform (version 1.24.5849) according to the manufacturer’s guidelines. Subio Platform can convert FASTQ data into raw count and Transcripts Per Million (TPM) matrix of gene expression, using a pipeline composed of fastp (version 0.20.0), HISAT2 (version 2.1.0), and StringTie (version 2.1.1) having a graphical user interface on both Windows 10 and Mac.

Figure 3. UMAP visualization for hiPSCs and NPCs

(A–C) UMAP plot based on (A) only the scRNA-seq data and (B) only the scGlycan-seq, and (C) both scRNA-seq and scGlycan-seq (scGR-seq) data of hiPSCs (n = 53, red) and NPCs (n = 43, green). Figure reprinted with permission from Minoshima et al. (2021).
Quality control

Extremely low count data may induce a data bias in scGlycan-seq; it is necessary to check whether the total number of barcode count influence component 1 or component 2 obtained from principal component analysis (Figure 5A). If the total count-dependent bias is detected, then a cut-off value of the total barcode count is determined by Otsu’s method using R with tidyverse packages as follows (Otsu, 1979) (Figure 5B).

1. Function declaration of Otsu’s method

Figure 4. Cell marker expression in scRNA-seq and scGlycan-seq data
(A) Violin plots of scGlycan-seq data.
(B) Violin plots of 41 selected cell marker gene expression in scRNA-seq data. Red rectangle: iPSC marker, Green Rectangle: NPC marker. Figure reprinted with permission from Minoshima et al. (2021).

Figure 5. Example of quality control of scGlycan-seq
(A) Example of total barcode-biased scGlycan-seq dataset. Principal component analysis (PCA) plot of scGlycan-seq data from 96 fibroblasts (left). Scatter plot of component 1 (PC1) or component 2 (PC2) from principal component analysis and total barcode count (middle, right). Component 1 shows total count-dependent bias.
(B) Histogram of total barcode count. Black line indicates cut-off value which is calculated by Otsu’s method using R. Figure reprinted with permission from Minoshima et al. (2021).
2. Load total count data

```r
threshold_otsu <- function(x, log10transform=FALSE){
  if(log10transform){
    x_original <- x
    x <- log10(x)
  }
  n <- length(x)
  second_max <- sort(x, partial=n-1)[n-1]
  search_space <- sort(x)[-length(x)]
  sigma <- 0
  th <- 0
  for(th_now in search_space){
    class0 <- x[x <= th_now]
    class1 <- x[x > th_now]
    w0 <- length(class0)
    w1 <- length(class1)
    mu0 <- sum(class0) / w0
    mu1 <- sum(class1) / w1
    sigma_now <- w0 * w1 * (mu0 - mu1)^2
    if(sigma < sigma_now){
      sigma <- sigma_now
      th <- th_now
    }
  }
  if(log10transform){
    th <- x_original[match(th, x)]
  }
  return(list(sigma=sigma, threshold=th))
}

df1 <- read_tsv("rawdata.tsv") #Replace rawdata.tsv with your data file name
df1 %>%
  filter(! BarcodeID %in% c("mIgG", "gIgG") ) %>%
  select(- BarcodeID) %>%
  as.matrix() -> mat1
dft <- tibble(Sample_ID = colnames(mat1), Total_count = colSums(mat1))
```

2. Load total count data
3. Perform binarization in log10-transformed total count data with Otsu’s method

```r
res_otsu <- threshold_otsu(dft$Total_count, log10transform = TRUE)
print(res_otsu$threshold)
table(dft$Total_count > res_otsu$threshold)
```

4. Visualization of binarized total count data

```r
n_accepted = sum(dft$Total_count > res_otsu$threshold)
n_rejected = sum(dft$Total_count <= res_otsu$threshold)
dft %>%
  mutate(filtered = Total_count > res_otsu$threshold) %>%
  ggplot(aes(reorder(Sample_ID, Total_count), Total_count)) +
  geom_point(aes(colour = filtered)) +
  geom_hline(yintercept=res_otsu$threshold) +
  theme_bw() +
  scale_colour_discrete(
    name="Filtered",
    breaks=c(TRUE, FALSE),
    labels=c(sprintf("Total count > %d (n=%d)", res_otsu$threshold, n_accepted),
                sprintf("Total count <= %d (n=%d)", res_otsu$threshold, n_rejected)) +
  labs(x="") -> g
plot(g)
```

In scRNA-seq data, cells with low-quality data are determined by several parameters, such as read count, gene count, or mitochondrial read ratio with Seurat R package (version 4.02). Dead/damaged cells exhibited low read count and increased mitochondrial read ratio whilst aggregated cells showed abnormally high read count. Since an optimum cut-off value to exclude low-quality cells depends on cell type, read depth and read quality, it needs to be determined by individual data set.

**Integrated data analysis**

The Seurat R package performs dimensionality reduction, cellular clustering, and identification of differential gene expression and thus can be used to analyze scGlycan-seq and scRNA-seq data. The Seurat platform also supports the integrated analysis of scGlycan-seq and scRNA-seq based on the weighted-nearest neighbor (wnn) workflow. A detailed protocol for the Seurat R package is described in (Hao et al., 2021).

**LIMITATIONS**

Like flow cytometry and lectin microarray, absolute amounts of glycans and accurate glycan structures cannot be determined directly from the signal intensities described above. Another limitation of the current system is the throughput. Since scGR-seq is a plate-based platform, processing of cell numbers is limited to hundreds of cells, while it can perform full-length total RNA sequencing (Hayashi et al., 2018). In contrast, droplet-based methods such as 10× Genomics (CITE-seq) can
sequence thousands of cells at once but target only the 3’ ends of poly(A) transcripts (Baran-Gale et al., 2018).

**TROUBLESHOOTING**

**Problem 1**
The molar ratio of DNA barcode relative to lectin is too low (”purification of DNA-barcoded lectins” step 23).

**Potential solution**
Increase the amount of PC-DBCO-NHS and incubation time.

**Problem 2**
Cells might be partially agglutinated after incubation with DNA-barcoded lectins (”single cell glycan-seq” step 19).

**Potential solution**
Cells might be agglutinated during incubation with DNA-barcoded lectins. The cell aggregates can be removed using 100 μm filter. In manual picking, single cells can be selected by visual inspection. In FACS, gating with FSC-H and FSC-W can exclude aggregated cells from the analysis.

**Problem 3**
No band is detected when DNA barcodes obtained from each single-cell were run on the microchip electrophoresis system, MultiNA (”single cell glycan-seq” step 28).

**Potential solution**
It is recommended to include bulk samples (1 x 10⁴ cells) as a positive control to validate PCR reactions. If the band is detected only in bulk samples, the amount of DNA barcodes obtained from each single-cell is likely too low. Even in that case, it may be detectable in a next-generation sequencer such as Miseq if you follow the protocol in “step 29: Denaturing library DNA if the concentration of library DNA is < 4 nM.”

**Problem 4**
Amount of cDNA library obtained from each single-cell is lower than 50–100 fmol (”single cell RNA-seq” step 35).

**Potential solution**
Keep an experimental space clean to prevent degradation of RNA by RNase contamination.

Confirm that ethanol is dried out after washing steps of AMPure beads, since the residential ethanol may inhibit the subsequent reactions.

Increase the number of PCR cycle.

Remove low yield samples from sequencing analysis with Next-generation sequencer.

Include whole volume of low yield samples into the mixed cDNA library.

**Problem 5**
High amount of primer dimers is detected around 110–130 bp when cDNA library obtained from each single-cell were run on the microchip electrophoresis system, MultiNA (”single cell RNA-seq” step 35).
Potential solution
It is recommended to remove primer dimers by size-selection of DNA fragments with AMPure XP beads because primer dimers will compete with cDNA to bind flow cell in Next-generation sequencer. The addition of 1.0–1.2 times the volume of AMPure XP to the PCR reaction solution is sufficient to remove 110–130 bp fragments. Note that this selection step may slightly reduce short cDNA fragments around 150 bp.

RESOURCE AVAILABILITY
Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Hiroaki Tateno (h-tateno@aist.go.jp).

Materials availability
Recombinant lectins are available from FUJIFILM Wako Pure Chemical Corporation or the lead contact upon request.

Data and code availability
The code of the barcode DNA counting system is available from github (https://github.com/bioinfo-tsukuba/barcode-dna-counting-system).

SUPPLEMENTAL INFORMATION
Supplemental information can be found online at https://doi.org/10.1016/j.xpro.2022.101179.

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AUTHOR CONTRIBUTIONS
H.T. designed research and wrote the paper. H. Odaka developed the experimental methods and wrote the paper. H. Ozaki developed the data analytical methods.

DECLARATION OF INTERESTS
The authors declare no competing interests.

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