Glycan Profiling of Crude Sample
Comparison of CHO and Lec1 mutant cell lines

As is well know, cells have specific glycomes depending on the origins: species, organs, differentiation stages, and/or health conditions. Therefore, glycan are usually called “the face of the cell”. Up until the present, there have not been any simple and high-sensitive ways to identify cells focusing on the difference of glycomes. In this technical note, we compare glycomes between CHO and Lec1 mutant cells, from which GlcNAc-transferase I was knocked out, using GlycoStation™. The differences in glycomes are detected from fluorescence patterns due to interactions of Cy3 labeled cell membrane glycoproteins with lectins immobilized on LecChip™.

**GlcNAc-transferase I**

As is shown in Fig.1, GlcNAc-transferase I (GlcNAc-T I) plays important role in adding GlcNAc onto the α 1-3 and α 1-6 Man of complex and hybrid type N-glycan core structure. Therefore, in a case of GlcNAc-T I deficient Lec1 mutant, we can theoretically expect that Lec1 lacks complex and hybrid type N-glycans, resulting in increase of high-mannose type N-glycans instead.

**Characteristic differences in lectin signals**

We observed characteristic differences in N-glycan binding lectins from the lectin microarray analyses. The signals of complex type N-glycan binders (PHA(L), PHA(E), ACG), that for α 2,3-Sia binder (MAL 1), and that for asialo binders (RCA120) decreased in Lec1 (see Fig.2(A)), whereas the signals of high-mannose binders (GNA, HHL, PWM, Calsepa, PSA, LCA) increased in Lec1 (see Fig.2(B)). These findings are quite reasonable taking into consideration that Lec1 is a GlcNAc-T I deficient mutant.

![Glycan Profiling Diagram](image-url)
- Protocol -

1. Sample preparation and fractionation
   1-1. Wash cells (5 × 10⁶) by PBA several times and store at -80°C.
   1-2. Fractionate the cells into membrane and cytoplasm fractions by using a commercial Kit¹.

2. Cy3 labeling
   2-1. Measure protein concentration with a commercial Micro BCA Protein Assay Reagent Kit² (reaction time = 2h).
   2-2. Prepare 20µL sample volume with a concentration of 50µg/ml using PBS³, then mix it with Cy3 Mono-Reactive dye 100µg labeling⁴.
   2-3. React it for 1h in a dark place at R.T.
   2-4. Add 300µL TBS⁵ into a gel filtration column⁶, then centrifuge at 1,500 × g, for 1 min at 4°C.
   2-5. Repeat it two times.
   2-6. Add the whole sample prepared at 2-3 and 25µL TBS into the gel filtration column prepared at 2-5, then centrifuge at 1,500 × g, for 2 min, at 4°C, and remove excess Cy3.

3. Measurement
   3-1. Dilute samples with a Probing Solution⁷ (in a range from 2µg/mL to 31.25ng/mL).
   3-2. Wash LecChip⁸ by a Probing Solution three times, then apply samples into wells (100µL/well).
   3-3. Incubate LecChips at 20°C over night.
   3-4. Measure fluorescence patterns without any washing of LecChips by GlycoStation™ Reader 1200⁹.
   3-5. Analyse the results by Array-Pro™ Analyzer ¹⁰ and GlycoStation™ Tools ¹¹.

Note
1) ProteoExtract® Subcellular Proteome Extraction Kit (Calbiochem, #539790)
2) Micro BCA Protein Assay Reagent Kit (PIERCE, #23235)
3) PBS(-) pH7.3
4) Use the equivalent amount of Cy3 Mono-Reactive dye pack (GE, #PA23011) to that used for 100µg protein labeling.
5) Zeba Desalt Spin Columns, 0.5ml (Thermo,#89883)
6) TBS pH7.5
7) Probing Solution (GlycoTechnica)
8) LecChip™ (GlycoTechnica)
9) GlycoStation™ Reader 1200 (GlycoTechnica)
10) Array-Pro™ Analyzer ver.4.5 (MEDIA CYBERNETICS)
11) GlycoStation™ Tools (GlycoTechnica)