

# Chapter 2

## Cloning, Expression, and Purification of Galectins for In Vitro Studies

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### Abstract

Galectins are best known for their ability to bind glycoconjugates containing  $\beta$ -galactose, but classification of these small proteins within the galectin family is also defined by amino acid homology within structural domains and exon/intron junctions within genes. As galectins are expressed by organisms as diverse as some fungi, *C. elegans*, fish, birds, and mammals, and biological activities attributed to galectins are equally diverse, it becomes essential to identify, clone, and characterize galectins from many sources. Glutathione S-transferase (GST) fused to the amino-terminus of galectin cDNAs has proven to be especially useful for preparation of recombinant galectins in bacteria for use on glycan arrays, in experiments with cultured or isolated cells, and in pull-down assays with immunopurified glycoproteins. Many galectins are stabilized by reducing reagents, such that binding and elution of GST-galectins from glutathione-conjugated Sepharose with excess glutathione is both efficient and innocuous. The ability to bind and elute GST-galectins from lactose-conjugated Sepharose with excess lactose provides a relatively easy means to insure that galectins are competent for glycoconjugate binding prior to experimentation. This chapter focuses primarily on the varied approaches to use GST-galectin binding to glutathione- and lactose-conjugated Sepharose to purify recombinant galectins and then develop effective experimental protocols to characterize the specificity, interactions, and function of galectins cloned from any source. We provide one example where a pull-down assay with all the GST-tagged canine galectins reveals that the C-terminal carbohydrate recognition domain of galectin-9 (Gal-9C) specifically recognizes the glycan-dependent apical targeting signal from the glycoprotein MUC1.

**Key words** Galectin, GST-galectin, Recombinant galectin, Pull-down assay

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## 1 Introduction

Our interest in the role of galectins (Gal) in glycan-dependent apical targeting of transmembrane MUC1, in the well-characterized model system of polarized Madin Darby canine kidney (MDCK) epithelial cells, led us down the unforeseen path of cloning and characterizing all of the canine galectins [1, 2].

At the start of our odyssey, Gal-3 and Gal-4 were implicated in glycan-dependent and lipid raft-dependent sorting of apical proteins, respectively, in polarized epithelial cells [3–7]. Gal-3 was

purported to cross-link glycoproteins while Gal-4 was thought to cross-link glycolipids, thereby producing sorting platforms for clustering of proteins. As our preliminary data were most consistent with glycan-dependent targeting of MUC1 [2], we focused on knockdown of Gal-3 with siRNAs in MDCK cells but found no effect on MUC1 apical targeting [8]. In order to consider a role for other galectins in apical targeting in MDCK cells, we had to both determine which galectins were actually expressed by dogs and which were present in MDCK cells [9].

Using Nucleotide BLAST and TBLASTN programs with both the dog genome and the NCBI Nucleotide Database, we identified sequences which aligned with nucleotide sequences and exon profiles, or amino acid sequences, for mammalian Gal-1, -2, -3, -4, -7, -8, -9, and -12, as well as the galectin-related HSPC159. HSPC159 lacks essential residues in the carbohydrate recognition domains for sugar binding. We found no canine genes homologous to rat Gal-5, mouse Gal-6, human Gal-10, human Gal-13, ovine Gal-14, ovine Gal-15, human PP13, or human PPL13.

We estimated the levels of transcripts for the canine galectins in MDCK cells using RT-PCR and primers designed to yield ~200 bp sequences. RNA from canine jejunum was used as a positive control. Amplification of RNA for Gal-1, -3, -8, and -9 was readily achieved by this approach while amplification of RNA for Gal-4 and -7 required nested primers to produce a band on an agarose gel with ethidium bromide staining. Based on these data we concluded that transcript levels varied (Gal-3 > Gal-9 > Gal-8 > Gal-1 >>> Gal-4 > Gal-7). While we did not obtain evidence for Gal-2 or Gal-12 transcripts in MDCK cells, a subsequent study by Friedrichs et al. [10] using real-time PCR reported that transcripts for Gal-3 were 100 times more abundant than transcripts for Gal-1, -8, and -9, and 1,000 times more abundant than for Gal-12, essentially consistent with our findings [9].

We first attempted to clone the canine galectins by RT-PCR from RNA isolated from MDCK cells using primers designed to match nucleotide sequences overlapping the predicted translational start and stop codons. Full-length cDNAs for canine Gal-1, -3, and -8 were obtained by this approach, while cloning of Gal-4 and -7 required amplification with nested primers where external primers matched nucleotide sequences within the 3' or 5' UTR, and internal primers overlapped the start and stop codons. The cDNAs for Gal-2 and -9 required amplification from a commercial source of canine jejunum RNA, and Gal-12 cDNA was eventually obtained by amplification from canine heart RNA. The cDNAs were cloned and sequenced for alignment with the canine genome and determination of the exon/intron structure of the genes for comparison to other mammalian galectin genes. We found 73–86 % identity

between canine and human amino acid sequences of the galectin with missing or extra exons representing previously described short (Gal-8 and Gal-12) and long (Gal-9) isoforms, respectively. We found five residues within exon 1 of Gal-7 as described for cow, but unique from that of mouse (two) and human (six), and six 9-mer amino acid repeats in Gal-3 as compared to cow (four), mouse (three), pig (three), human (two), rat (two), and rabbit (one). Although these differences are small, they could affect galectin biological activities and we did proceed to isolate the GST-tagged recombinant canine galectins for characterization on glycan arrays of Core H of the NIH Consortium for Functional Glycomics. This involved expression of GST-galectins in bacteria and the realization that GST-Gal-9 and -12 formed aggregates in bacteria and were not useful constructs. We subsequently subcloned the two different carbohydrate recognition domains from Gal-9 as it was prevalent in MDCK, while Gal-12 was not, producing GST-Gal-9N and GST-Gal-9C. GST-Gal-1, -3, -4, -7, -8, -9N, and -9C were purified from bacterial extracts by affinity binding to glutathione-conjugated and then lactose-conjugated Sepharose. Binding of GST-galectins on glycan arrays was then followed with an Alexa 488-conjugated anti-GST antibody [9].

Finally, to assess which endogenous galectins are likely interacting with MUC1 expressed in polarized MDCK cells, we carried out preliminary experiments where human MUC1 immunopurified from stably transfected MDCK cell extracts was incubated with recombinant GST-tagged Gal-1, -3, -4, -7, -8, -9N, and -9C in pull-down assays. Interestingly, we found that MUC1, whether metabolically labeled with [ $^{35}\text{S}$ ]Met/Cys or followed by immunoblotting, interacted best with Gal-3 and Gal-9, the two most abundant galectins expressed in MDCK cells [9]. In subsequent experiments we found that core-glycosylated mucin-like tandem repeats (OTR) from MUC1 are an apical targeting signal in polarized MDCK cells when appended to a non-polarized protein (Tac) [8]. We now present data from galectin pull-down assays comparing interactions with Tac and OTR-Tac that clearly shows that Gal-9C specifically binds to the apical targeting motif of MUC1.

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## 2 Materials

### 2.1 Cloning of Galectins

1. RNA from specified organisms and/or tissues (Zyagen) (*see Note 1*).
2. RNA Superscript II reverse transcriptase and Taq DNA polymerase (Invitrogen).
3. PCR primers (Integrated DNA Technologies).

4. pCR2.1 TOPO vector for DNA cloning (Invitrogen).
5. pGEX-6P-1 for production of glutathione S-transferase (GST) fusion galectins (GE Health Care Life Sciences) (*see Note 2*).
6. Bacteria XL-1 Blue (Agilent Technologies).

## **2.2 Expression and Purification of GST-Galectins**

1. *E. coli* strain BL21 (EMD Millipore).
2. Isopropyl  $\beta$ -D-1-thiogalactopyranoside (IPTG) (Sigma).
3. Luria Bertani (LB) broth.
4. Ampicillin (Sigma).
5. Lysis-Sonication buffer: 300 mM NaCl, 5 mM MgCl<sub>2</sub>, 1 mM dithiothreitol, 0.2 % Triton X-100 and 50 mM Tris-HCl, 0.1 mM phenylmethanesulfonylfluoride (PMSF), pH 8.0.
6. Glutathione conjugated to Sepharose (GE Healthcare).
7. PD-10 gel filtration columns (GE Healthcare).
8. Sepharose CL-6B (Sigma) (*see Note 3*).
9. Lactose-conjugated Sepharose (Sigma).
10. Elution buffer (with glutathione): 100 mM Tris-HCl, pH 8.0, 15 mM glutathione, 1 mM dithiothreitol (*see Note 4*).
11. Tris buffered saline: 0.1 M Tris-HCl, pH 8, 150 mM NaCl, 14 mM beta-mercaptoethanol ( $\beta$ ME).
12. Elution buffer (with lactose or sucrose): 0.1 M Tris-HCl, pH 8, 150 mM NaCl, 14 mM beta-mercaptoethanol ( $\beta$ ME), 0.1 mM  $\alpha$ -lactose (Sigma) (*see Note 4*).
13. 1-cm diameter column (8 cm tall) containing a sintered glass filter.
14. 15-ml conical plastic tubes.

## **2.3 Preparation of GST-Galectins for Pull-Down Assays**

1. Lactose-conjugated Sepharose (Sigma).
2. Glutathione conjugated to Sepharose (GE Healthcare).
3. Sepharose CL-6B (Sigma).
4. Hamilton syringe, 100  $\mu$ l size with a 22S-gauge needle (Gastight).
5. PreScission protease (GE Healthcare).
6. 15-ml conical tubes.

## **2.4 Preparation of Immunopurified Proteins**

1. Glutathione conjugated to Sepharose (GE Healthcare).
2. Protein G or Protein A conjugated to Sepharose (Invitrogen).
3. [<sup>35</sup>S]Met/Cys (Perkin Elmer).
4. Hepes-buffered saline” (10 mM Hepes, pH 7.4, 150 mM NaCl) with 1 % Triton X-100 or 0.1 % SDS.

5. Hamilton syringe, 100  $\mu$ l size with a 22S-gauge needle (Gastight).
6. Criterion Precast Gels (4–15 % Tris-HCl, 1 mm).
7. BioRad Criterion Gel system.
8. SDS-PAGE sample buffer (BioRad).
9. Nitrocellulose (Millipore, 0.45  $\mu$ m).
10. Bio-safe Coomassie (BioRad).
11. 1.5-ml tubes with snap cap.

### **2.5 Special Equipment**

1. Thermocycler.
2. BioRad Phosphorimager.
3. Quantity One Software.

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## **3 Methods**

### **3.1 Cloning**

1. First strand cDNA is synthesized from 1  $\mu$ g RNA using RNA Superscript II reverse transcriptase and amplified using Taq DNA polymerase as described by the manufacturer.
2. Primers for PCR to amplify the full-length transcripts are designed to overlap the predicted start and stop codons using nucleotide sequence information from database queries (*see* **Note 5**).
3. Amplify DNA using standard PCR technique.
4. Amplified DNA is cloned into pCR2.1 TOPO vector as described by the supplier.
5. Transform bacteria XL-1 Blue by the heat shock protocol.
6. Culture bacteria and isolate colonies.
7. Plasmid preps from multiple colonies should be generated.
8. Sequence isolated cDNAs from individual colonies in order to verify the predicted amino acid sequence of new galectins.

### **3.2 Expression and Purification of GST-Galectins**

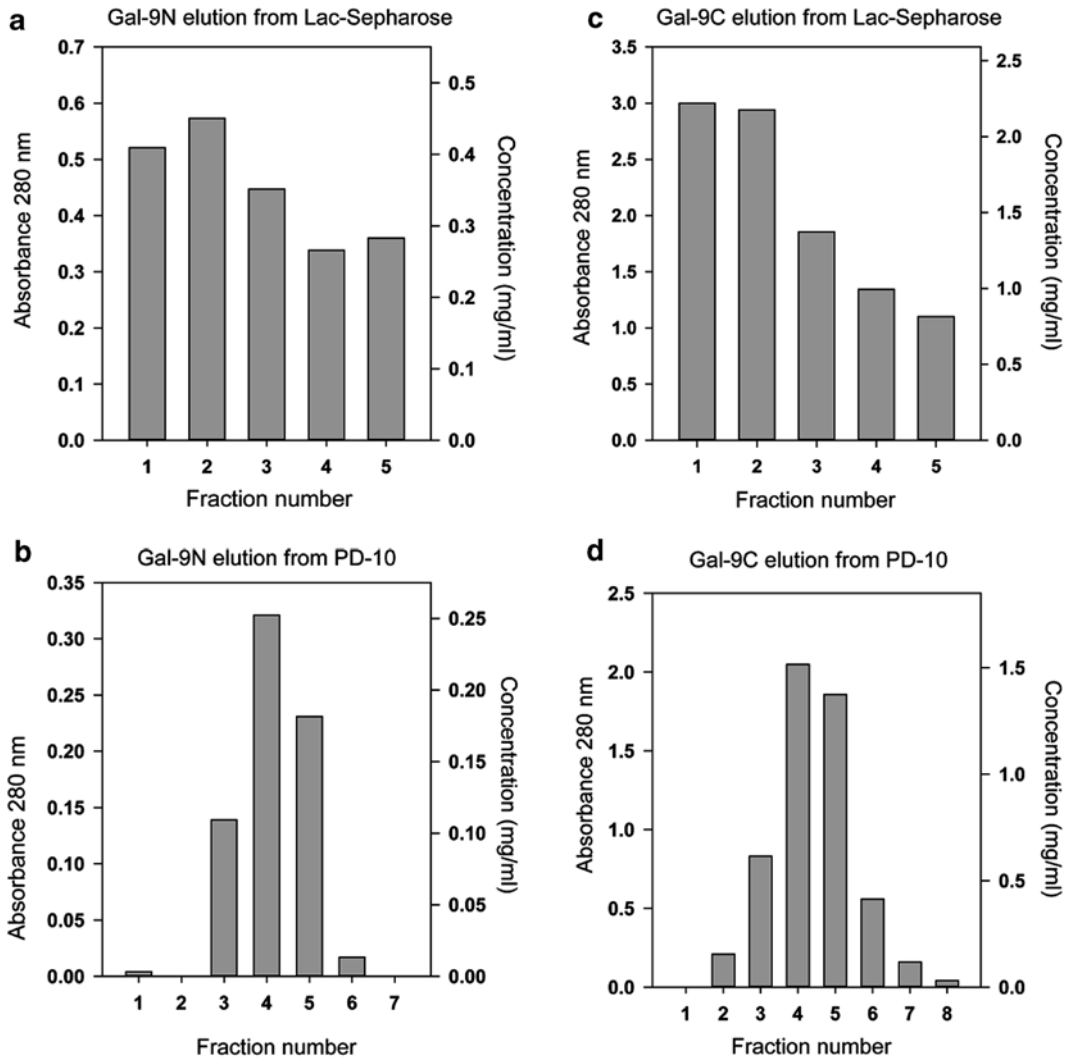
1. Galectin cDNAs are subcloned into pGEX-6P-1 for expression in bacteria as N-terminal GST-fusion proteins based on a previous protocol [11]. Details are found below.
2. LB medium (20 ml) containing ampicillin is inoculated with 200  $\mu$ l of glycerol stock of *E. coli* strain BL21 transformed with pGEX-6P-1 encoding GST-galectin and grown overnight with shaking at 37 °C.
3. This overnight 20-ml starter culture is used to inoculate 2 l of culture medium with ampicillin (divided between two 2 l flasks) and grown with shaking for 2–3 h at 37 °C until it reaches an OD<sub>600</sub> of 0.8.

4. Isopropyl  $\beta$ -D-1-thiogalactopyranoside is then added to 0.1 mM to induce expression of the fusion protein, and bacteria are grown with shaking overnight at room temperature.
5. Bacteria are collected by centrifugation at  $25,000\times g$  for 12 min at 4 °C in 250-ml centrifuge bottles. Pellets of bacteria can be frozen at -20 °C at this step for later use.
6. Pellets of bacteria are subsequently resuspended in 10 ml of Lysis-Sonication buffer (with PMSF) using a glass rod and transfer pipettes.
7. Bacteria are further lysed by sonication on ice with a Fisher Sonic Dismembrator Model 100 (five times, at setting 5 (8–12 W) for 30 s with 30 s intervals) (see **Note 12**).
8. The lysate is centrifuged for 30 min at  $10,000\times g$  at 4 °C to pellet cell debris.
9. The supernatant from the lysed bacteria is incubated at 4 °C overnight with 0.75 ml of a 50 % slurry of glutathione-conjugated Sepharose in a 15-ml conical tube with end-over-end mixing.
10. Beads are subsequently pelleted after centrifugation for 2 min at  $500\times g$  (Eppendorf model 5702 swinging bucket centrifuge) and the supernatant removed with a transfer pipette.
11. Beads are washed three times by addition of 5 ml of Lysis-Sonication buffer, mixing and centrifugation, as already described.
12. GST-galectins are released from the washed glutathione-conjugated Sepharose beads by adding 5 ml of Elution buffer (with glutathione), mixing, and incubation at 4 °C for 15 min.
13. After centrifugation for 2 min at  $500\times g$ , supernatant (eluate) is removed with a transfer pipette and saved.

*Alternative protocol for purifying galectin from glutathione eluate (see **Notes 6** and **7**).*

14. The entire eluate from the glutathione-conjugated Sepharose beads is incubated with 1 ml of a 50 % slurry of lactose-conjugated Sepharose in a 15-ml conical tube at 4 °C for 30 min with end-over-end mixing.
15. The slurry is then poured into a 1-cm diameter column containing a sintered glass filter and the packed column bed is overlaid with 1 ml of a 50 % slurry of Sepharose 6B. The column bed is washed with 5 ml Tris buffered saline.
16. To elute the GST-galectins from the column, 2 ml of elution buffer (with lactose).
17. 0.5 ml is collected from the bottom, and the column closed and capped for 30 min at 4 °C.

18. Fractions of 0.25 ml are subsequently collected after adding buffer to the top of the column.
19. The absorbance at 280 nm is determined with a spectrophotometer for each fraction.
20. The peak fractions are pooled and lactose is removed on a PD-10 gel filtration column as described by the manufacturer (*see* Fig. 1 for representative elution profiles) (*see* **Note 8**).



**Fig. 1** Affinity purification of GST-galectins on Lac-Sepharose. After batch elution of recombinant GST-galectins from glutathione conjugated to Sepharose, the GST-galectins can be stored at  $-80^{\circ}\text{C}$ . Before experimental use, GST-galectins are affinity purified by incubation with lactose conjugated to Sepharose (Lac-Sepharose) and eluted after incubation in a column equilibrated with  $\alpha$ -lactose (**a** and **c**). Lactose is removed from the GST-galectins using a PD-10 gel filtration column (**b** and **d**). See text for protocol details. Example profiles for GST-Gal-9N (**a** and **b**) and GST-Gal-9C (**c** and **d**) are shown here using calculated extinction coefficients of 55010M-1 and 56620M-1, respectively ( $A_{280}$  on *left* axis and concentration on *right* axis).

### **3.3 Preparation of GST-Galectins for Pull-Down Assays**

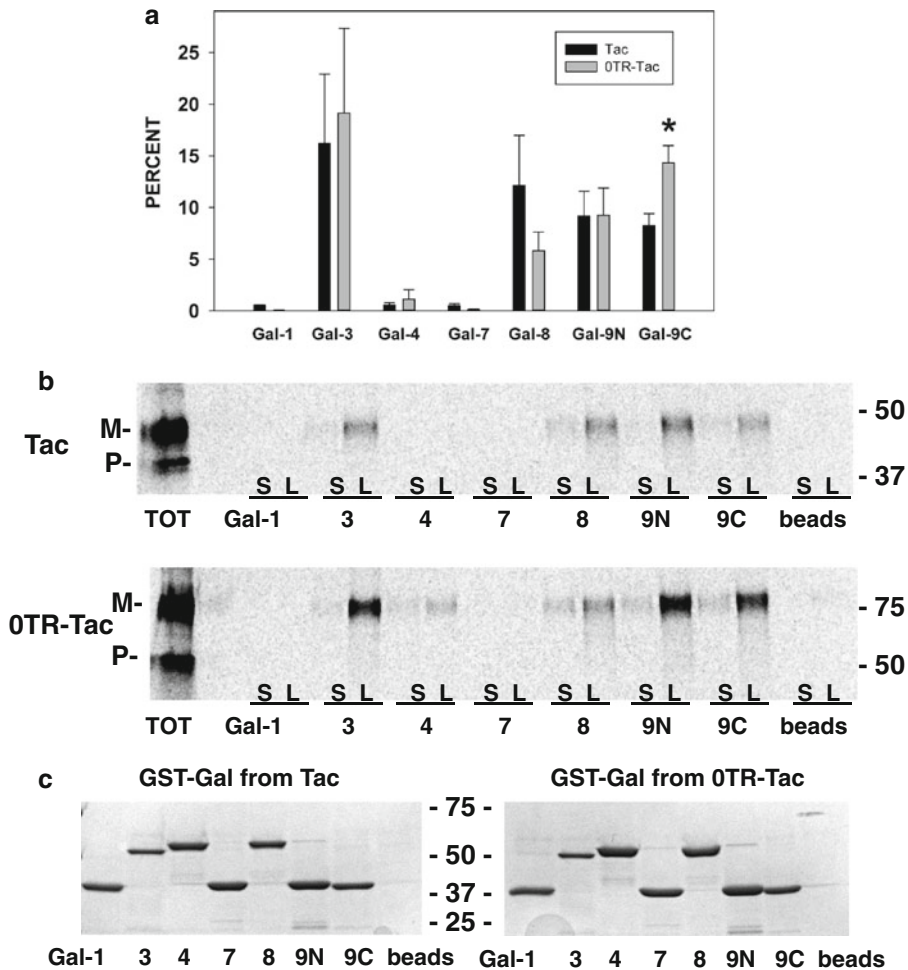
1. Aliquots of thawed GST-galectins (*see Note 7*) for pull-down assays are first affinity purified on lactose-conjugated Sepharose before binding to glutathione beads and incubation with immunopurified proteins (*see Subheading 3.4*). Tris buffered saline containing  $\beta$ ME is used throughout the protocol (*see Note 4*).
2. An aliquot of GST-galectin (1  $\mu$ g) is added to 30  $\mu$ l of a mixed slurry of lactose-conjugated Sepharose and carrier Sepharose CL-6B in a 1.5-ml tube with snap cap and incubated for 30 min at 4 °C with end-over-end mixing (*see Note 9*).
3. After centrifugation at 500 $\times g$  in a table-top microcentrifuge for 2 min, the supernatant is removed and discarded and the pellet is washed two times with 150  $\mu$ l Tris buffered saline to remove any unbound GST-galectin that is potentially “inactive.” Liquid is always removed from the beads with a Hamilton syringe (100  $\mu$ l size with a 22S-gauge needle) (*see Note 10*).
4. GST-galectin is eluted from the lactose-conjugated Sepharose by incubation with 30  $\mu$ l of Elution buffer (with lactose) for 15 min at 4 °C with end-over-end mixing.
5. After centrifugation at 500 $\times g$  in a table-top microcentrifuge for 2 min, the supernatant containing the “active” GST-galectin is transferred to a mixed slurry of glutathione-conjugated Sepharose and carrier Sepharose CL-6B in a 1.5-ml tube with snap cap, and incubated for 15 min at 4 °C with end-over-end mixing (*see Note 11*).
6. After centrifugation at 500 $\times g$  in a table-top microcentrifuge for 2 min, the beads are washed twice with 200  $\mu$ l of Tris buffered saline and all liquid removed from the pellet with a Hamilton syringe.
7. The final pellet of GST-galectin bound to glutathione-conjugated beads is subsequently mixed with immunopurified protein already prepared from cell extracts (*see Subheading 3.4*). Alternatively, the beads can be resuspended into 100  $\mu$ l of Tris buffered saline and 30–45  $\mu$ l can be transferred to different tubes of immunopurified proteins to produce duplicate or triplicate samples.
8. The GST tag on GST-galectin prepared in the pGEX-6P-1 vector can also be removed by treatment of GST-galectin pre-bound to glutathione-conjugated beads using PreScission protease to cut at the single engineered consensus sequence LEVLFQ/GP between the GST and galectin as directed by the manufacturer (where / is the cleavage site and letters are abbreviations for amino acids).

### **3.4 Pull-Down Assays with GST-Galectins**

1. Pull-down assays can be carried out either by following protein binding by immunoblotting or by following binding of [ $^{35}$ S] proteins recovered by metabolic labeling of cell cultures



- (described here). The exact protocol for preparation of each immunopurified protein should be optimized by the researcher.
2. Multiple wells (or permeable supports) of cultured cells expressing the protein of interest are metabolically labeled with [ $^{35}\text{S}$ ]Met/Cys for 15–60 min and chased in medium containing Met and Cys for 60–120 min (*see Note 12*). Cells are extracted from each well in Hepes buffered saline with a mild detergent such as octyl-glucoside or Triton X-100, centrifuged to remove cell debris and nuclei, and the supernatants combined.
  3. [ $^{35}\text{S}$ ]Proteins are recovered by immunoprecipitation from the combined supernatants in 1.5-ml tube with snap cap using specific antibodies and either Protein G or Protein A conjugated to Sepharose beads. Proteins are eluted from the beads by heating at 90 °C for 2 min in 200  $\mu\text{l}$  Hepes buffered saline containing 0.1 % SDS (heating is optional). The samples are centrifuged at  $9,300\times g$  in a table-top microcentrifuge twice for 30 s to pellet the beads.
  4. Equal aliquots of eluant (20  $\mu\text{l}$ ) are transferred to clean snap cap tubes. One of the aliquots is retained as the *input* sample and mixed with 10  $\mu\text{l}$  SDS gel sample buffer. The remainder of the aliquots is diluted tenfold with Hepes buffered saline containing  $\beta\text{ME}$  and 1 % Triton X-100 to neutralize the 0.1 % SDS prior to addition of freshly prepared GST-galectins prebound to glutathione-conjugated Sepharose beads. One tube receives Sepharose CL-6B beads as a negative control (i.e., there should be no binding to the beads alone). *See Fig. 2* for a representative experiment with eight different GST-galectins, beads alone and *input* sample (total).
  5. [ $^{35}\text{S}$ ]Proteins are incubated with the GST-galectins prebound to glutathione beads overnight at 4 °C with end-over-end mixing. After centrifugation at  $500\times g$  in a table-top microcentrifuge for 2 min, the supernatant is removed and the pellet washed two times with 200  $\mu\text{l}$  Tris buffered saline containing  $\beta\text{ME}$  to remove any unbound [ $^{35}\text{S}$ ]proteins.
  6. Beads are incubated for 30 min at 4 °C with 10  $\mu\text{l}$  Elution buffer (with sucrose)  $\beta\text{ME}$  and 100 mM *sucrose* to elute proteins bound nonspecifically. After centrifugation at  $500\times g$  in a table-top microcentrifuge for 2 min, the eluant is removed with a Hamilton syringe and saved.
  7. Beads are incubated for 30 min at 4 °C with 10  $\mu\text{l}$  Elution buffer (with lactose) containing  $\beta\text{ME}$  and 100 mM *lactose* to elute bound proteins. After centrifugation at  $500\times g$  in a table-top microcentrifuge for 2 min, the eluant is removed with a Hamilton syringe and saved.
  8. Beads are incubated for 30 min at 4 °C with 10  $\mu\text{l}$  Elution buffer (with glutathione) containing  $\beta\text{ME}$  and 15 mM *glutathione*



**Fig. 2** Pull-down experiments with GST-galectins reveal specific binding of GST-Gal-9C to the apical targeting signal from MUC1. Core-glycosylated mucin-like tandem repeats (OTR) from MUC1 are an apical targeting signal in polarized MDCK cells when appended to a non-polarized protein (Tac) [8]. To identify specific galectins that bind to OTR, we compared Tac and OTR-Tac binding to GST-galectins in pull-down assays. (a) Equal aliquots of immunopurified [ $^{35}$ S]Tac or [ $^{35}$ S]OTR-Tac were incubated overnight with fresh GST-galectins bound to GSH-beads (or beads alone) and eluted with sucrose (S), then lactose (L), and analyzed after SDS-PAGE with a BioRad imager. The percent [ $^{35}$ S]Tac or [ $^{35}$ S]OTR-Tac eluted with lactose was calculated from the input total (TOT) aliquot and is presented as the mean and SEM for three experiments. [ $^{35}$ S]OTR-Tac binding to only GST-Gal-9C was consistently greater than [ $^{35}$ S]Tac binding ( $p < 0.05$ ). Note that mature (M), but not precursor (P), forms were bound. One representative experiment is presented in (b) showing a side-by-side comparison of [ $^{35}$ S]Tac or [ $^{35}$ S]OTR-Tac binding. The GST-galectins from each sample were eluted with glutathione for SDS-PAGE, and analyzed by scanning the Coomassie-stained SDS-gel. Mobility of MW markers in kDa is noted between the two gels (c). See text for protocol details

to elute GST-galectins. After centrifugation at  $500\times g$  in a table-top microcentrifuge for 2 min, the eluant is removed with a Hamilton syringe and saved.

9. Eluants from *sucrose*, *lactose*, and *glutathione* incubations are subjected to SDS-PAGE by mixing 10  $\mu$ l of sample with 10  $\mu$ l SDS sample buffer and heating at 90 °C for 2 min.
10. After electrophoresis, the proteins eluted by *sucrose* and *lactose* can be electrophoretically transferred to nitrocellulose. Nitrocellulose is dried for 1 h at room temperature, stored 0.5–3 days with a Kodak TR screen and bands analyzed with a BioRad Phosphorimager and Quantity One Software. The SDS-gel containing proteins eluted with *glutathione* (GST-galectins) is stained with Bio-safe Coomassie. The stained gel is scanned and bands analyzed with Quantity One Software. See Fig. 2 for a representative experiment with immunopurified [ $^{35}$ S]proteins and pull-down with eight different GST-galectins.
11. The dry nitrocellulose can be subsequently hydrated and used for immunoblotting after blocking using standard protocols. Bands are visualized with a BioRad Versadoc or by scanning bands on exposed film, and quantified using Quantity One Software.
12. The fraction of immunopurified protein bound and specifically eluted from each GST-galectin is calculated using the *input* aliquot as 100 %. These values are normalized with the amount of each GST-galectin bound to the glutathione-conjugated beads (based on the Coomassie stained gel and divided by the individual formula weight of the GST-galectin).

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## 4 Notes

1. Alternatively, RNA can be isolated from tissues or cell lines using commercial kits (Ambion RNAqueous 4PCR Kit) following the directions of the manufacturer.
2. It may be necessary to express carbohydrate binding domains from tandem repeat galectins separately to assess binding specificities individually. On the other hand, we found that canine GST-Gal-9 repeatedly aggregated in the bacteria, so we subcloned the N-terminal and C-terminal CRDs separately as GST-Gal-9N (residues 1–148) and GST-Gal-9C (residues 225–355), respectively, for further study. In more recent studies it appears that GST on the C-terminus rather than the N-terminus of Gal-9 permits expression of the full-length galectin.
3. Sepharose CL-6B is used because it is cross-linked and will not be altered by heating in 1 % SDS.

4. Beta-mercaptoethanol or dithiothreitol is included in buffers to stabilize the galectins as many have reactive Cys residues.
5. When either the start or stop codon cannot be located in the database, the full-length cDNA should be obtained using standard approaches such as RACE-PCR [12]. Amplification of rare transcripts may also require the use of nested primers whereby an aliquot (1 %) of the amplified DNA obtained by PCR with external primers after 28 cycles is amplified a second time for 28 cycles with internal primers.
6. At this stage, the GST-galectin can be divided into aliquots and frozen at  $-80^{\circ}\text{C}$  after addition of  $\beta\text{ME}$  to 14 mM. Before any further use, GST-galectins should be affinity purified on lactose-conjugated Sepharose to be sure that the galectin is active (i.e., competent for binding glycans). Protein concentration of GST-galectin is estimated from Coomassie staining after SDS-PAGE as glutathione interferes with spectrophotometer readings at  $A_{280}$ .
7. GST-galectins can be batch eluted from the lactose-conjugated Sepharose. Elution from the column allows retrieval of the most concentrated fractions as needed for protocols such as screening glycan arrays. Sufficient GST-galectin for pull-down assays can be obtained from a 50 ml culture of bacteria ( $\sim 5\text{ }\mu\text{g}$  is eluted from the glutathione-conjugated Sepharose).
8. Protein concentrations of GST-galectins are calculated from  $A_{280}$  using individual extinction coefficients determined from the amino acid content of each GST-Gal using the Peptide Property Calculator found online (<http://www.basic.northwestern.edu/biotools/proteincalc.html>). Be sure to include the amino acid content of GST in the calculation. Pooled peak samples (1–1.5 ml) routinely contain  $\sim 0.25$ –1 mg GST-galectin. This active concentrated GST-galectin is optimal for use on glycan arrays.
9. A 50 % slurry of lactose-conjugated Sepharose (25  $\mu\text{l}$ ) is mixed with a 50 % slurry of carrier Sepharose CL-6B (225  $\mu\text{l}$ ), and 30  $\mu\text{l}$  of the mixed slurry is transferred to a 1.5-ml conical tube with snap cap for subsequent addition of 1  $\mu\text{g}$  of GST-galectin.
10. This size of Hamilton syringe allows removal of all the liquid while excluding the Sepharose beads when the tip is placed at the bottom of the tube in the middle of the pellet.
11. A 50 % slurry of glutathione-conjugated Sepharose (90  $\mu\text{l}$ ) is mixed with a 50 % slurry of Sepharose CL-6B (270  $\mu\text{l}$ ). A 40  $\mu\text{l}$  aliquot of the mixed slurry is used for each aliquot of GST-galectin eluted from the mixed slurry of lactose-conjugated Sepharose.
12. Our investigation of MUC1 binding to canine GST-galectins was carried out by metabolic labeling of polarized cultures of

MDCK epithelial cells with [ $^{35}\text{S}$ ]Met/Cys for 30 min and a chase in normal culture medium for 90 min, a time profile previously determined for efficient surface expression of MUC1. [ $^{35}\text{S}$ ]MUC1 eluted from GST-galectins (or the input control) was subjected to SDS-PAGE and transferred to nitrocellulose such that we were able to analyze both newly synthesized radiolabeled MUC1 and then steady-state MUC1 by immunoblotting. Data can also be obtained from analysis of a dried SDS-gel.

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