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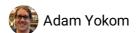
## ♠ Expression and purification GST-tagged ATG13:ATG101 constructs

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ABSTRACT

Expression and purification of GST-tagged ATG13/ATG101 constructs

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**KEYWORDS** 

**ASAPCRN** 



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## Expression 2d 12h 50m

- 1 Transfect HEK GNTI cells at concentration of  $2 \times 10^6$  cells/ml
- 2 Dilute PEI with Warm Hybridoma-SFM(1X)
- 3 In a separate tube, dilute DNA with Hybridoma-SFM(1X)

- 30m 4 Add PEI to DNA dilution. Incubate mixture for © 00:30:00 at § 37 °C 2d 5 Add mixture to cells. Let cells grow for 48:00:00 10m Harvest Cells \$\mathbb{G}\$500 rpm, 4°C, 00:10:00 Wash pellet with cold PBS. Store pellet at -80C until purification or lyse immediately Purification 2d 12h 50m Resuspended pellet in lysis buffer (25 mM HEPES pH 7.5, 200 mM NaCl, 2 mM MgCl<sub>2</sub>, 1 mM TCEP, 5 mM EDTA, 10% Glycerol) with 1% Triton X-100 and protease inhibitor cocktail (Thermo Scientific, Waltham, MA) 35m 9 Clarify lysate for **17000 rpm**, **4°C**, **00:35:00** 10 Wash GST Sepharose 4B resin into lysis buffer (without Triton) 11 Load supernatant onto resin using a gravity column setup 1h 12 Rock supernatant with equilibrated resin for © 01:00:00 at & 4 °C
  - Wash with 5CV lysis buffer (25 mM HEPES pH 7.5, 200 mM NaCl, 2 mM MgCl<sub>2</sub>, 1 mM TCEP, 5 mM EDTA, 10% Glycerol)

14	Elute with I	ysis buffer	plus 25 mM	glutathione	for GST resin

- Concentrate elution and inject onto pre-equilibrated S200 10/30 column (25 mM HEPES pH 7.5, 200 mM NaCl, 2 mM MgCl<sub>2</sub>, 1 mM TCEP, 5 mM EDTA)
- 16 Pool peak fractions, concentrate, snap freeze, and store at -80C