Please cite this article as: Ualiyeva, S. *et al.* (2021). Isolation of Nasal Brush Cells for Single-cell Preparations. *Bio-protocol* 11(18): e4163. DOI: 10.21769/BioProtoc.4163.

bio-protocol www.bio-protocol.org/e4163 Bio-protocol 11(18): e4163. DOI:10.21769/BioProtoc.4163

Isolation of Nasal Brush Cells for Single-cell Preparations

Saltanat Ualiyeva, Amelia A. Boyd, Nora A. Barrett and Lora G. Bankova*

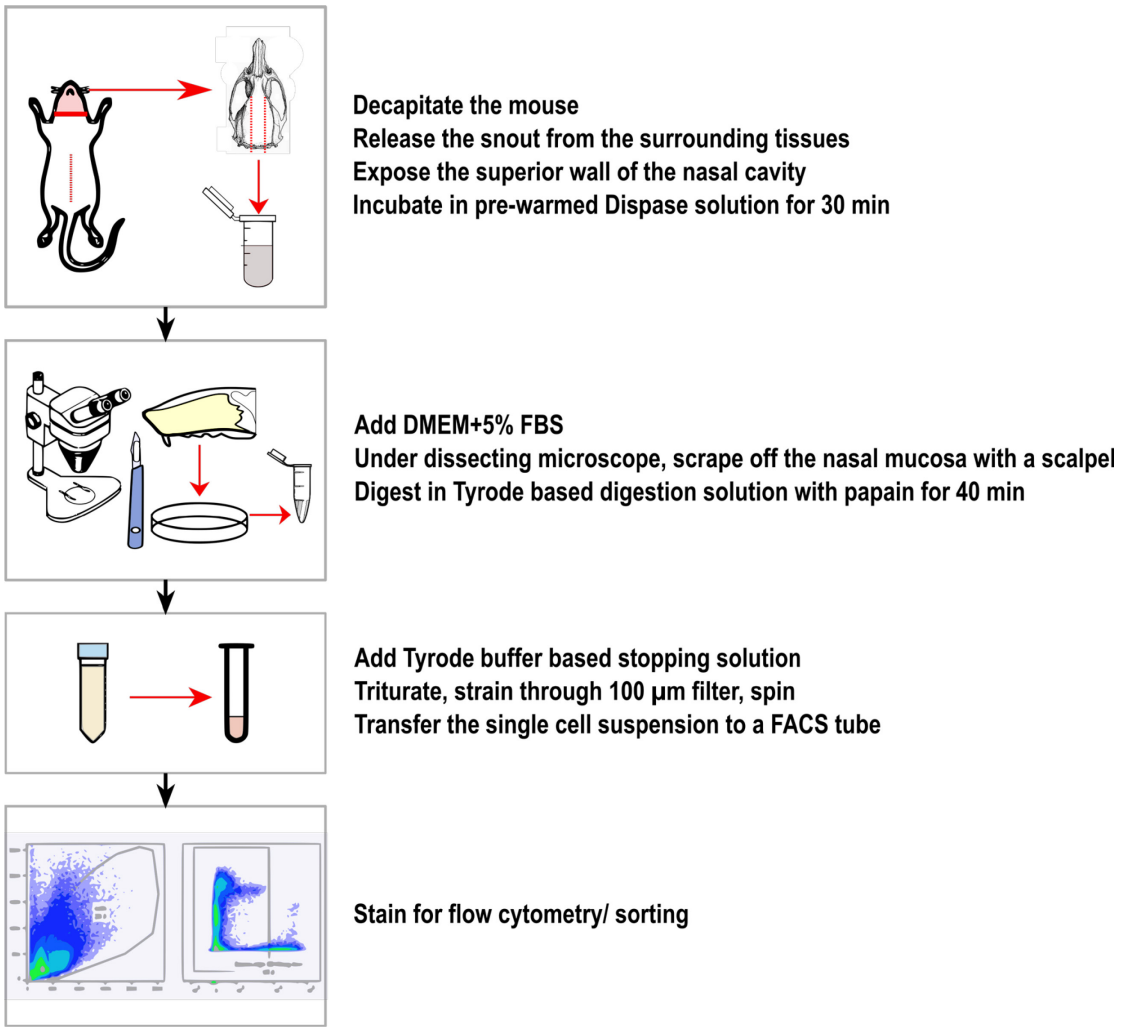
Division of Allergy and Clinical Immunology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

*For correspondence: lbankova@bwh.harvard.edu

[Abstract] Solitary chemosensory epithelial cells are scattered in most mucosal surfaces. They are referred to as tuft cells in the intestinal mucosa, brush cells in the trachea, and solitary chemosensory and microvillous cells in the nasal mucosa. They are the primary source of IL-25 in the epithelium and are also engaged in acetylcholine generation. We recently demonstrated that nasal solitary chemosensory (brush) cells can generate robust levels of cysteinyl leukotrienes in response to stimulation with calcium ionophore, aeroallergens, and danger-associated molecules, such as ATP and UTP, and this mechanism depends on brush cell expression of the purinergic receptor P2Y2. This protocol describes an effective method of nasal brush cell isolation in the mouse. The method is based on physical separation of the mucosal layer of the nasal cavity and pre-incubation with dispase, followed by digestion with papain solution. The single cell suspension obtained this way contains a high yield of brush cells for fluorescence-activated cell sorting (FACS), RNA-sequencing, and *ex vivo* assays.

Please cite this article as: Ualiyeva, S. *et al.* (2021). Isolation of Nasal Brush Cells for Single-cell Preparations. *Bio-protocol* 11(18): e4163. DOI: 10.21769/BioProtoc.4163.

Graphic abstract:



Workflow of nasal digestion for brush cell isolation.

Keywords: Nasal brush cells, Cholinergic epithelial cells, Microvillous cells, Solitary chemosensory cells, Bitter taste sensing cells, Bitter taste receptors, Tuft cells

[Background] Brush cells are chemosensory epithelial cells best known for their expression of bitter taste receptors (Finger *et al.*, 2003; Krasteva and Kummer, 2012). Brush cells are also be referred to as tuft cells and have been found in the airways (Krasteva and Kummer, 2012), gastrointestinal tract (Howitt *et al.*, 2016), and urinary epithelium (Deckmann *et al.*, 2014). Morphologically, brush cells are characterized by apical microvilli that form a tuft-like projection on the cell surface, extending to the mucosal lumen (Rhodin and Dalhamn, 1956; Reid *et al.*, 2005). Protective functions of brush cells have been most closely linked to their generation of acetylcholine leading to activation of peptidergic sensory nerve fibers (Krasteva *et al.*, 2011). We have previously reported a role for brush cell-derived IL-25 in eliciting type 2 inflammation and epithelial cell remodeling in the lung (Bankova *et al.*, 2018). We recently reported robust generation of pro-inflammatory lipid mediators termed cysteinyl leukotrienes by nasal

Please cite this article as: Ualiyeva, S. *et al.* (2021). Isolation of Nasal Brush Cells for Single-cell Preparations. *Bio-protocol* 11(18): e4163. DOI: 10.21769/BioProtoc.4163.

brush cells in response to aeroallergens and ATP through the purinergic receptor P2Y2 (Ualiyeva *et al.*, 2020), suggesting potent functions for these cells in directing immune responses in the airways.

In the nose, the mucosal lining is composed of olfactory and respiratory epithelia (Adams, 1972). In mice, the olfactory epithelium lines up to 50% of the total nasal mucosa, overlying the middle and superior turbinates and the caudal/posterior area of the nasal septum, while the respiratory epithelium covers around 45% of the nasal cavity in more rostral/anterior parts (Gross *et al.*, 1982; Chamanza and Wright, 2015). Solitary chemosensory cells in the respiratory mucosa of the nose respond to irritants and bacteria and are established as the nasal equivalent of tracheal brush cells in mice (Finger *et al.*, 2003; Sbarbati and Osculati, 2003) and humans (Lee *et al.*, 2014; Kohanski *et al.*, 2018). Microvillous cells are related cells found in the apical olfactory epithelium, above the layer of olfactory sensory neurons, and supporting sustentacular cells (Hansen and Finger, 2008). Both microvillous cells and solitary chemosensory cells express *Chat,* choline acetyltransferase, an enzyme necessary for the synthesis of the neurotransmitter acetylcholine (Krasteva *et al.*, 2011), *Trpm5*, a transient receptor potential gene ubiquitous in chemosensory cells (Pérez *et al.*, 2002; Kaske *et al.*, 2007; Zhang *et al.*, 2007; Ogura *et al.*, 2011; Genovese and Tizzano, 2018), and depend on *Pou2f3*, a transcription factor required for differentiation of all chemosensory cells in the nose, trachea, and intestine (Ohmoto *et al.*, 2013; Yamaguchi *et al.*, 2014; von Moltke *et al.*, 2016).

Our recent study using fluorescence-activated cell sorting (FACS) based on the fluorescent reporter expression of *Chat* (ChAT-eGFP) and size and granularity characteristics [forward scatter (FSC) and side scatter (SSC)] allowed us to distinguish the olfactory-enriched microvillar cells from the respiratory-enriched solitary chemosensory cells. We confirmed by bulk RNA-sequencing (RNA-seq) that both populations belong to the larger family of chemosensory tuft/brush cells and are mostly distinguished by their varied expression of taste receptors. This protocol describes our method for isolation of a mixed population of nasal chemosensory brush cells providing high yields of ~20,000/mouse, which can be further characterized by FACS and RNA-seq for investigation of the similarities and differences between these cell types in the nasal cavity.

Several groups have reported successful isolation of solitary chemosensory cells from the respiratory nasal mucosa and microvillar cells from the olfactory mucosa. Gulbransen *et al.* (2008) used a Tyrode-based solution supplemented with 20 U/ml of papain to isolate solitary chemosensory cells from the respiratory mucosa of TRPM5-GFP fluorescent reporter mice. Several hundred viable cells were used for calcium flux studies in response to bitter tasting agonists. Lin *et al.* (2008) isolated solitary chemosensory cells from the anterior respiratory epithelium with a Ringer-based solution with 10-30 U/ml of papain to measure changes in intracellular calcium levels in single cells in response to odorous irritants. Ogura *et al.* (2011) isolated microvillar cells from the main olfactory epithelium of ChAT-eGFP and TRPM5-GFP mice with 4 U/ml of papain for 3-5 min at room temperature and showed increased calcium flux from freshly isolated cells in response to ATP, soil bacterium lysate, and denatonium benzoate. Although no information was provided for the total yields of recovered solitary chemosensory or microvillous cells with the described protocols, each used <100 cells for live imaging for calcium flux. We tested a comparable concentration of papain (26 U/ml) to isolate brush cells from the trachea and

Please cite this article as: Ualiyeva, S. *et al.* (2021). Isolation of Nasal Brush Cells for Single-cell Preparations. *Bio-protocol* 11(18): e4163. DOI: 10.21769/BioProtoc.4163.

found that pre-incubation of the trachea with high dose dispase (16 U/ml) results in a 20-fold increase of isolated brush cell yields compared to digestion with papain alone (Ualiyeva *et al.*, 2019).

Here, we describe a step-by-step protocol for isolation of brush cells from the murine snout of ChAT-eGFP fluorescent transgenic mice, with enhanced green fluorescent protein expression dictated by *Chat*. Choline acetyltransferase is highly expressed in both subsets of nasal chemosensory brush cells, in tracheal, intestinal, and urethral brush/tuft cells and specifically enriched in these chemosensory epithelial cells compared to other epithelial cells. This allows for identification of the fluorescent cholinergic brush cells by FACS. The single-cell solution preparation procedure is based on initial incubation of the snout with dispase solution, which allows for easy mechanical separation of the mucosa from the underlying bones and cartilages. The sample is subsequently incubated with 26 U/ml papain solution for 40 min at 37°C with agitation, which delivers fine dissociation of tight and adherens junctions between epithelial cells. This method grants access to a significant number of viable cells in a single-cell suspension for FACS sorting, RNA-seq, and functional assays.

Materials and Reagents

1. 20, 200, and 1,000 µl Pipette tips (no specific brand)
2. Thermo Scientific™ screw cap microtubes (2.0 ml) (Fisher Scientific, catalog number: 21-403-202)
3. 3 ml syringes (BD Biosciences, catalog number: 309657)
4. 18 G 1.5 in needles (BD Biosciences, catalog number: 305196)
5. 21 G 1.5 in needles (BD Biosciences, catalog number: 305167)
6. 50 ml conical tubes (Crystalgen, catalog number: 23-2263)
7. 12 × 75 mm (5 ml) round bottom polystyrene tubes (Corning, catalog number: 352052)
8. Petri dish (Falcon, catalog number: 351029)
9. Aluminum foil
10. Dispase powder (Gibco, catalog number: 17105041)
11. ChAT^{BAC}-eGFP mice (B6.Cg-Tg (RP23-268L19-EGFP) 2Mik/J) (The Jackson Laboratory, catalog number: 7902)
12. 200 Proof Ethanol (Koptec, catalog number: V1001)
13. DNase I (Sigma, catalog number: 10104159001)
14. Dulbecco's Phosphate-Buffered Saline (PBS) (Boston BioProducts, catalog number: BSS-220DM-C)
15. DMEM/F-12 (Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12) (ThermoFisher Scientific, catalog number: 11320033)
16. Heat-inactivated fetal bovine serum (FBS) (ThermoFisher Scientific, catalog number: 10082139)
17. Tyrode's Solution (with bicarbonate, HEPES, and 0.25% BSA, without calcium) (Boston BioProducts, catalog number: PY-912)
18. Papain from papaya latex (Sigma, catalog number: P3125)

19. L-cysteine (Sigma, catalog number: C7352)
20. Tyrode's Solution (with HEPES and calcium) (Boston BioProducts, catalog number: BSS-355)
21. Leupeptin trifluoroacetate salt (leupeptin) (Sigma, catalog number: L2023)
22. HBSS, 1× without calcium, magnesium, and phenol red (Hank's Balanced Salt Solution) (Corning, catalog number: 21-022-CV)
23. EDTA (0.5 M), pH 8.0. RNase-free (Thermo Fisher, catalog number: AM9260G)
24. TruStain FcX™ (anti-mouse CD16/32) antibody (Biolegend, catalog number: 101320)
25. Pacific Blue anti-mouse CD45 monoclonal antibody (Biolegend, catalog number: 103126)
26. Allophycocyanin (APC) anti-mouse CD326 (EpCAM) monoclonal antibody (Biolegend, catalog number: 118214)
27. Propidium iodide (PI) (Sigma, catalog number: P4170)
28. 70% EtOH (500 ml) (see Recipes)
29. Dispase solution (see Recipes)
30. DMEM-based stopping solution (see Recipes)
31. Tyrode buffer-based digesting solution with papain (see Recipes)
32. Tyrode buffer-based stopping solution (see Recipes)
33. FACS buffer (washing buffer for flow cytometry) (see Recipes)

Equipment

1. Dissection scissors (straight) (Fisher Scientific, catalog number: 08-951-5)
2. Forceps (straight, serrated) (Fisher Scientific, catalog number: 13-812-36)
3. Pipettes (P10, P200, P1000) (no specific brand)
4. Fisherbrand™ Isotemp™ General Purpose Deluxe Water Bath (Fisher Scientific, catalog number: FSGPD05)
5. Fisherbrand™ Multiplatform Shaker (Fisher Scientific, catalog number: 88-861-021)
6. Dissecting microscope (Leica, catalog number: M165FC)
7. Scalpel (No.10) (ThermoFisher Scientific, catalog number: 3120032)
8. Fisher Vortex Genie 2 (Fisher Scientific, catalog number: 12-812)
9. 100 µM Fisherbrand™ Sterile Cell Strainers (Fisher Scientific, catalog number: 22-363-549)
10. Sorvall Legend X1R Centrifuge (ThermoFisher Scientific, catalog number: 75004261)
11. BD LSRFortessa™ Flow Cytometer (BD Biosciences)

Software

1. FlowJo v.8 (FlowJo, LLC, https://www.www.flowjo.com)
2. Prism 7 (GraphPad Software, https://www.graphpad.com/scientific-software/prism/)

Procedure

Please cite this article as: Ualiyeva, S. *et al.* (2021). Isolation of Nasal Brush Cells for Single-cell Preparations. *Bio-protocol* 11(18): e4163. DOI: 10.21769/BioProtoc.4163.

A. Isolation of mouse snouts

Mice used: ChAT(BAC)-eGFP (B6.Cg-Tg(RP23- 268L19-EGFP)2Mik/J), 3-6 months of age of both sexes. All animal care and procedures are approved by the Animal Care and Use Committees (IACUC) of Brigham and Women's Hospital.

1. Euthanize the mouse. Spray the fur over the abdomen with 70% EtOH (see Recipe 1). Make a midline abdominal incision with straight scissors to open the abdominal cavity and bleed the mice through puncture of the abdominal aorta. Allow the mouse to bleed for 1-2 min, for blood outflow from the head and avoidance/minimization of further necessity to lyse red blood cells.
2. Decapitate the mouse by cutting off the mandible and separating the head from the rest of the body.
3. Using straight scissors, cut off the tip of the nose and the front incisor teeth. Remove the skin of the head and release the skull from the surrounding muscle and brain tissues.
4. Cut off the zygomatic arches (**Figure 1A**). Remove the parietal bone. Make longitudinal incisions along the frontal bone, exposing the nasal cavity from the top (**Figures 1A-1C**).

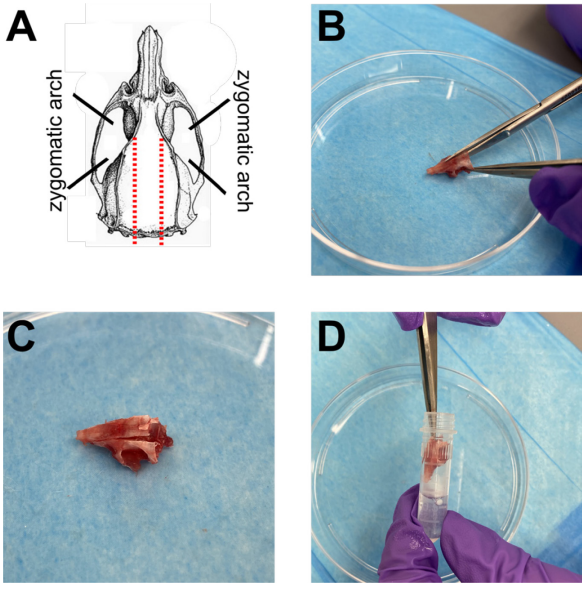


Figure 1. Representative images of nasal snout preparation for first digestion with dispase solution. A. The mouse head, released from the surrounding tissues and brain. The bilateral zygomatic arches need to be removed (black lines). The red dotted lines represent the longitudinal lines for further incision along the frontal bone. **B.** Longitudinal incision along the frontal bone. **C.** The frontal and nasal bones lifted, and the top of the nasal cavity exposed. **D.** The snout placed in 2 ml tube with pre-warmed dispase solution.

B. Nasal epithelial cell dissociation

1. Pre-warm 750 µl of dispase solution (see Recipe 2) in 2 ml tube at 37°C water bath for 5-10 min. Place the isolated snout into the solution (**Figure 1D**) and incubate on a shaker at 100 rpm for

Please cite this article as: Ualiyeva, S. *et al.* (2021). Isolation of Nasal Brush Cells for Single-cell Preparations. *Bio-protocol* 11(18): e4163. DOI: 10.21769/BioProtoc.4163.

30 min at room temperature. Keep the tubes protected from light with foil covers.

2. Reduce the digestion by adding 750 µl of cold DMEM-based stopping solution (see Recipe 3). Place the tubes on ice for 2-5 min.
3. Remove the digested snout out of the tube and place on Petri dish under a dissecting microscope with the palate facing down.
4. Remove the nasal bone comprising the superior wall of the nasal cavity. Now you can visualize the nasal cavity with the overlying mucosa (**Figure 2A**). Split the nasal cavity into left and right halves through a longitudinal incision along the palate (**Figure 2B**).

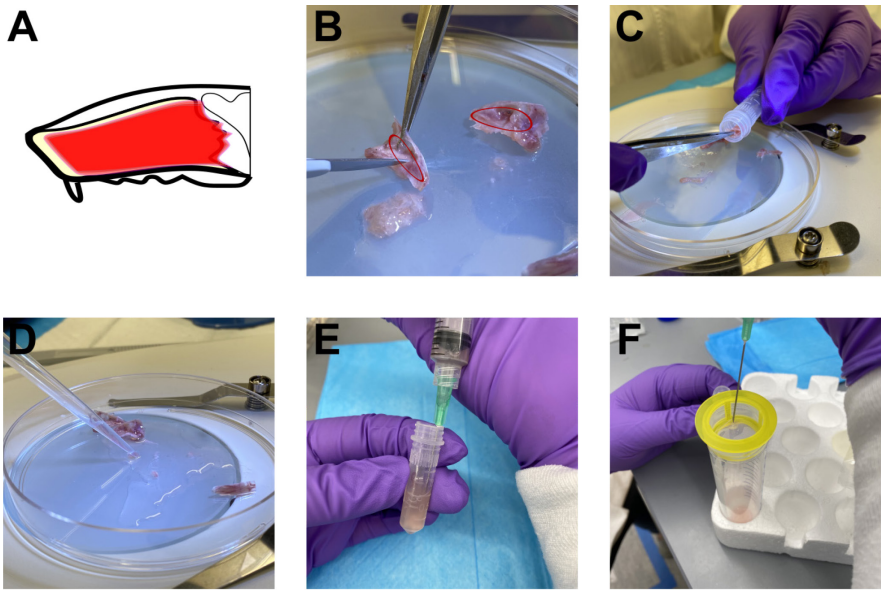


Figure 2. Representative images of single cell preparations from isolated nasal mucosa of the snout. A. A scheme of the lateral wall of the nasal cavity, with red color indicating the nasal mucosa coverage. B**.** The nasal cavity split into right and left halves on a Petri dish. The mucosa was scraped off with scalpel under a dissecting microscope. The red outlines represent the area of the nasal mucosa to be isolated. **C.** Transfer of the scraped mucosa to a 2 ml tube. **D.** Flushing of the Petri dish with 750 µl of Tyrode buffer based digesting solution with papain. **E.** After stopping the digestion and vortexing the sample, trituration of the suspension with 18-gauge needle 8-10 times, followed by trituration with 21-gauge needles for 15-20 times. **F.** The cell suspension strained through 100 µm filter into a 50 ml conical tube.

5. Scrape the nasal mucosa with a scalpel until no tissue can be visualized under the dissecting microscope except for the bone. Transfer the separated mucosa with the small underlying bones and cartilages to a 2 ml tube (**Figure 2C**).
6. Flush the Petri dish with 750 µl of Tyrode buffer based digesting solution with papain (see Recipe 4) and transfer the flushed fluid into the tube containing the nasal mucosa (**Figure 2D**).
7. Incubate the isolated nasal mucosa in Tyrode based digestion buffer for 40 min at 37°C, shaking at 210 rpm.

8. Vortex the digested tissue at 300 rpm for 10 s. Terminate the digestion by adding cold Tyrode buffer-based stopping solution (see Recipe 5) and placing the sample on ice.
9. Vortex the tissue at 1,000 rpm for 20 s. Triturate the solution with a syringe attached to an 18-gauge needle 8-10 times. Remove the remaining bones and cartilages to avoid clogging the syringe. Switch the needle on the syringe to 21-gauge needle and triturate 15-20 more times (**Figure 2E**).
10. Pass the suspension through a 100 µm filter into a 50 ml conical tube (**Figure 2F**). Add 20-25 ml of cold FACS buffer (see Recipe 6).
11. Spin the tube at 350 × *g* for 15 min at 4°C. Check the tube for the visible pellet. Discard the supernatant without disturbing the cell pellet and resuspend the pellet in 1 ml of FACS buffer and transfer to a 5 ml polystyrene tube. Fill the polystyrene tube with 4 ml of FACS buffer.
12. Spin the tube at 350 × *g* for 10 min at 4°C. Discard the supernatant and resuspend the pellet in 100 µl of FACS buffer.
13. To block Fc receptors, pre-incubate the cell suspension with TruStain FcX™ (anti-mouse CD16/32) antibody; use 1.0 µg of antibody per 10^6 cells in 100 µl volume for 5-10 min on ice. Washing after this step is not necessary.
14. Incubate the cells with antibodies for 45 min on ice, protected from light. The panel of antibodies used for brush cell identification includes pacific blue anti-mouse CD45 monoclonal antibody at 0.25 µg per 10^6 cells in 100 µl volume and allophycocyanin (APC) anti-mouse EpCAM monoclonal antibody at 0.5 µg per 10^6 cells in 100 µl volume.
15. Wash the cells by resuspending them with 1 ml of FACS buffer and add an additional amount of 3.8-4 ml of cold FACS buffer. Spin at 350 × *g* for 10 min at 4°C. Discard the supernatant and resuspend the pellet in 300 µl of cold FACS buffer.
16. Perform manual cell counts if not planning on running the whole sample on FACS machine. If the whole sample is collected on the machine, the counting step can be omitted.
17. Add propidium iodide (PI) 5 µg/ml for viability for 2-3 min before FACS.

Data analysis

After isolation of the nasal mucosa and digestion with this two-step protocol (see the graphical abstract), label the cell suspension fluorescently with EpCAM and CD45. Cells are gated by FSC-A/SSC-A to exclude debris and by SSC-H/SSC-W and FSC-H/SSC-W to select single cells (**Figure 3A**). Propidium iodide (PI) is used as a dead cell exclusion marker. From 1.2 × 10^6 to 3.1 × 10^6 live cells can be obtained with this method (**Figure 3B**). In our analyses, $CD45^{low/neg}$ and $EpCAM^{high}$ cells constituted 14-18% of all live cells. Brush cells were defined as $EpCAM^{high}$ and GFP^+ cells, and comprised 14-24% of the $EpCAM^{high}$ population and 1.3% of all live cells. This method allows isolation of 16,000-30,000 GFP^+ brush cells per mouse. Nasal brush cells can further be subdivided into SSC^{low} and SSC^{high} populations based on their size, and represent microvillous cells and solitary chemosensory cells, respectively. SSC^{low} comprised the majority of GFP^+ cells (94%), while SSC^{high}

Please cite this article as: Ualiyeva, S. *et al.* (2021). Isolation of Nasal Brush Cells for Single-cell Preparations. *Bio-protocol* 11(18): e4163. DOI: 10.21769/BioProtoc.4163.

cells represented 4% of brush cells.

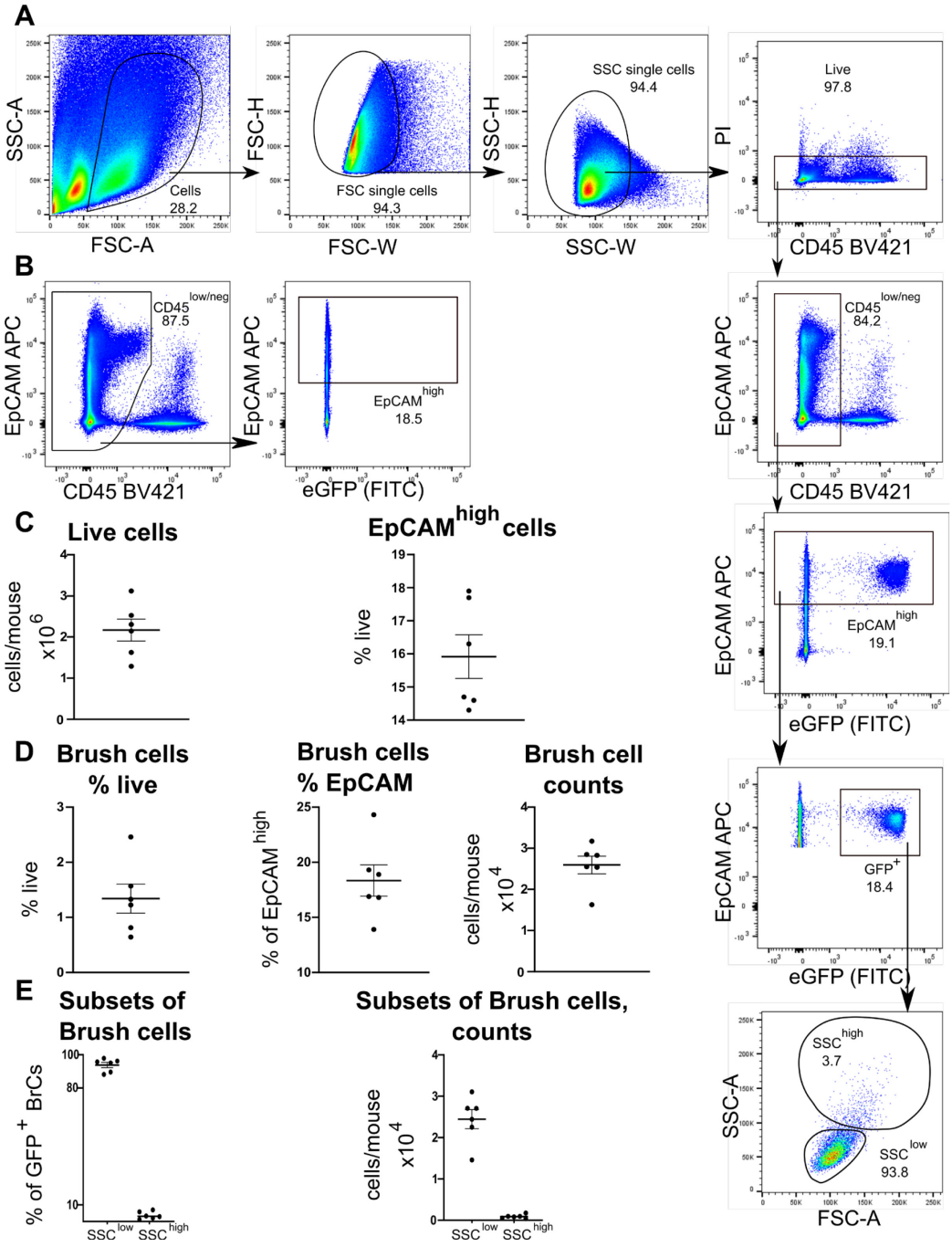


Figure 3. Flow cytometry analysis of nasal brush cells. The cell suspensions were stained with fluorescent-conjugated antibodies against EpCAM and CD45 and examined by flow cytometry. The gating strategy was previously published (Ualiyeva *et al.*, 2020). **A.** Representative flow cytometry gating strategy. Cells were gated based on their size and granularity on forward and side scatter features. Doublets were further excluded, and live cells were selected based on absence of staining with Propidium Iodide. $CD45^{low/neg}$ were evaluated on their expression of EpCAM. Brush cells were defined as $EpCAM^{high}$ GFP^{+} cells and were

further specified on forward scatter and side scatter characteristics. SSC^{high} population represents solitary chemosensory cells, and SSC^{low} brush cells correspond to microvillous cells. **B.** Flow cytometry analysis of cell suspensions from C57BL6 mice. The sample was pre-gated on single live cells, and $CD45^{low/neg}$ cells were evaluated on their expression of EpCAM. **C**. Number of live cells recovered from nasal mucosa and frequency of $EpCAM^{high}$ epithelial cells as percent of live cells. **D.** Frequency of nasal brush cells presented as percent of live cells, percent of $EpCAM^{high}$ population and number of nasal brush cells per mouse nose. **E**. Frequency of SSC^{high} (solitary chemosensory cells) and SSC^{low} (microvillous cells) populations as percent of all $EpCAM^{high}$ GFP^{+} cells and counts. Each dot represents a separate mouse. Data are from 3 separate experiments with 2 mice each.

This protocol has been successfully applied to isolate nasal brush cells for RNA sequencing and functional assays (Ualiyeva *et al.*, 2020).

Recipes

1. 70% EtOH (500 ml)
   350 ml 200 proof pure ethanol
   150 ml distilled H_2O
   Mix well
   This solution is flammable.
2. Dispase solution, prepare fresh prior to isolation
   16 U/ml dispase powder
   Dulbecco's Phosphate-Buffered Saline (PBS)
   Distilled H_2O
   20 µg/ml DNase I
   Prepare 1× PBS
   Dissolve 16 U/ml dispase powder and 20 µg/ml DNase I in 1× PBS
   Store solution at 4°C. Pre-warm in a water bath to 37°C before adding the snout.
3. DMEM-based stopping solution (DMEM with 5% FBS) (525 ml) (can be stored for up to 2 months at 4°C)
   500 ml Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM)
   25 ml Heat-inactivated fetal bovine serum (FBS)
   Prepare solution in a sterile biosafety cabinet and store at 4°C
4. Tyrode buffer-based digesting solution with papain (prepare fresh prior to isolation)
   Tyrode's Solution (with bicarbonate, HEPES, and 0.25% BSA, without calcium)
   20 µl/ml papain (28 U/mg)
   10 µl/ml L-cysteine (25 mg/ml)
   Prepare solution in a sterile biosafety cabinet and store at 4°C

5. Tyrode buffer-based stopping solution, prepare fresh prior to isolation
   Tyrode's Solution (with HEPES and calcium)
   2 µl/ml leupeptin (5 mg/ml)
6. FACS buffer (washing buffer for flow cytometry), can be stored up to 1 month at 4°C
   HBSS, 1× without calcium, magnesium, and phenol red (Hank's Balanced Salt Solution)
   2% Heat-inactivated fetal bovine serum (FBS)
   2 mM Ethylenediaminetetraacetic acid (EDTA, 0.5 M, pH 8.0)
   Prepare solution in a sterile biosafety cabinet and store at 4°C

Acknowledgments

This protocol was adapted with minor modifications from a previous study published by Ualiyeva *et al*. (2020). This methodology was based on a previous protocol for tracheal brush cell isolation used in our lab (Ualiyeva *et al.*, 2019). We thank Adam Chicoine at the Brigham and Women's Human Immunology Center Flow Core for his help with flow cytometric sorting. This work was supported by National Institutes of Health Grants R01 HL120952 (N.A.B.), R01 AI134989 (N.A.B), U19 AI095219 (N.A.B., L.G.B), K08 AII32723 (L.G.B), and T32 AI00730634 (S.U.), and by the American Academy of Allergy, Asthma, and Immunology (AAAAI)/American Lung Allergic Respiratory Disease Award (N.A.B.), by the AAAAI Foundation Faculty Development Award (L.G.B.), by the Steven and Judy Kaye Young Innovators Award (N.A.B.), by the Joycelyn C. Austen Fund for Career Development of Women Physician Scientists (L.G.B.), and by a generous donation by the Vinik family (L.G.B.).

Competing interests

The authors declare no competing interests.

Ethics

All procedures performed in animal study were guided by Brigham and Women's Hospital IACUC protocol 2016N000517.

References

1. Adams, D. R. (1972). Olfactory and non-olfactory epithelia in the nasal cavity of the mouse, Peromyscus. *Am J Anat* 133(1): 37-49.
2. Bankova, L. G., Dwyer, D. F., Yoshimoto, E., Ualiyeva, S., McGinty, J. W., Raff, H., von Moltke, J., Kanaoka, Y., Frank Austen, K. and Barrett, N. A. (2018). The cysteinyl leukotriene 3 receptor regulates expansion of IL-25-producing airway brush cells leading to type 2 inflammation. *Sci Immunol* 3(28).

3. Chamanza, R. and Wright, J. A. (2015). A Review of the Comparative Anatomy, Histology, Physiology and Pathology of the Nasal Cavity of Rats, Mice, Dogs and Non-human Primates. Relevance to Inhalation Toxicology and Human Health Risk Assessment. *J Comp Pathol* 153(4): 287-314.
4. Deckmann, K., Filipski, K., Krasteva-Christ, G., Fronius, M., Althaus, M., Rafiq, A., Papadakis, T., Renno, L., Jurastow, I., Wessels, L., Wolff, M., Schutz, B., Weihe, E., Chubanov, V., Gudermann, T., Klein, J., Bschleipfer, T. and Kummer, W. (2014). Bitter triggers acetylcholine release from polymodal urethral chemosensory cells and bladder reflexes. *Proc Natl Acad Sci U S A* 111(22): 8287-8292.
5. Finger, T. E., Böttger, B., Hansen, A., Anderson, K. T., Alimohammadi, H. and Silver, W. L. (2003). Solitary chemoreceptor cells in the nasal cavity serve as sentinels of respiration. *Proc Natl Acad Sci U S A* 100(15): 8981-8986.
6. Genovese, F. and Tizzano, M. (2018). Microvillous cells in the olfactory epithelium express elements of the solitary chemosensory cell transduction signaling cascade. *PLoS One* 13(9): e0202754.
7. Gross, E. A., Swenberg, J. A., Fields, S. and Popp, J. A. (1982). Comparative morphometry of the nasal cavity in rats and mice. *J Anat* 135(Pt 1): 83-88.
8. Gulbransen, B. D., Clapp, T. R., Finger, T. E. and Kinnamon, S. C. (2008). Nasal solitary chemoreceptor cell responses to bitter and trigeminal stimulants *in vitro*. *J Neurophysiol* 99(6): 2929-2937.
9. Hansen, A. and Finger, T. E. (2008). Is TrpM5 a reliable marker for chemosensory cells? Multiple types of microvillous cells in the main olfactory epithelium of mice. *BMC Neurosci* 9: 115.
10. Howitt, M. R., Lavoie, S., Michaud, M., Blum, A. M., Tran, S. V., Weinstock, J. V., Gallini, C. A., Redding, K., Margolskee, R. F., Osborne, L. C., Artis, D. and Garrett, W. S. (2016). Tuft cells, taste-chemosensory cells, orchestrate parasite type 2 immunity in the gut. *Science* 351(6279): 1329-1333.
11. Kaske, S., Krasteva, G., König, P., Kummer, W., Hofmann, T., Gudermann, T. and Chubanov, V. (2007). TRPM5, a taste-signaling transient receptor potential ion-channel, is a ubiquitous signaling component in chemosensory cells. *BMC Neurosci* 8: 49.
12. Kohanski, M. A., Workman, A. D., Patel, N. N., Hung, L. Y., Shtraks, J. P., Chen, B., Blasetti, M., Doghramji, L., Kennedy, D. W., Adappa, N. D., Palmer, J. N., Herbert, D. R. and Cohen, N. A. (2018). Solitary chemosensory cells are a primary epithelial source of IL-25 in patients with chronic rhinosinusitis with nasal polyps. *J Allergy Clin Immunol* 142(2): 460-469 e467.
13. Krasteva, G., Canning, B. J., Hartmann, P., Veres, T. Z., Papadakis, T., Muhlfeld, C., Schliecker, K., Tallini, Y. N., Braun, A., Hackstein, H., Baal, N., Weihe, E., Schutz, B., Kotlikoff, M., Ibanez-Tallon, I. and Kummer, W. (2011). Cholinergic chemosensory cells in the trachea regulate breathing. *Proc Natl Acad Sci U S A* 108(23): 9478-9483.
14. Krasteva, G. and Kummer, W. (2012). "Tasting" the airway lining fluid. *Histochem Cell Biol*

138(3): 365-383.

15. Lee, R. J., Kofonow, J. M., Rosen, P. L., Siebert, A. P., Chen, B., Doghramji, L., Xiong, G., Adappa, N. D., Palmer, J. N., Kennedy, D. W., Kreindler, J. L., Margolskee, R. F. and Cohen, N. A. (2014). Bitter and sweet taste receptors regulate human upper respiratory innate immunity. *J Clin Invest* 124(3): 1393-1405.
16. Lin W, Ogura T, Margolskee RF, Finger TE, Restrepo D (2008). TRPM5-Expressing Solitary Chemosensory Cells Respond to Odorous Irritants. *J Neurophysiol* 99(3):1451-1460.
17. Ogura, T., Szebenyi, S. A., Krosnowski, K., Sathyanesan, A., Jackson, J. and Lin, W. (2011). Cholinergic microvillous cells in the mouse main olfactory epithelium and effect of acetylcholine on olfactory sensory neurons and supporting cells. *J Neurophysiol* 106(3): 1274-1287.
18. Ohmoto, M., Yamaguchi, T., Yamashita, J., Bachmanov, A. A., Hirota, J. and Matsumoto, I. (2013). Pou2f3/Skn-1a is necessary for the generation or differentiation of solitary chemosensory cells in the anterior nasal cavity. *Biosci Biotechnol Biochem* 77(10): 2154-2156.
19. Pérez, C. A., Huang, L., Rong, M., Kozak, J. A., Preuss, A. K., Zhang, H., Max, M. and Margolskee, R. F. (2002). A transient receptor potential channel expressed in taste receptor cells. *Nat Neurosci* 5(11): 1169-1176.
20. Reid, L., Meyrick, B., Antony, V. B., Chang, L. Y., Crapo, J. D. and Reynolds, H. Y. (2005). The mysterious pulmonary brush cell: a cell in search of a function. *Am J Respir Crit Care Med* 172(1): 136-139.
21. Rhodin, J. and Dalhamn, T. (1956). Electron microscopy of the tracheal ciliated mucosa in rat. *Z Zellforsch Mikrosk Anat* 44(4): 345-412.
22. Sbarbati, A. and Osculati, F. (2003). Solitary chemosensory cells in mammals? *Cells Tissues Organs* 175(1): 51-55.
23. Ualiyeva, S., Hallen, N., Kanaoka, Y., Ledderose, C., Matsumoto, I., Junger, W. G., Barrett, N. A. and Bankova, L. G. (2020). Airway brush cells generate cysteinyl leukotrienes through the ATP sensor P2Y2. *Sci Immunol* 5(43).
24. Ualiyeva, S., Yoshimoto, E., Barrett, N. A. and Bankova, L. G. (2019). Isolation and Quantitative Evaluation of Brush Cells from Mouse Tracheas. *J Vis Exp*(148).
25. von Moltke, J., Ji, M., Liang, H. E. and Locksley, R. M. (2016). Tuft-cell-derived IL-25 regulates an intestinal ILC2-epithelial response circuit. *Nature* 529(7585): 221-225.
26. Yamaguchi, T., Yamashita, J., Ohmoto, M., Aoude, I., Ogura, T., Luo, W., Bachmanov, A. A., Lin, W., Matsumoto, I. and Hirota, J. (2014). Skn-1a/Pou2f3 is required for the generation of Trpm5-expressing microvillous cells in the mouse main olfactory epithelium. *BMC Neurosci* 15: 13.
27. Zhang, Z., Zhao, Z., Margolskee, R. and Liman, E. (2007). The transduction channel TRPM5 is gated by intracellular calcium in taste cells. *J Neurosci* 27(21): 5777-5786.

