Chapter 12

Fluorescence-Activated Cell Sorting (FACS) Protocol for Podocyte Isolation in Adult Zebrafish

Thomas J.D. Bates, Uta Naumann, and Christoph Englert

Abstract

Zebrafish is becoming a very important model for studying human diseases. The conserved structure of the nephrons in the kidney allows the user to answer questions relating to study human kidney disorders. *Wt1a*-expressing podocytes are the most important cells within the glomeruli of adult zebrafish. In order to understand the molecular characteristics of these cells, within damage models, we have established a method for isolating them.

Key words Podocytes, Wt1a, FACS, RNA isolation, qPCR

1 Introduction

When analyzing kidney function in adult zebrafish, it is sometimes necessary to perform tissue-specific gene expression analysis. This protocol was developed to allow the isolation of GFP-positive cells from the adult zebrafish kidney (*wt1a:EGFP*) and thus gives the user the ability to obtain podocytes for RNA isolation and qPCR. Here you will find a detailed explanation of the tissue isolation, FACS sample preparation, and subsequent analysis.

2 Materials

1. Fish water: 25 l ddH_2O, 2.5 g sea salt, 4.95 g $CaSO_4$, 1 mg $NaHCO_3$.
2. Tricaine stock: 2.5 g Tricaine, 50 ml dH_2O.
3. Tricaine solution: 2 ml Tricaine stock, 250 ml fish water.
4. HBSS.
5. Sterile cell culture PBS.
6. Collagenase II (20 mg/ml).

Nicholas Hastie (ed.), *The Wilms' Tumor (WT1) Gene: Methods and Protocols*, Methods in Molecular Biology, vol. 1467, DOI 10.1007/978-1-4939-4023-3_12, © Springer Science+Business Media New York 2016

7. Dispase 4%.
8. FACS buffer: Sterile PBS, 2% FCS, 2 mM EDTA.
9. Fetal calf serum (FCS).

3 Methods

3.1 Kidney Isolation

1. Fish are euthanized in tricaine solution for 5 min, until the heart stops beating.
2. Firstly, remove the head, with a cut just behind the eyes (*see* **Note 1**).
3. Lay fish ventral side up, and make a cut from the cloaca towards the left gill, along the body wall. Repeat for the right side of the body.
4. Next fold ventral back towards head, taking attached organs. Remove remaining organs with fine forceps (*see* **Note 2**).
5. The zebrafish kidney adheres to the dorsal wall of the zebrafish body cavity. It is distinguishable from muscle, by its distinctive grey color. Podocytes can be visualized on a simple fluorescent microscope (Fig. 1a).
6. Kidney tissue is removed using two pairs of fine forceps (*see* **Note 3**). Starting at the most posterior region, start to remove the kidney tissue.
7. When you have isolated the whole kidney, place onto a glass petri dish (*see* **Note 4**). Using a sterile scalpel blade, macerate the kidney tissue lightly, and carefully transfer to a sterile 5 ml Eppendorf tube.

3.2 FACS Sample Preparation

1. Add 1 ml HBSS (+100 μl Collagenase) and incubate tubes for 1 h at 37 °C.
2. Place a 100 μl cell strainer on top of a sterile 50 ml Falcon tube.
3. Using a 1 ml pipette, add the whole sample (plus any remaining tissue) onto the cell strainer. Using a plunger from a 2 ml syringe, gently push any remaining tissue through the cell strainer.
4. Add 1 ml of sterile PBS to the filter, to wash the filter (*see* **Note 5**).
5. Centrifuge samples at 300 × *g* for 5 min at 4 °C.
6. Remove supernatant, and resuspend pellet in 1 ml sterile PBS (+25 μl Collagenase II and 3 μl Dispase) and incubate tubes for 1 h at 37 °C.
7. Add 100 μl FCS to samples, to stop digestion.
8. Again, centrifuge samples at 300 × *g* for 5 min at 4 °C.
9. Resuspend pellet in 500 μl of FACS buffer. 10 μl of cell suspension is put onto a cytometer to check the quality of population (Fig. 1b).

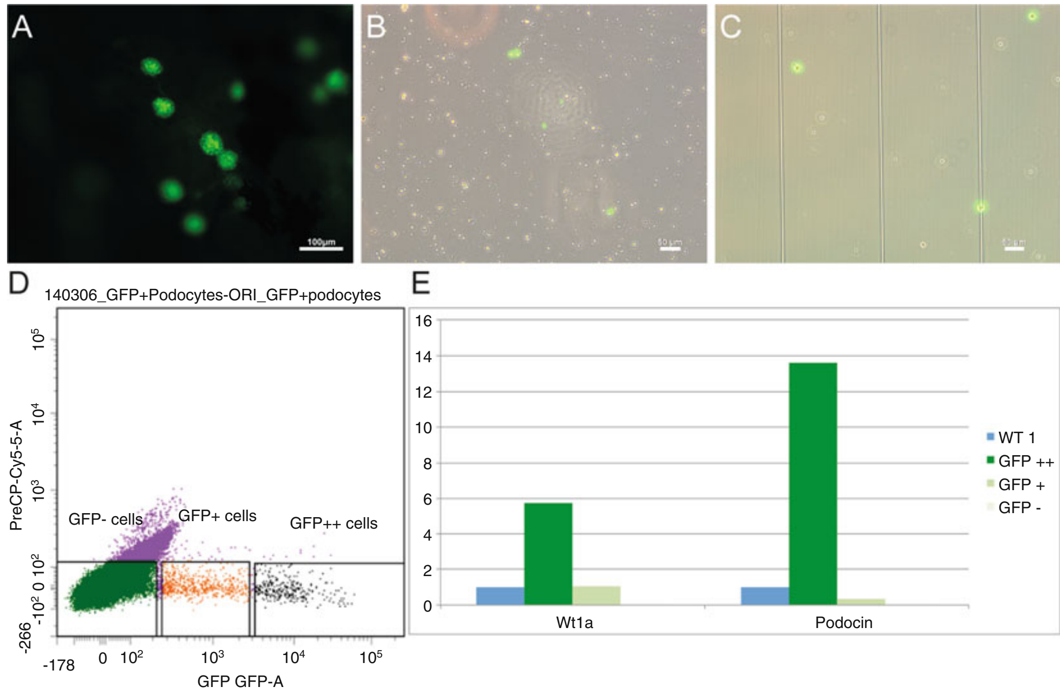

Fig. 1 FACS of Wt1a podocytes. (**a**) *wt1aE:GFP* fish exhibit a positive signal in the glomeruli of adult zebrafish. (**b**) Very few GFP+ve cells are seen when analyzing unsorted whole-kidney mass. (**c**) Following stringent FACS, a higher proportion of GFP+ve cells is achieved. (**d**) Sorting gates are shown that can distinguish cells into three groups, based upon GFP intensity. (**e**) qPCR data suggests that GFP++ are podocytes, as they express high levels of *wt1a* and *podocin*

3.3 FACS Analysis

1. Samples were sorted based on GFP fluorescence (*see* **Note 6**) using a 100 μl nozzle at 20 psi.
2. Isolated cells were checked under a normal fluorescent microscope (Fig. 1c, d), and then centrifuged at 300 × *g* for 5 min at 4 °C.
3. Total RNA was isolated from the cells with a MagMax Total RNA Isolation kit (*see* **Note 7**), and standard qPCR (Fig. 1e) was possible after this.

4 Notes

1. After decapitation, it is best to blot any blood away. This often hinders dissection of kidney tissue.
2. This should be done delicately; if possible, one should not rip/tear organs.

3. This takes some practice, and is often initially learned using fixed tissue.
4. Place the petri dish on ice, to keep samples cool at all times.
5. There is normally an accumulation of sample on the underside that does not drip into the Falcon tube. This can be retrieved with a 1 ml pipette after the 1 ml of PBS has been added.
6. To set up the FACS machine for detecting, a GFP–ve kidney is initially analyzed. This allows for correct identification of GFP+ve cells.
7. This kit is from Ambion, but any kit that allows RNA isolation from low cell numbers should suffice.