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Protocol

# Dissociated Prostate Regeneration under the Renal Capsule

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Tissue recombination models are useful for studying cancer initiation, progression, and metastasis. They also provide an in vivo environment in which to investigate the functional role of stem cells in tissue repair. In this protocol, we describe in detail the dissociated prostate regeneration assay. Dissociated adult murine prostate cells are combined with embryonic urogenital sinus mesenchymal cells and implanted under the renal capsule. Morphological tissue structures with appropriate epithelial–stroma interactions are reconstituted in the grafts.



## MATERIALS

**It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous material used in this protocol.**

**RECIPES: Please see the end of this protocol for recipes indicated by <R>. Additional recipes can be found online at http://cshprotocols.cshlp.org/site/recipes.**

### Reagents

BFS growth medium <R>
Collagenase type I (1900 units/mL in RPMI medium)
DNase I
Dulbecco's Modified Eagle Medium (DMEM)
Ethanol
Fetal bovine serum (FBS)
Ketamine/xylazine
Mice (C57/BL6, male)
Mice (severe combined immunodeficient [SCID], male)
Neutralized collagen (freshly prepared, ice-cold) <R>
Povidone-iodine solution
Testosterone pellets (12.5 mg/pellet, 90-d release) (Innovative Research of America)

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Trypan blue
Trypsin (0.05%)
Urogenital sinus mesenchymal cells (see Protocol: **Preparation of Urogenital Sinus Mesenchymal Cells for Prostate Tissue Recombination Models** [Zong et al. 2014])

## Equipment

Cell-culture plates (six well)
Cell strainer (40 µm)
Centrifuge (benchtop)
Dissecting macroscope
Dissecting tools (scissors and fine forceps)
Glass pipette (fire-polished)
Hemocytometer
Incubator
Razor blade
Shaker
Shaver
Surgical supplies (sutures and metal clips)
Syringes with 18-, 20-, and 22-gauge needles
Tissue-culture dishes
Tubes (1.5-mL microcentrifuge)
Tubes (50-mL conical)
Warming plate
Vortex



## METHOD

### Dissociation of Mouse Prostate Tissues

1. Harvest and dissect the prostate tissues from male C57/BL6 mice as previously described in detail (Lukacs et al. 2010).
2. Place the prostate tissue into a culture dish and mince thoroughly using a razor blade. Transfer the minced prostate fragments into a 50-mL conical tube by flushing the dish with DMEM + 10% FBS, and add more fresh DMEM + 10% FBS to bring the final volume to 27 mL.
3. Add 3 mL of collagenase solution (1900 units/mL in RPMI medium) to the tissue and incubate the tube on a shaker at 37°C for 2 h.

   *These conditions are suitable for the digestion of prostates from as many as five adult mice. To dissociate more prostate tissue, simply split the tissue to ensure efficient digestion.*
4. Centrifuge the prostate chunks at 550*g* for 5 min at 25°C. Discard the supernatant and add 2 mL of 0.05% trypsin to the pellet, mix briefly by vortex, and incubate at 37°C for 5 min.
5. Add 3 mL of DMEM + 10% FBS containing 500 units of DNase I to quench the trypsin and break up the genomic DNA of the dead cells. Serially pass the suspension through 18-gauge, 20-gauge, and 22-gauge syringes by pipetting up and down five times per syringe.
6. Filter the cells through a 40-µm cell strainer into a 50-mL conical tube, and then wash the cell strainer with 40 mL of DMEM + 10% FBS.
7. Centrifuge the tube at 550 g for 5 min at 25°C to collect the cell pellet. Resuspend the cells in 1 mL of DMEM + 10% FBS. Count the live cells using a hemocytometer after trypan blue staining.


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### Preparation of Prostate Cell Recombinants in Collagen Plugs

8. Aliquot 1.5–2 × $10^5$ dissociated prostate cells into a 1.5-mL microcentrifuge tube and add 2 × $10^5$ UGSM cells. Centrifuge the combined cells at 550 g for 3 min at room temperature and remove the supernatant completely.
9. Resuspend the cell pellet thoroughly in 15 µL of ice-cold neutralized collagen and place the cell/collagen slurry into one well of a six-well cell-culture plate.

   *Caution should be taken to avoid introducing air bubbles when resuspending the cells in collagen.*
10. Incubate the plate at 37°C for 20 min to allow the collagen plug(s) to solidify. Add 3 mL of BFS medium to cover the plugs and incubate at 37°C until the engrafting surgery.

    *Surgery can be performed the same day or after overnight incubation. Occasionally, the collagen plugs will lift off the plate and become condensed after incubation; this is unlikely to affect the regeneration.*

### Engraftment of Prostate Cell Recombinants into the Subrenal Capsules

11. Anesthetize the SCID mice via intraperitoneal injection of ketamine/xylazine (100 mg/kg and 10 mg/kg body weight, respectively).
12. Prepare the surgical area by shaving and sterilization with povidone-iodine and ethanol.

    *Ensure that the mice are fully anesthetized, and increase the dose of ketamine/xylazine if necessary.*
13. With a mouse lying on its side on a warming plate, make a skin incision (~6–8 mm) on the middle flank above the kidney region. Cut through the peritoneal wall and expose the kidney. Exteriorize the kidney out of the abdominal cavity by applying gentle pressure on the abdomen using the thumb and forefinger.

    *See Troubleshooting.*
14. Gently pinch and lift the thin fibrous membrane of the kidney with fine forceps, and then puncture it and create a pocket between the lifted membrane and the renal parenchyma by blunt dissection. Transfer the collagen plug(s) from the six-well plate onto the cut edge of the renal capsule, and insert the grafts into the pocket using a sterile fire-polished glass pipette. Take care not to damage the parenchyma, which can cause severe bleeding.

    *Two to three prostate cell recombinants can be placed under the renal capsule of one kidney.*
15. Gently push the kidney back into the abdominal cavity while dilating the incision on the peritoneal wall. Suture the peritoneal wall and insert a testosterone pellet subcutaneously into the dorsal neck and shoulder region. Close the skin incision using metal clips, which should be removed 7 d after surgery.

    *See Troubleshooting.*

## TROUBLESHOOTING

***Problem (Step 13):*** The kidney cannot be slipped out of the abdominal cavity using gentle pressure on the abdomen.

***Solution:*** Alternatively, grab the fat tissue surrounding the tip of the kidney with fine forceps, and pull it and the kidney out.

***Problem (Step 15):*** Prostate cell recombinants fall out of the subrenal capsules.

***Solution:*** Before pushing the kidney back into the abdominal cavity, confirm that the grafts are inserted into the subrenal capsules and completely covered with the fibrous membrane of the kidney.

## RECIPES

### *BFS Growth Medium*

| Reagent | Final concentration |
|---|---|
| Dulbecco's Modified Eagle Medium (DMEM) | 1× |
| Fetal bovine serum (FBS) | 5% (v/v) |
| Nu-Serum IV | 5% (v/v) |
| Glutamine | 2 mM |
| Penicillin | 100 units/mL |
| Streptomycin | 100 µg/mL |
| Insulin | 5 µg/mL |

Store medium at 4°C for up to 1 mo.

### *Neutralized Collagen*

| Reagent | Volume |
|---|---|
| Collagen I, rat tail | 500 µL |
| NaOH (1 N) | 11.5 µL |
| PBS (10×) | 56.8 µL |

The final pH value should be 7.0. Store the solution on ice for up to 1 h.

## REFERENCES

Lukacs RU, Goldstein AS, Lawson DA, Cheng D, Witte ON. 2010. Isolation, cultivation and characterization of adult murine prostate stem cells. *Nat Protoc* **5:** 702–713.

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