expansion conditions [6]. They were able to expand the nephron progenitors and maintain nephron progenitor markers (**Figure 8**) without causing nephron progenitor differentiation (data not shown).

3. Experimental Approach:

A. Collection of fresh tissue for immediate shipment: In our preliminary data we show that we can collect genitourinary organs and produce high quality histological images and cell culture material both in house and via shipment to GUDMAP laboratories. In this aim, we will develop a collaborative relationship with the successful GUDMAP projects to provide high quality fresh samples based on individual needs. The tissue will be dissected by (b)(6) and a designated technician, and inspected for mechanical damage. The tissue will then be sent to the validation laboratories for further analysis before shipment. A small piece of the tissue will be used for histological evaluation to confirm tissue integrity. **The remaining tissue will be sent directly to the GUDMAP Atlas project investigators, arriving within 24 hours of acquisition**. The ages and number of samples that are required will be determined via interactions between the HUB and the individual Atlas projects (the current HSTB approach for all projects). We will work with each investigator to ensure they receive the highest quality fresh samples and will adjust collection, based on individual project requirements and feedback.

B. Isolation of distinct cell populations for shipment: We have developed in house techniques for the isolation of distinct cellular populations. Based on consultation with the Atlas projects we will determine the specific needs and cellular compartments that are needed to complete the projects and will isolate these cell types using Dynabeads®. In brief, we will utilize the Dynabeads® FlowComp™ Flexi kit (Invitrogen). This kit contains Dynabeads® that are conjugated to streptavidin. We will then biotinylate with the specific antibody needed to separate the required cell type. We will incubate isolated cells from the required tissues with the specific biotinylated antibody; then incubate these cells with bound antibodies with the streptavidin conjugated Dynabeads®. These will then be placed into a magnet to separate the cells. We will utilize a release buffer to remove the beads from the cells; by placing the cells back into the magnet to remove the beads. We will then verify the purity of these cells prior to shipment to the individual atlas projects.

C. Provide Laser capture and microdissection (LCM) services**:** HSTB provides LCM for specific projects. We have experience using the Leica LMD6000 LCM platform. If a specific GUDMAP project requires micro-dissected samples, the HUB will be glad to work with the PI of that project to provide this service.

4. Anticipated Results, Pitfalls and Alternatives: In this aim we anticipate being able to harvest and distribute high quality tissue and cells from the various fetal organ tissues to the successful Atlas projects. A pathologist and anatomist will evaluate all the tissues before shipment. We will validate each sample by taking a small piece for histology. We do not anticipate any major problems related to the acquisition and distribution of the tissues. Some tissues might need different processing to maintain tissue integrity; e.g. with the bladder urothelium. In these instances we will work with the individual projects to maximize the quality of the material they require. However, if we are unable to meet the demands of the various Atlas projects via the volume of our tissue hub and collection site, we will utilize additional identified tissue collection sites such as IIAM. Secondly, we will harvest isolated cell populations from the various developing genitourinary tissues based on the needs of the Atlas projects. We have extensive experience with this isolation technique and do not anticipate difficulties with this isolation. If we have problems with particular antibodies to bind to cell populations, we will work with the individual Atlas projects to optimize the isolation process for the cell type of interest.

D. Plans for banked tissue beyond grant period: A premise of this proposal is that the University of Pittsburgh HUB would collect and store specimens in addition to those immediately required by the GUDMAP investigators. This includes duplicate and consortium priority specimens; HSTB would retain custody of these residual specimens. In order to sustain the availability of residual specimens after the award period, the costs for ongoing storage, record keeping, and the effort required to disburse the specimens and associated data would be invoiced to the requesting investigator. The utility of sustaining such a valuable resource would depend on charging reasonably affordable rates. HSTB would need to cover the costs to maintain storage of residual specimens and provide sufficient and timely follow-up to requests. Therefore this plan presupposes that some cost to maintain this resource could fall to the HSTB. In addition to residual specimens, GUDMAP investigators may want to request prospective collection after the award period; we would make efforts to fulfill such requests assuming regulatory and financial agreements can be completed.