Conditional Hoxb8 for the conditional immortalization of bone marrow myeloid progenitors

Dear colleague,

Thank you for your interest in the ER-HoxB8 system of conditionally immortalizing myeloid progenitors. The system is currently used in more than 30 labs worldwide, and has been used to conditionally immortalize the bone marrow myeloid progenitors from dozens of transgenic and knock-out backgrounds. The system is ideal for the *ex vivo* production and study of neutrophils and macrophages, as well as myeloid-derived dendritic cells.

The system was originally described in the Nature Methods manuscript:

> Quantitative production of macrophages or neutrophils ex vivo using conditional Hoxb8. Wang GG, Calvo KR, Pasillas MP, Sykes DB, Häcker H, Kamps MP. Nat Methods. 2006 Apr;3(4):287-93.

I have since created a more detailed protocol (attached) for the derivation and propagation of these cells. It is quite a lengthy protocol but explains many of the steps along the way from virus production through to the freezing the cells.

We developed this system while I was a graduate student in Dr. Mark Kamps' lab at the University of California San Diego. Thus, I have had over 20 years of experience with the cells! I am very happy to help collaborate with the design and execution of your project as I truly love its potential as a model in studying normal and malignant myelopoiesis. This might involve simply consultant along the way, or helping to derive a new myeloid progenitor cell line.

On the other hand, I also can understand if you rather move forward without input or collaboration, and I would ask simply that the system is cited appropriately in your manuscript.

I have attached a description of available components on the next page, and a Material Transfer Agreement on the third page. I believe strongly that science needs to be shared! However, if you feel so moved, we would appreciate a small reimbursement for keeping the plasmids, conditioned media, virus, and cell lines in stock and availably to the community.

Sincerely yours,

David B. Sykes

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There are several components of the system, most notably:

- Plasmids
  - MSCVneo-HA-ER-Hoxb8
  - MIG-Flag-ER-Hoxb8
  - ECO-PAK (Ecotropic packaging construct)

- Virus
  - Ecotropic retrovirus derived from the plasmids

- Cells
  - Conditionally-immortalized myeloid progenitors derived from transduced marrow (i.e. the ER-Hoxb8 cells derived from marrow of a wild-type, or other, mouse)

  - CHO-SCF cells
    - This is a Chinese Hamster Ovary cell line that releases SCF into the supernatant to produce conditioned media.

  - B16-GM-CSF cells
    - This is B16 melanoma cell line that releases GM-CSF into the supernatant to produce conditioned media.

- Conditioned media
  - We prepare large batches of conditioned media that can be used at a final 1-2% concentration to supply the necessary amount of SCF or GM-CSF. Of course, recombinant SCF or GM-CSF can also be used, though for large quantities of cells the costs can quickly become prohibitive.

- Beta-estradiol (Sigma E2758)

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Material Transfer Agreement

Name of scientist requesting material	
Institution	
Address	
Phone number	
E-mail	

Name and E-mail of person at receiving institution or company to whom MTA correspondence should be directed (if different than above)

Material being requested	
Planned use of the material	

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Length of time the material will be used (i.e. how long will the research take? If 'indefinite', please explain)	

Is this a collaboration?	

Federal Express account (or other) for shipping and handling	

Optional reimbursement (Similar to Addgene and the ATCC, $100 per plasmid and $300 per cell line)	

Please email the completed MTA to	