ER-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 >150 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 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 cell freezing.

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 >20 years of experience with the cells! I am very happy to help collaborate with the design and execution of your project as I love its potential as a model in studying normal and malignant myelopoiesis. 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 components on the next page, and a Material Transfer Agreement on the third page. I believe strongly that science needs to be shared!

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)

- Cells
  - 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

| Scientist requesting material | |
|---|---|
| Name | |
| Institution | |
| Address | |
| Phone number | |
| E-mail | |

| Person at receiving institution or company to whom MTA correspondence should be directed (if different) | |
|---|---|
| Name | |
| E-mail | |

| | |
|---|---|
| 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 # | |

| | |
|---|---|
| Please email the completed MTA to: | Jacob Reynolds (jreynolds4@partners.org)<br>David Sykes (dbsykes@partners.org) |