Gene Trap, NMD, and the Knockout Mouse Project

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COMMENTARY

The Knockout Mouse Project

Mouse knockout technology provides a powerful means of elucidating gene function *in vivo*, and a publicly available genome-wide collection of mouse knockouts would be significantly enabling for biomedical discovery. To date, published knockouts exist for only about 10% of mouse genes. Furthermore, many of these are limited in utility because they have not been made or phenotyped in standardized ways, and many are not freely available to researchers. It is time to harness new technologies and efficiencies of production to mount a high-throughput international effort to produce and phenotype knockouts for all mouse genes, and place these resources into the public domain.

The Knockout Mouse Project

- **All mouse genes in ES cells will be inactivated within *five years* using *random* and *targeted* insertional mutagenesis techniques.**

- **Random gene trapping will be the first choice because it is simple, rapid, and cost-effective.**

- ***Genes not expressed in ES cells* are difficult to trap for some reasons.**

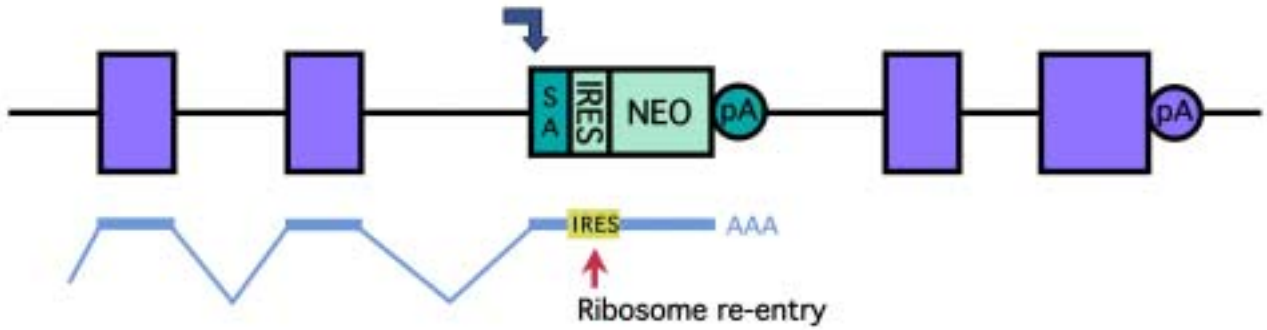
  ↓

  Laborious gene-targeting experiments might be required for the inactivation of such genes?

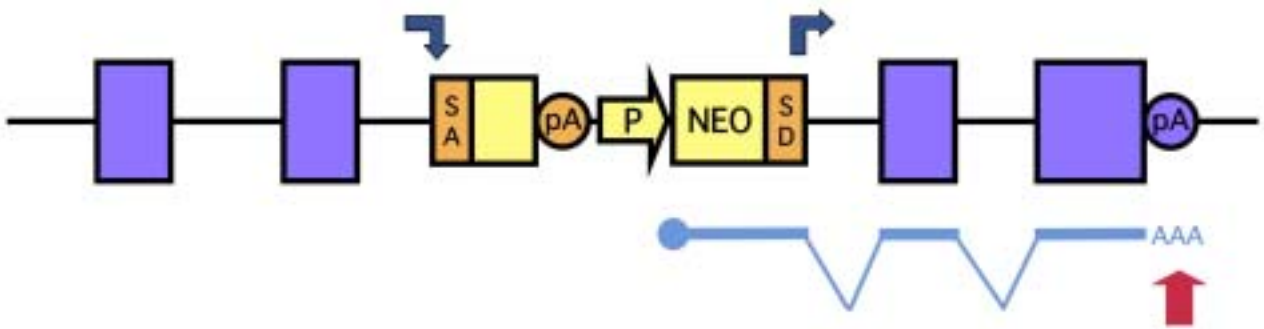
Two types of gene-trap techniques

- **Promoter trapping:** *only active genes*
- **Poly-A trapping:** *any genes*

Promoter



Poly-A



Two types of gene-trap techniques

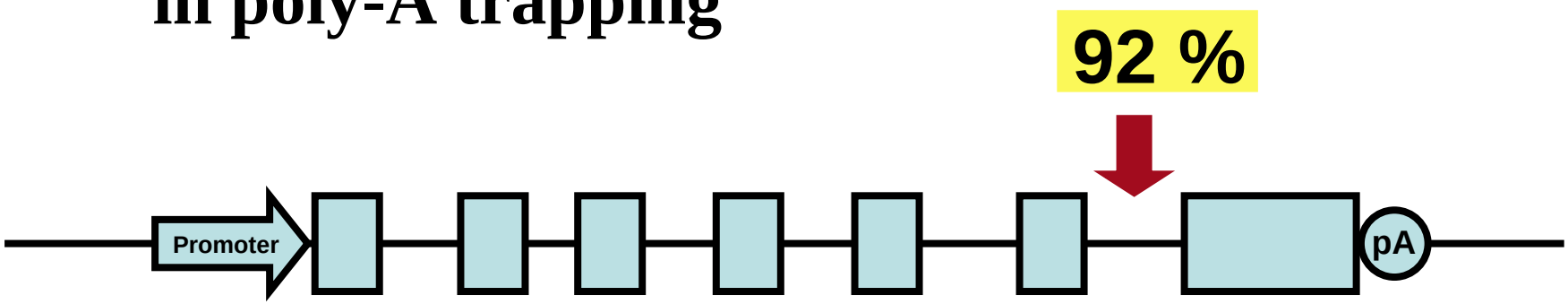
- **Promoter trapping:** ***only active genes***
- **Poly-A trapping:** ***any genes***

- **In theory, *poly-A trapping* should be much better than promoter trapping.**
- **In practice, *only a limited number of research groups* are currently engaged in poly-A trapping.**
- **Even *Lexicon Genetics Inc.* gave up their poly-A-trap project some years ago and switched back to promoter trapping.**

Two types of gene-trap techniques

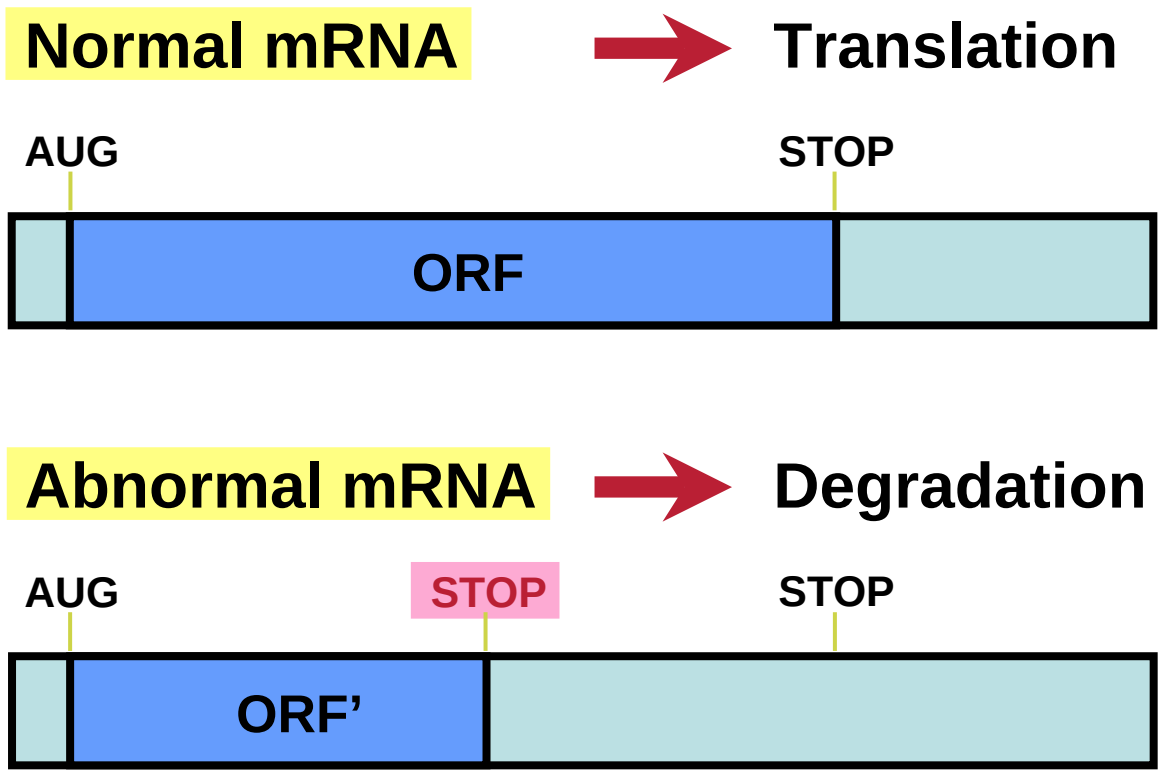
- **Promoter trapping:** *only active genes*
- **Poly-A trapping:** *any genes*

Strongly biased vector-integration site in poly-A trapping

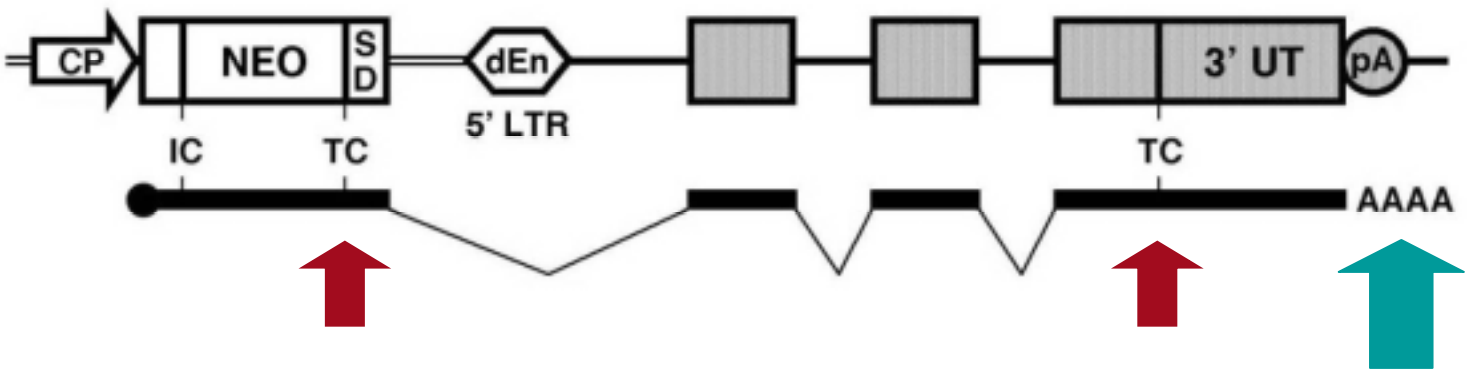


The same is also true for the CMHD poly-A trap

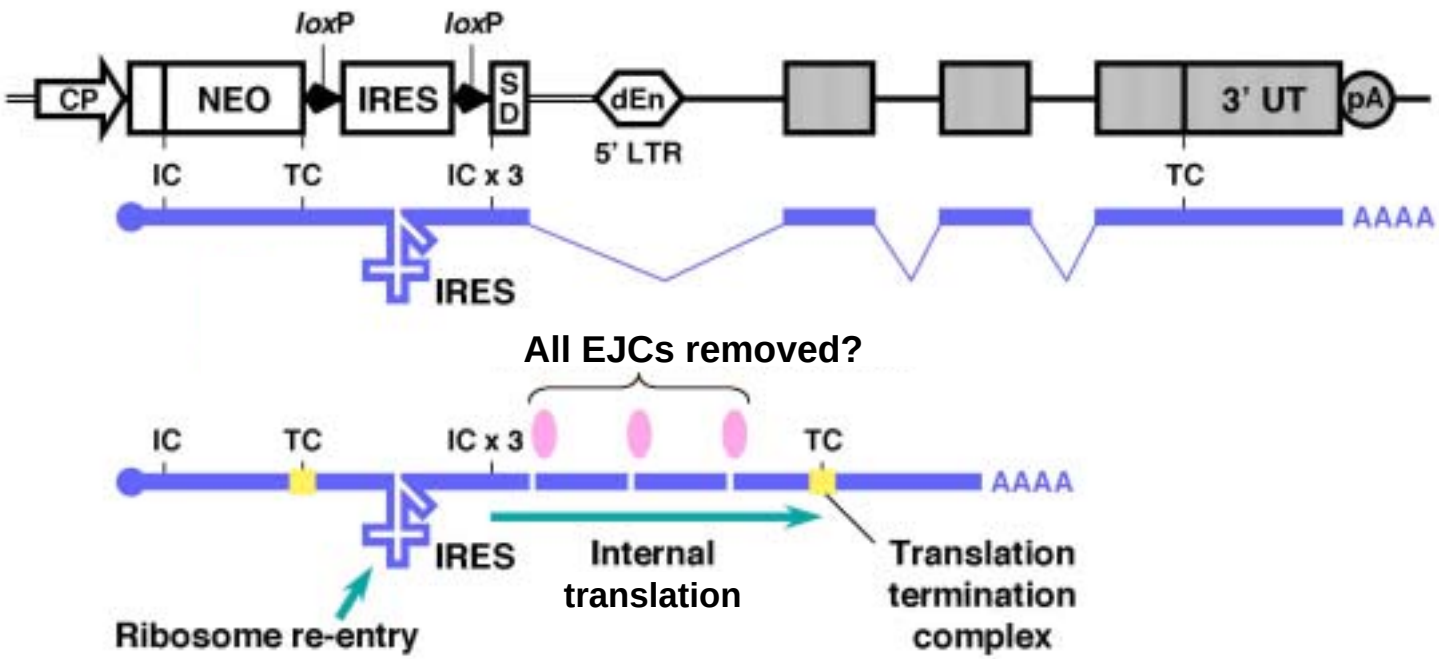
Nonsense-mediated mRNA decay (NMD): an mRNA surveillance mechanism



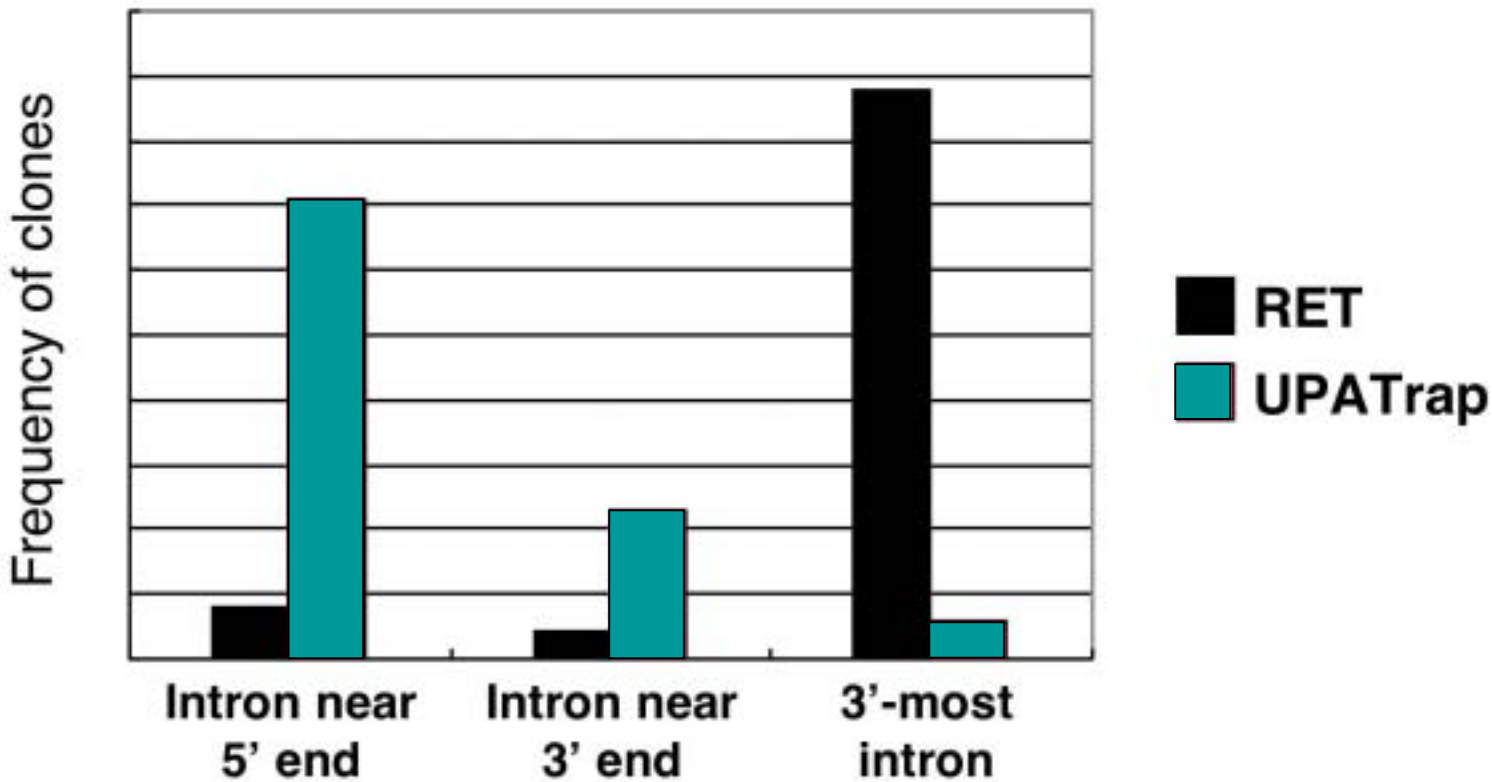
Presence of NEO PTC makes the cell nervous



The UPATrap vector



Strongly biased vector-integration site is completely corrected for in the UPATrap system



Summary of this part

A poly-A-trap vector:

Strongly biased vector-integration site

IRES insertion

Suppression of NMD

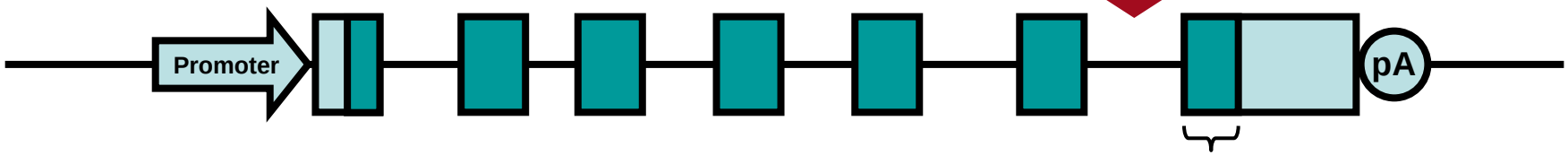
The UPATrap vector:

Unbiased vector-integration site

Poly-A trap

Strongly biased integration site

92 %

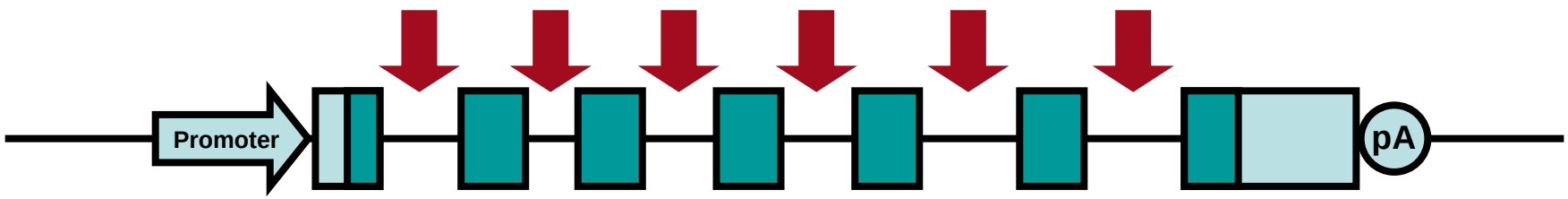


Small
C-terminal
deletion

Suppression
of NMD

UPATrap

Randomly distributed integration site



Geography of the Knockout Mouse Project

USA:

No big fund available yet

Canada:

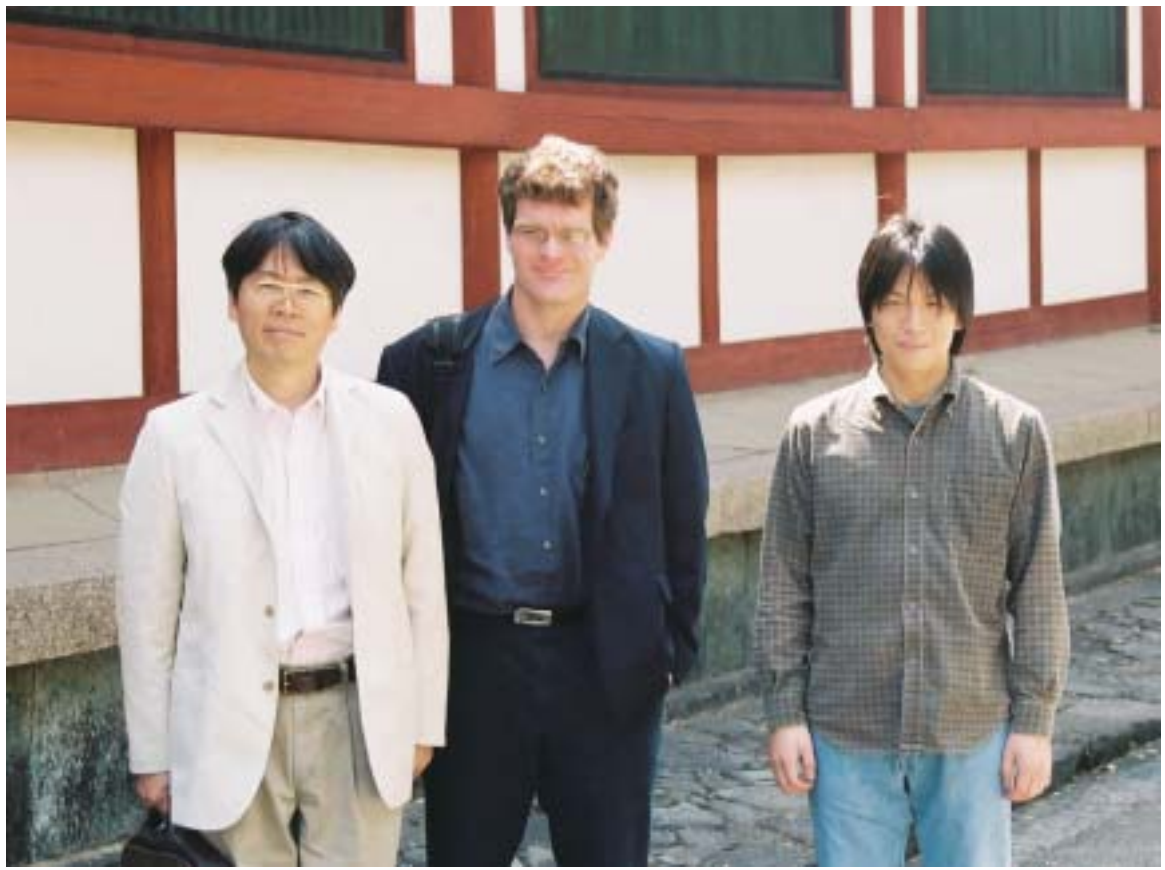
J. Rossant
CMHD & NorCOMM

EU:

A. Bradley
W. C. Skarnes
The Sanger Center, UK

W. Wurst
GGTC, Germany

Allan Bradley
Director
The Wellcome Trust Sanger Institute



Todaiji, Nara (April 21, 2005)

The Canadian Gene Trap Team

Mount Sini Hospital, University of Toronto (May 6, 2005)

The Japanese version of the Knockout Mouse Project

“Mouse Liaison”

University of Tokyo (April 15, 2005)

Contributors

- Toshiaki Shigeoka
- Nao Ekawa
- Yumi Hirose
- Masashi Kawaichi
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