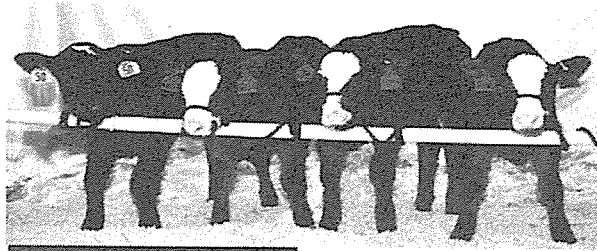
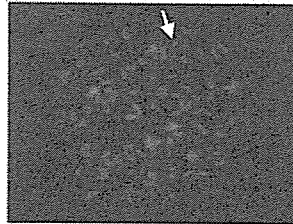


## Kirin Human Antibody Project

Kirin has been developing human antibody producing cows in collaboration with Hematech who owns bovine cloning technology.



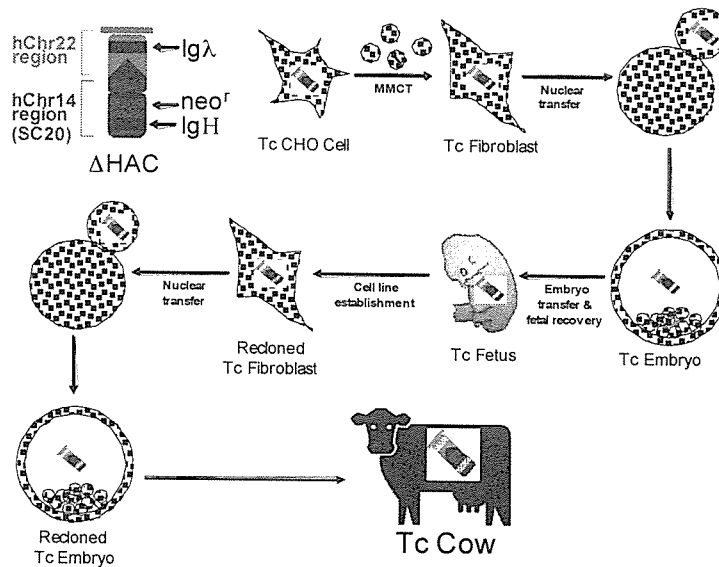
Cloned TransChromo Cow retaining a human chromosome fragment and expressing human immunoglobulin



A human chromosome in the Cloned TransChromo Cow

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## Generation of TC-cows



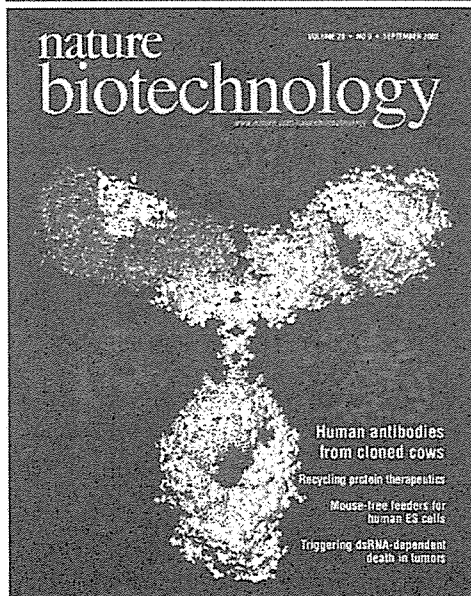
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## Kirin Human Antibody Project



We have generated more than 70 TC cows who express human immunoglobulin. The TC cows express various levels of the human Igs in their blood. Those TC cows produce antigen specific human Ig response upon immunization with the antigen. Endogenous bovine Ig- and PrP-gene KOs are now in progress to improve the human immunoglobulin expression for commercialization of the antibody products.<sup>11</sup>

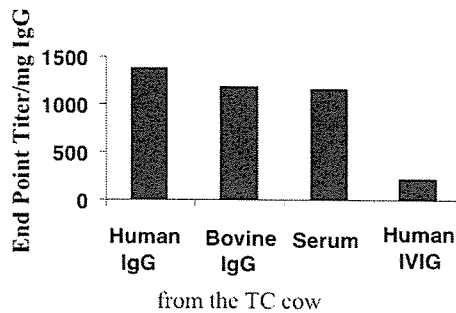
## Publication in Nature Biotechnology



100 Top Science Stories of 2002  
Published in DISCOVER Vol.  
24 No. 1 (January 2003).

1. The Year of Cloning  
Word in 2002 of new clones—including rabbits, cows, and a housecat—brings the tally of successfully cloned species to seven. The cloned cows had a human antibody gene added; the cloned pigs had a troublesome pig gene subtracted. And there was more than one boast that a human clone is in the works. How and why have we come this far? <sup>12</sup>

### Antigen specific immune response in the TC cow



A TC cow was immunized with bio-terro related pathogen. Human IgG or Bovine IgG was purified from the serum. The antigen specific titer of the human IgG or bovine IgG was measured by ELISA coated with the antigen.

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### Feasibility study using TC mouse (1)

#### Antisera against *Pseudomonas aeruginosa*

PBS (300 µl)	Human Plasma	TC-derived Serum (1/4 dil)	Human IgG (20 µg)	TC-derived Human IgG (20 µg)
5/5	5/5	<u>1/5</u>	5/5	<u>2/5</u>

The TC Mice were immunized by ip injection with about 100 mg per mouse of *Pseudomonas aeruginosa* (*Pa*) that had been fixed with 1% formalin for more than 24 h and washed with phosphate-buffered saline (PBS). Challenge involved inoculation of *Pa* ( $6.6 \times 10^7$  pfu/treated mice) was determined 4 days after *Pa* inoculation.

Ref: Cloning & Stem Cells, 4, 91-102, 2002.

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## Feasibility study using TC mouse (2)

### *In vitro* neutralization activity of antisera against Japanese Encephalitis Virus

	Titer (dilution)	Titer compensated <sup>a</sup>
Human plasma	29	<u>2.8</u>
TC-derived serum	971	<u>224.8</u>

<sup>a</sup>The titer values were compensated with the concentration of IgM and IgG contained in the serum samples.

The TC mice were immunized four times at 2-week intervals with Japanese encephalitis virus (JEV) vaccine prepared by the Chemo-Sero-Therapeutic Research Institute. Freund's adjuvant was included with the immunogen. Immobilized human or TC serum (56C for 30 min) diluted with buffer was mixed with the same volume of JEV (2,000PFU/ml) solution and incubated at 37C for 90 min, and then titrated using a plaque assay on vero cells. The neutralizing titer represents the dilution of the serum sample showing 50% plaque reduction compared to the control.

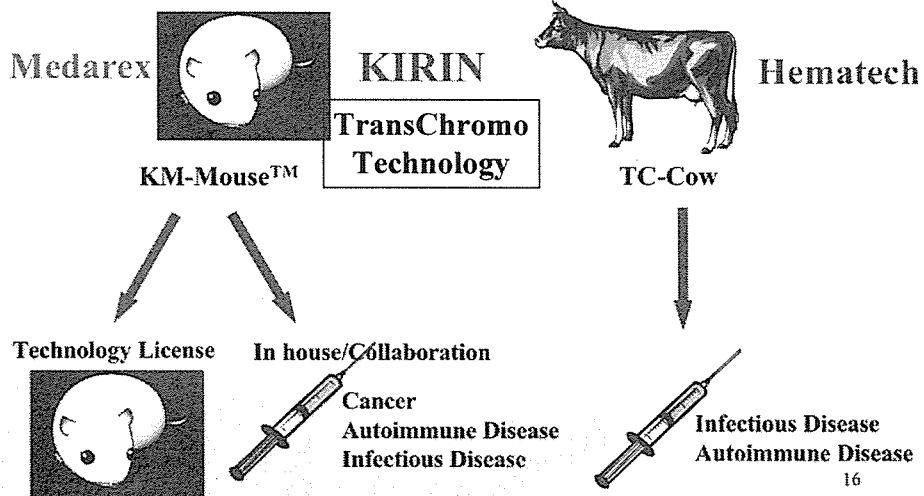
Ref: Cloning & Stem Cells, 4, 91-102, 2002.

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## Human Antibody Business

### Human monoclonal antibody

### Human polyclonal antibody



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