BTX HARVARD APPARATUS
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CELL TRANSFECTION & CELL FUSION PRODUCTS

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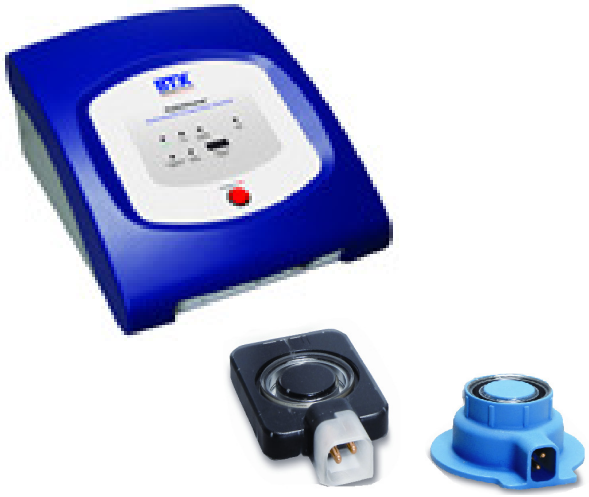
Electrofusion generates stable hetero-hybridoma cell lines from EBV-transformed B-CLL cells

ABSTRACT
The efficiency of primary B-CLL transformation is improved after EBV (Epstein-Barr Virus) infection by coculturing patient peripheral blood mononuclear cells (PBMCs) with irradiated mouse feeder cells (J774A.1 cells). When these clones were hybridized by electrofusion with an appropriate partner, stable hetero-hybridoma B-CLL cell lines of defined specificity were generated.

DISCUSSION
Hwang, K. et al., improved the efficiency of EBV B-CLL transformation of CpG oligonucleotide-stimulated cells by incubating patient peripheral blood mononuclear cells in the presence of an irradiated mouse macrophage cell line, J774A.1. Using this approach, peripheral blood mononuclear cells isolated from 13 of 21 B-CLL patients were transformed as documented by *IGHV-D-J* sequencing. Four clones grew and retained CD5 expression in culture for 2 to 4 months.

METHOD
K6H6/B5 myeloma partner cells and EBV-transformed B cells were washed twice with Cytofusion Medium C (BTX, Holliston, MA) before fusion. A 1:2 B cell to myeloma cell ratio was used in fusion. Electrofusion was achieved using a PA-4000/PA-101 apparatus (predecessor to the Hybrimune System, BTX, Holliston, MA) with FE-20/800 electrode fusion chamber (9 ml Chamber part #47-0020, BTX, Holliston, MA). Pre-fusion dielectrophoresis was performed with an alternating current voltage of 75 V at 0.8 MHz for 15 seconds. Cells were fused with a single square-wave direct current voltage of 300 V for 0.04 ms. Post fusion dielectrophoresis was performed with an alternating current voltage of 20 V at 0.2 MHz for 30 seconds. After fusion, cells were harvested and distributed into 96-well flat-bottom plates at 4000 B cells per well and incubated in culture medium.

CONCLUSION
This more reproducible and efficient system of EBV-induced growth transformation should help define the antigen reactivities of B-CLL clones as well as providing a replenishable source of B-CLL cells and DNA for genetic analyses.



Hybrimune® Hybridoma Production System
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Source: Enhanced outgrowth of EBV-transformed chronic lymphocytic leukemia B cells mediated by coculture with macrophage feeder cells
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