Visualizing Immunoglobulin Heavy Chain Gene Locus in Live B-Lymphocytes

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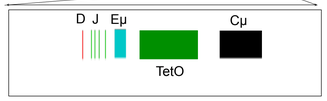
Abstract

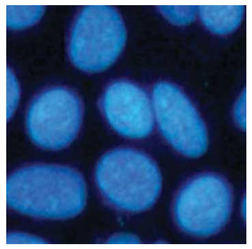
B lymphocytes (B cells) produces antibodies or immunoglobulins, which comprise of a pair of heavy chains linked to a pair of light chains. During B cell development these heavy and light chains are created from three gene segments: a variable (V) segment, a diversity (D) segment, and a joining (J) segment by VDJ recombination. For this project we aim to localize the immunoglobulin heavy chain (IgH) gene locus in live B lymphocytes, so that it can be tracked through live-cell imaging to study its motion. DNA Fluorescent In Situ Hybridization (DNA FISH) is a widely used technique to visualize specific DNA sequences, but a major drawback of this technique is that it kills cells in the fixation step. To overcome this challenge, we have utilized the specific binding of bacterial Tet Operator (TetO)-Tet Repressor (TetR) as a probe to mark the immunoglobulin heavy chain (IgH) gene. We transduced the mouse B lymphocytes, which have TetO inserted in the IgH locus with EGFP tagged Tet repressor (TetR-EGFP) and observed specific localization of IgH gene locus as visualized by two bright spots under fluorescent microscope.

Introduction

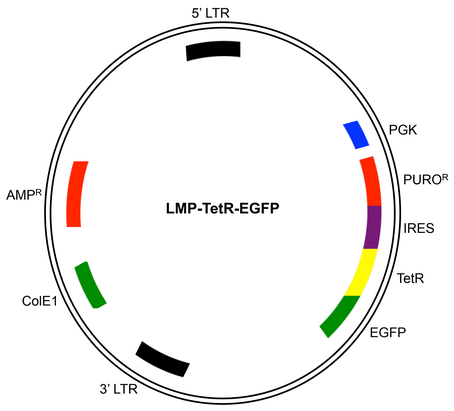
The immunoglobulin gene heavy chain locus is structured into distinct domains comprised of distal V_H, proximal V_H and the D_HJ_H regions. Previously, in Dr. Murre's laboratory, the TetO binding sites were inserted adjacent to D_HJ_H regions, which can be used as a marker to track immunoglobulin heavy chain gene (IgH) motion.



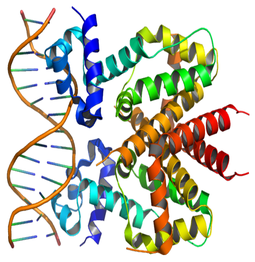
Tet operator (TetO) inserted in mouse IgH locus



DAPI labeled DNA:
DNA is labeled indiscriminately



TetR-EGFP –expressing retroviral LMP vector

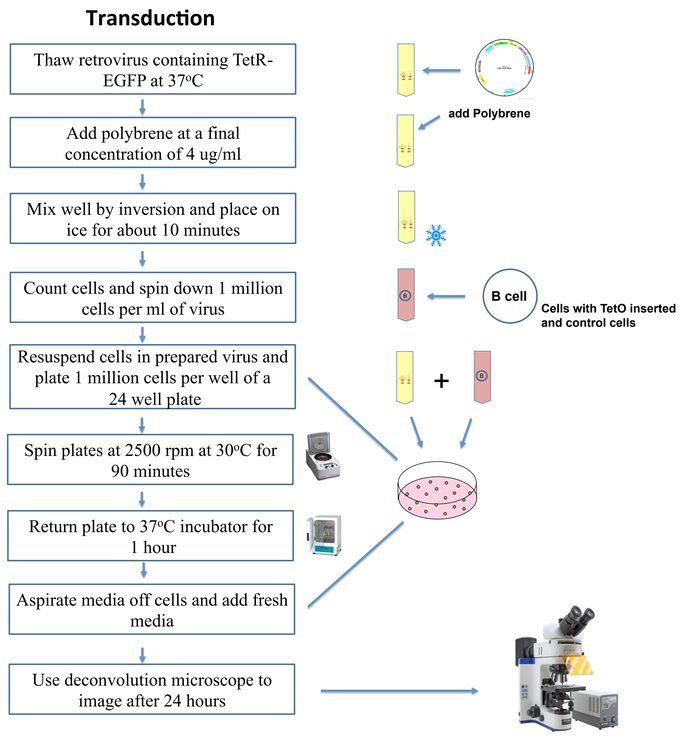


Specific binding of TetO and TetR

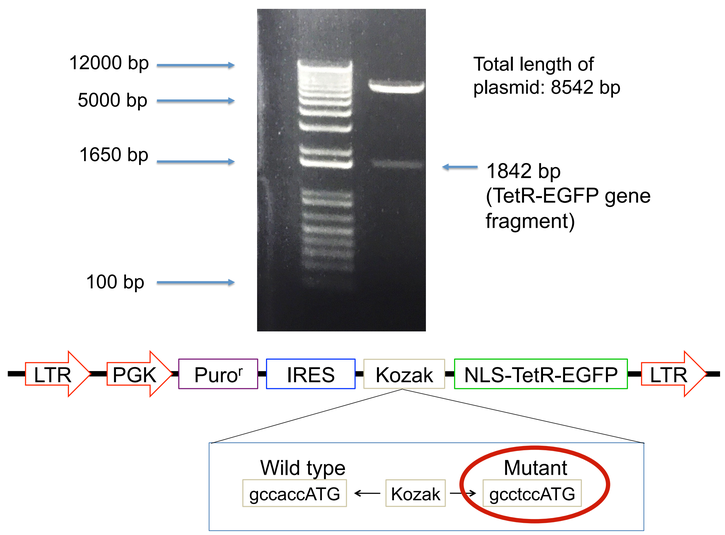
TetR-EGFP expressing retroviral LMP vector was used to transduce B cells. The kozak sequence was mutated to obtain optimal expression level of TetR-EGFP.

TetO and TetR are naturally found in bacteria. They are quite similar to LacO and LacR.
240 copies of TetO are required for a good signal.

Methods

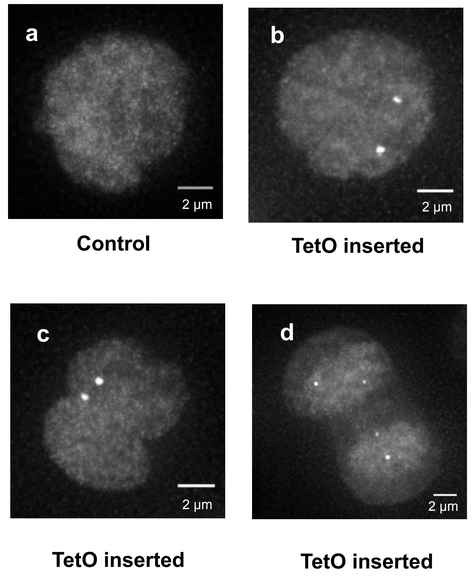


Gel Electrophoresis of TetR-EGFP Containing Plasmid



We used agarose gel electrophoresis to illustrate the components of our probe plasmid. TetR-EGFP recombinant plasmid was digested by AvrII/ SalI restriction enzyme. The TetR-EGFP gene fragment is depicted in the gel image.

Results



Figures:
a. Wild- type B cell, no bright spot was observed.
b. TetO was inserted, two bright spots were observed.
c. TetO was inserted, two bright spots were observed.
d. TetO was inserted, two brightspots were observed. Cell undergoing division.

Discussion and Conclusions

We optimized a technique that allows us to visualize a specific gene locus in mouse B lymphocytes. In our project we were able to localize the D-J segments of the IgH gene loci as indicated by two bright spots. This technique keeps the cells alive and enables us to track gene motion in live cells. This provides a deeper understanding of gene movement and its functional implication compared to DNA FISH, where we get still images of dead cells. Scientists can further use this technique to localize different segments of the IgH gene and can gain a fuller picture of how antibodies assemble in B lymphocyte.

References

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Acknowledgments

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