Cloning bovine immunoglobulin VH and VL genes from single B cells.

Immunize cow with antigen.

Collect blood and isolate PBMCs.

Sort PBMCs by FACS for IgG-presenting B cells.

Amplify VH and VL genes separately using nested primers with restriction sites for cloning.

Clone VH and VL genes into expression vectors and sequence.

Figure 1. Schematic of heavy and light chain gene amplification from a single bovine B cell.

Figure 2. Primers for VH and VL PCR.

Figure 3. Specific primer product was formed at each reduced forward primer concentration.

Figure 4. Primer dimmer formation was reduced compared to previous combined primer set experiments. Figure 7. Primer dimmer formation was reduced compared to previous combined primer set experiments.

Figure 5. Primer dimmer formation was reduced compared to previous combined primer set experiments. Figure 6. Primer dimmer formation was reduced compared to previous combined primer set experiments.

Figure 6. Primer dimmer formation was reduced compared to previous combined primer set experiments.

Figure 7. Primer dimmer formation was reduced compared to previous combined primer set experiments.

Figure 8. The cell sorter impacts PCR success.

Table 1. Type of cell sorter used in pooled PCR reaction success rate.

Table 2. FACS machine used to sort cells.

Table 3. Results obtained from cells sorted by BD FACSAria II (A) or NanoCell's WOLF (B) cell sorter.

Conclusions

1. This is the first protocol to comprehensively optimize amplification of antibody genes from single bovine B cells.

2. Conditions for single cell PCR to amplify heavy and light chain genes can be optimized through nested PCR with this method.

3. A correlation exists between type of FACS machine used to sort cells and success rate of single cell PCR results, which may be due to lower stress on the B cells.

Future Directions

1. Application of this method for antigen-specific antibody discovery projects.

2. Little is known about bovine antibody gene repertoires, this method will enable us to evaluate bovine heavy and light chain genetic repertoires at the single cell level.

References

