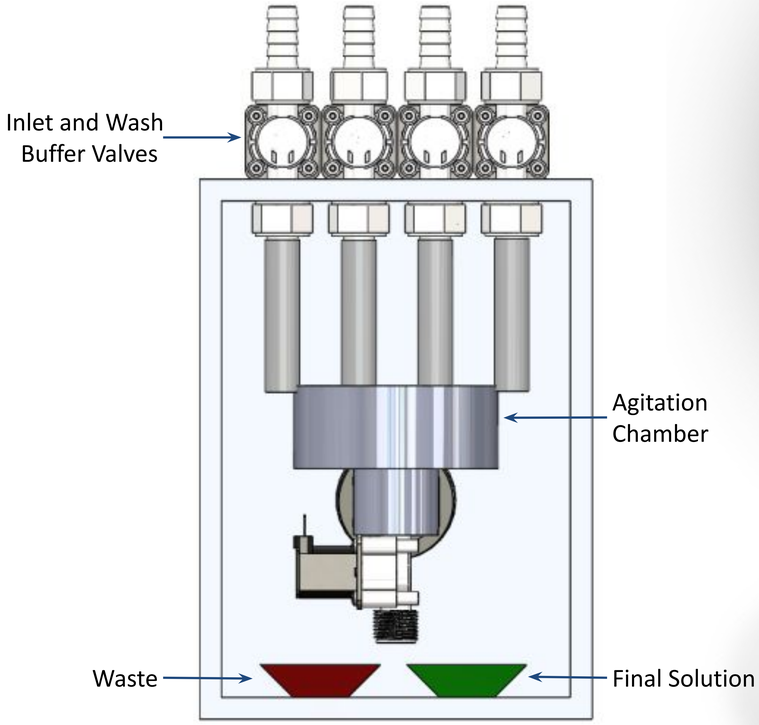
MilkGizmo DNA Extractor

Abstract

Bovine mastitis is the most common disease found in the dairy industry, resulting in economic loss due to diminished milk quality produced by infected cows. This condition arises from microbial infections and, in severe cases, can lead to systemic illness or even death. Currently, identifying these bacterial species requires labor-intensive processes, specialized lab equipment, and overall consumes valuable time, causing treatment delays, and potentially contributing to the spread of disease within the cow herd. This project aims to create a user-friendly device for on-site operation by dairy farmers. The device is engineered to extract bacterial DNA from milk, thereby streamlining the identification process through established diagnostic techniques. This innovation promises to significantly reduce treatment time, providing a timely and efficient solution to mitigate the impact of bovine mastitis on the dairy farming industry.



Device

Methods employed in the device for DNA extraction include fat and protein separation, bacterial lysis, DNA binding, DNA washing, and DNA elution.

Fat and Protein Separation: Using the one-pot lysis buffer and facilitating agitation of the solution, fat and proteins undergo emulsification.

Lysis: The one-pot lysis buffer facilitates chemical lysis, effectively breaking down the outer membrane of the bacteria and releasing DNA along with other intracellular components.

DNA Binding: DNA is captured using silica-coated beads.

DNA Washing: To remove contaminants released from the cell, three different wash buffers sourced from Qiagen, known as AW1, AW2, and AE, are employed.

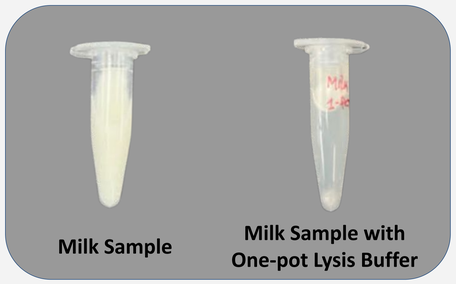
DNA Elution: The DNA is eluted using distilled water, resulting in an aqueous solution of bacterial DNA.

Discussion

With the innovative one-pot lysis buffer, multiple crucial steps are achieved. This buffer enables efficient fat separation, lysis of bacterial cells, and prepares released DNA for subsequent binding. It serves as the cornerstone of the device, significantly reducing the total runtime to 2 hours, ensuring swift results for farmers.

COMPOSITION

- **Guanidinium Hydrochloride** : 4 M
  - Necessary for binding to silica
- **Triton X-100**: 1% (v/v)
  - Helps lyse gram-negative bacteria
- **Isoamyl Alcohol**: 0.6% (v/v)
  - More potent to prepare DNA for binding
- **Ethanol**: 5% (v/v)
  - Necessary for silica binding
- **Sodium Acetate**: 0.1 M
  - Sodium helps with DNA binding
- **Acetic Acid**: Adjust the pH to approximately 4.5
  - Helps with precipitation at low pH
- **SDS**: 0.5% (w/v)
  - Inhibits nucleuses from working and helps with lysis
  - Emulsifies fats and proteins



Conclusion

At present, there are no convenient method available for testing bovine mastitis in cows. This device enables farmers to swiftly and effortlessly extract DNA from cow milk, offering a novel approach for detecting and testing bovine mastitis with ease. This innovation not only saves farmers time but also provides a financially advantageous solution by reducing costs associated with traditional testing methods.

REFERENCES

- W. N. Cheng and S. G. Han, “Bovine mastitis: Risk factors, Therapeutic strategies, and Alternative Treatments — a Review,” Asian-Australasian Journal of Animal Sciences, vol. 33, no. 11, pp. 1699–1713, Nov. 2020, doi: https://doi.org/10.5713/ajas.20.0156.
- "MP Biomedicals," Bead Beating Technology Explained, MP Biomedicals. [Online]. Available: https://www.mpbio.com/ca/bead-beating-technology-explained.
- B. Shi, Y. K. Shin, A. Hassanali, and S. J. Singer, “DNA binding to the silica surface,” The Journal of Physical Chemistry B, vol. 119, no. 34, pp. 11030–11040, Jun. 2015, doi: 10.1021/acs.jpcb.5b01983.
- L. J. A. Beckers, M. Baragona, S. Shulepov, T. Vliegenthart, and A. R. Van Doorn, “Mechanical Cell Lyses device,” Philips Applied Technologies, Oct. 2010, [Online]. Available: https://www.researchgate.net/publication/267416072_Mechanical_cell_lysis_device#fullTextFileContent

AUTHORS

Vlad Buiac
Dein Dami-Fiberesima
Cara Matthews
Harmanvir Sibia
Zachary Xavier