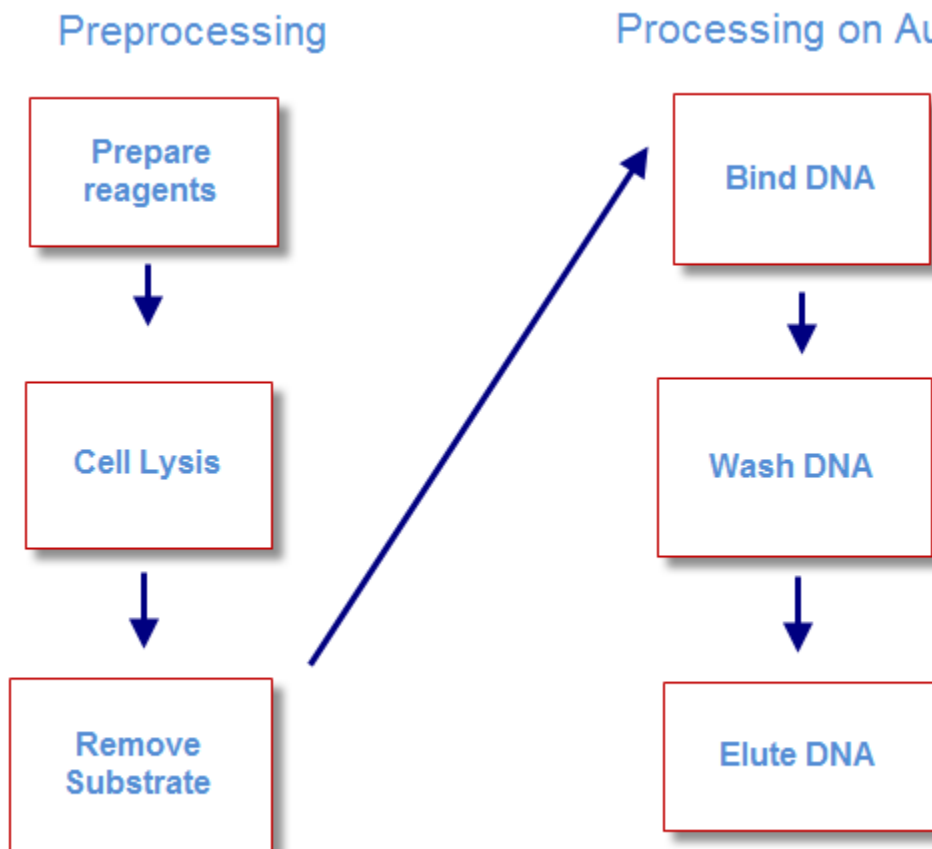


# Robotic Extraction

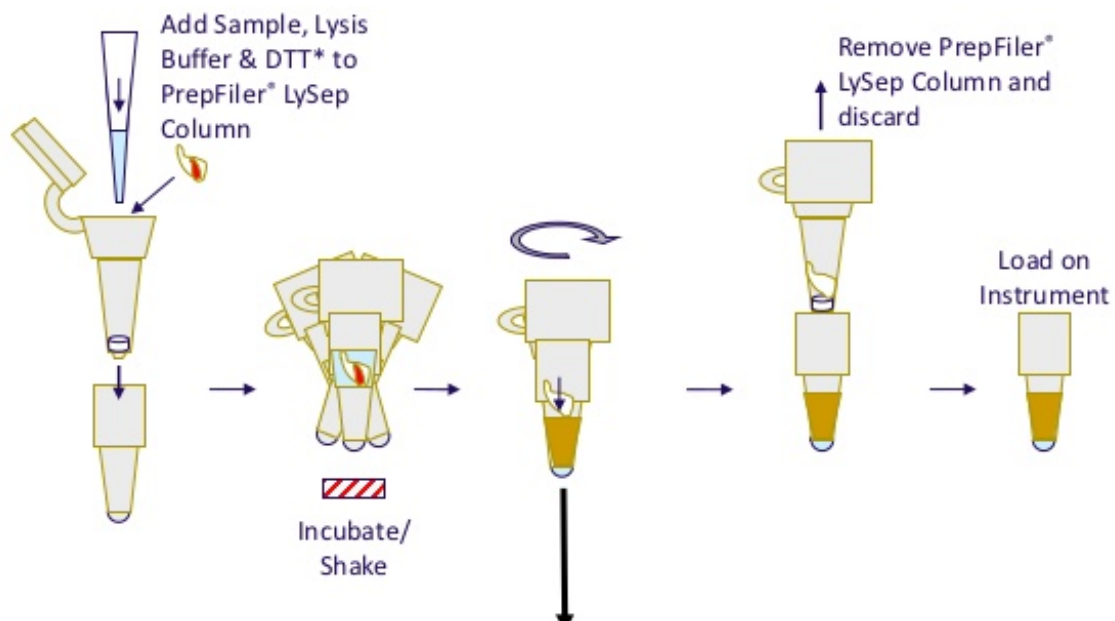
Using Automate Express™  
Instrument and PrepFiler Express™  
Chemistry

# Robotic Extraction



# Robotic Extraction

Summary of Preprocessing of sample using PrepFiler LySep™ Column



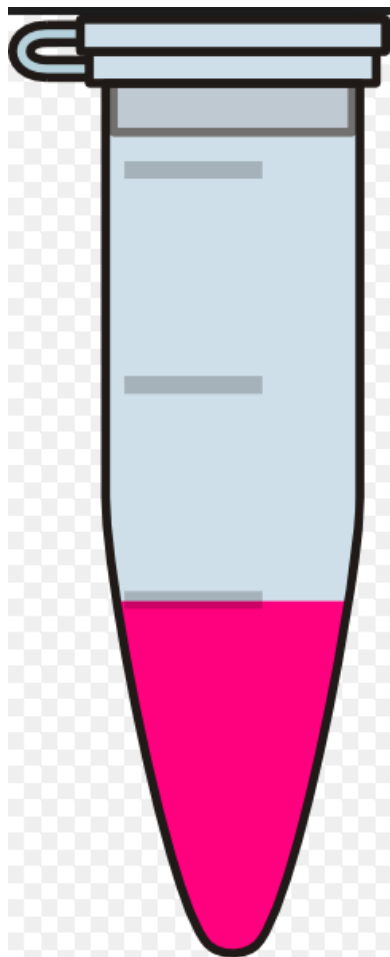
# Robotic Extraction

Lysep Column- Holds substrate and lysis buffer for cell lysis to occur during incubation.

Upon centrifugation the lysis buffer with DNA is pulled down into the tube.

The LySep column can then be removed with the substrate and discarded.

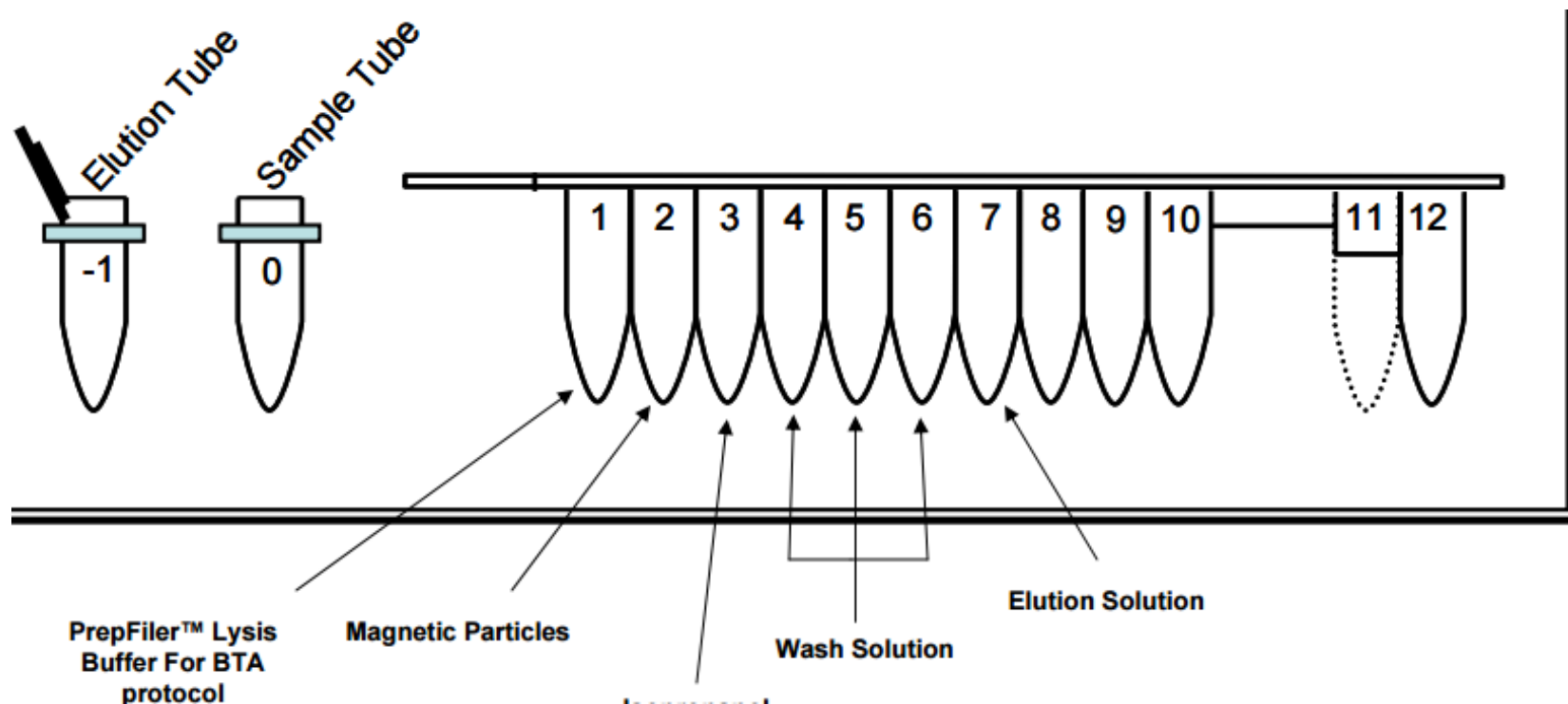
# Robotic extraction



- Liquid containing DNA is called Lysate.
- There should be at least 300 $\mu$ l of lysate remaining in tube for the following reasons
  - 1- Allows for effective binding of DNA to magnetic beads
  - 2- Allows for Proper mixing
  - 3- Prevents formation of air bubbles in tip
  - 4- Prevents liquid handling problems

# Robotic Extraction

## What happens on the Automate



# Robotic Extraction

## On Automate

- 1-Sample mixes with magnetic particles and other reagents for subsequent DNA binding to magnetic particles in tips.
- 2-Using magnetic separation the DNA bound to the magnetic particles are separated from the Lysate.
- 3-PCR inhibitors are removed by washing with Wash buffers
- 4-Magnetic particles are dried to remove ethanol
- 5- Concentrated purified DNA is eluted in elution buffer.

# Robotic Extraction

## How DNA Binds to Magnetic Beads

- A chaotropic salt (guanidine thiocyanate) sarkosyl and a chelating salt act to bind DNA and chelate ions.
- DNA binds through presence of the chaotropic salt forming a salt bridge between negatively charged DNA and the polymer coated magnetic bead.
- Maximum binding of DNA occurs under conditions of high salt and low pH.



# Robotic Extraction

## Wash Step

After binding to Prepfil® beads wash buffers are used to remove degraded proteins and other inhibitors.

Wash buffers do not disrupt the salt bridge between the DNA and the magnetic particles.

# Robotic Extraction

## Elution Step

DNA is eluted using Prepfiler Elution Buffer.

The elution buffer is similar to TE Buffer.

DNA can be eluted using buffers with low salt concentration.

## Reference:

Ferguson, E. E. , "Effects of pH on Binding of DNA Using the PrepFiler BTA Forensic DNA Extraction Kit on the AutoMate Express Forensic DNA Extraction System" Fort Worth, Tx: University of North Texas Health Science Center; (2014). <http://digitalcommons.hsc.unt.edu/theses/746>