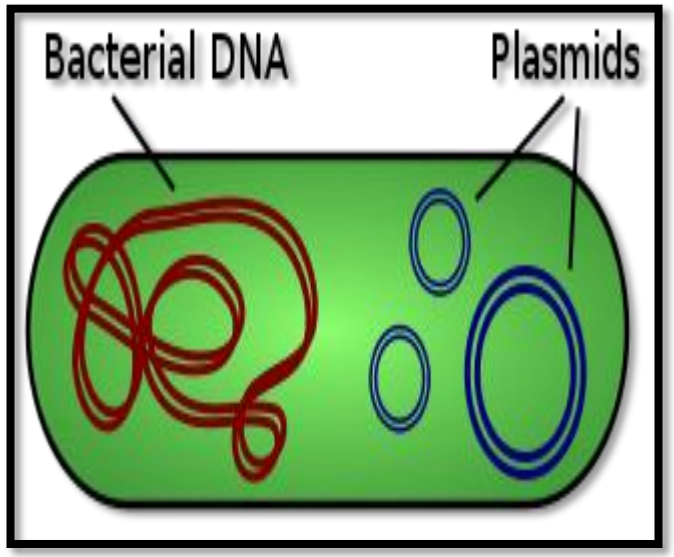
plasmid preparation

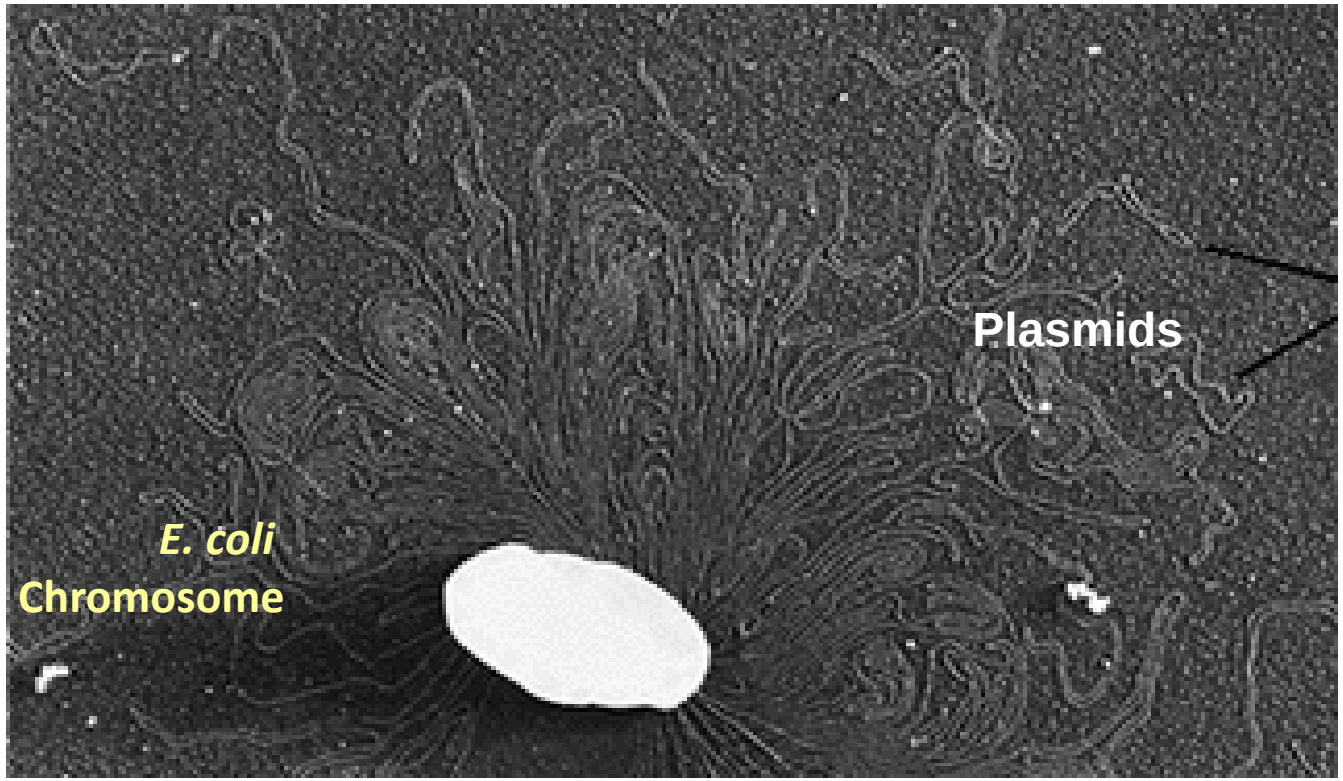
A **plasmid preparation** is a method used to extract and purify plasmid DNA. Many methods have been developed to purify plasmid DNA from bacteria. These methods invariably involve three steps:[*citation needed*]

Growth of the bacterial culture

Harvesting and lysis of the bacteria

Purification of plasmid DNA





plasmid

- A **plasmid** is a small DNA molecule within a cell that is physically separated from a chromosomal DNA and can replicate independently. They are most commonly found in bacteria as small, circular, double-stranded DNA molecules; however, plasmids are sometimes present in eukaryotic organisms.
- In nature, plasmids often carry genes that may benefit the survival of the organism, for example antibiotic resistance. While the chromosomes are big and contain all the essential information for living, plasmids usually are very small and contain only additional information.

Introduction

plasmids (positive clones). First, we will isolate plasmid DNA from two of the

transformed bacteria. Next we will cut the recombinant plasmid with the appropriate

restriction enzymes. After DNA digestion we will load an agarose gel and the results will

be examined during our next lab period.

LEARNING GOALS

1. Understand the biochemical and molecular effects of each reagent used in theminiprep protocol.

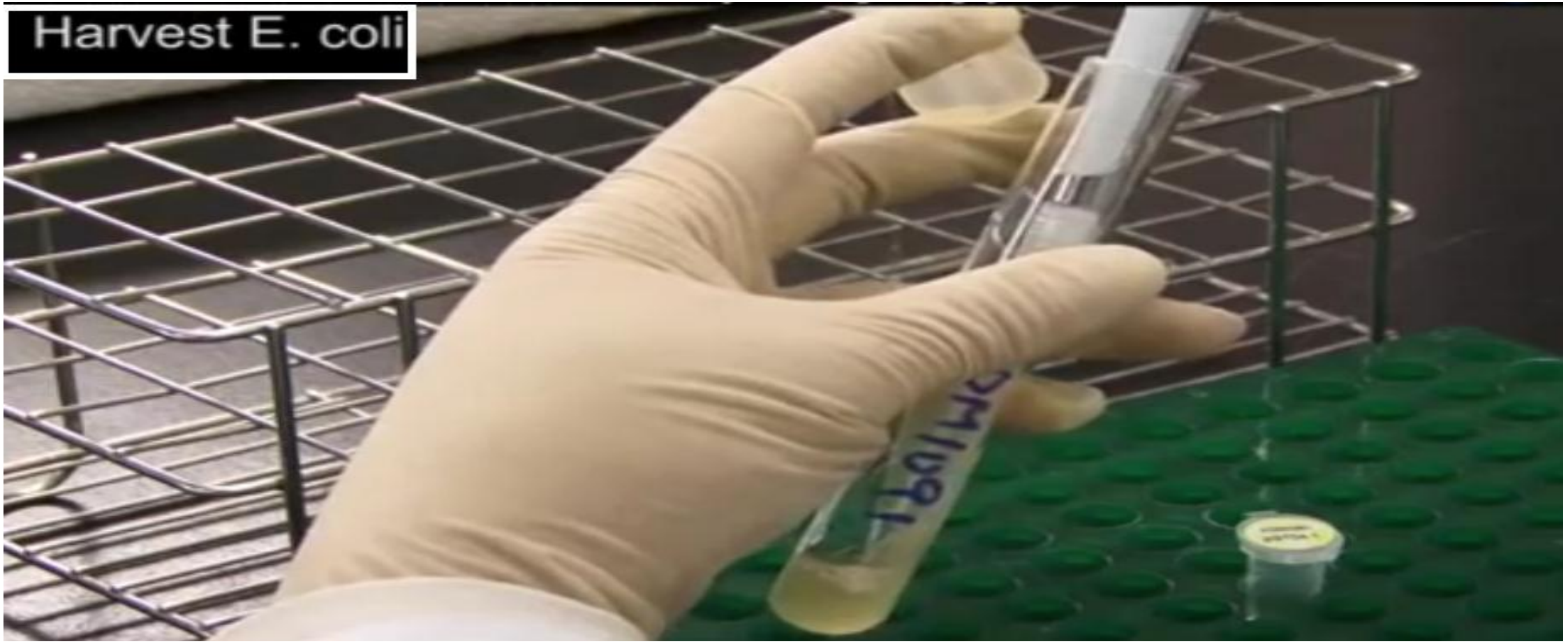
2. Understand the difference in mobility between a DNA fragment and an intactplasmid.

3. Know the main conformations of uncut plasmid DNA and be able to recognizethem on a gel.

4. Be able to recognize the major differences between bulk prep plasmid DNA (which is very pure) and miniprep plasmid DNA.

Plasmid DNA Isolation

1. Pour the culture into a 450ml centrifuge bottle. Balance the bottles



2. centrifuge in the Sorvall Centrifuge for 2 minutes at a speed of 13200 rpm.

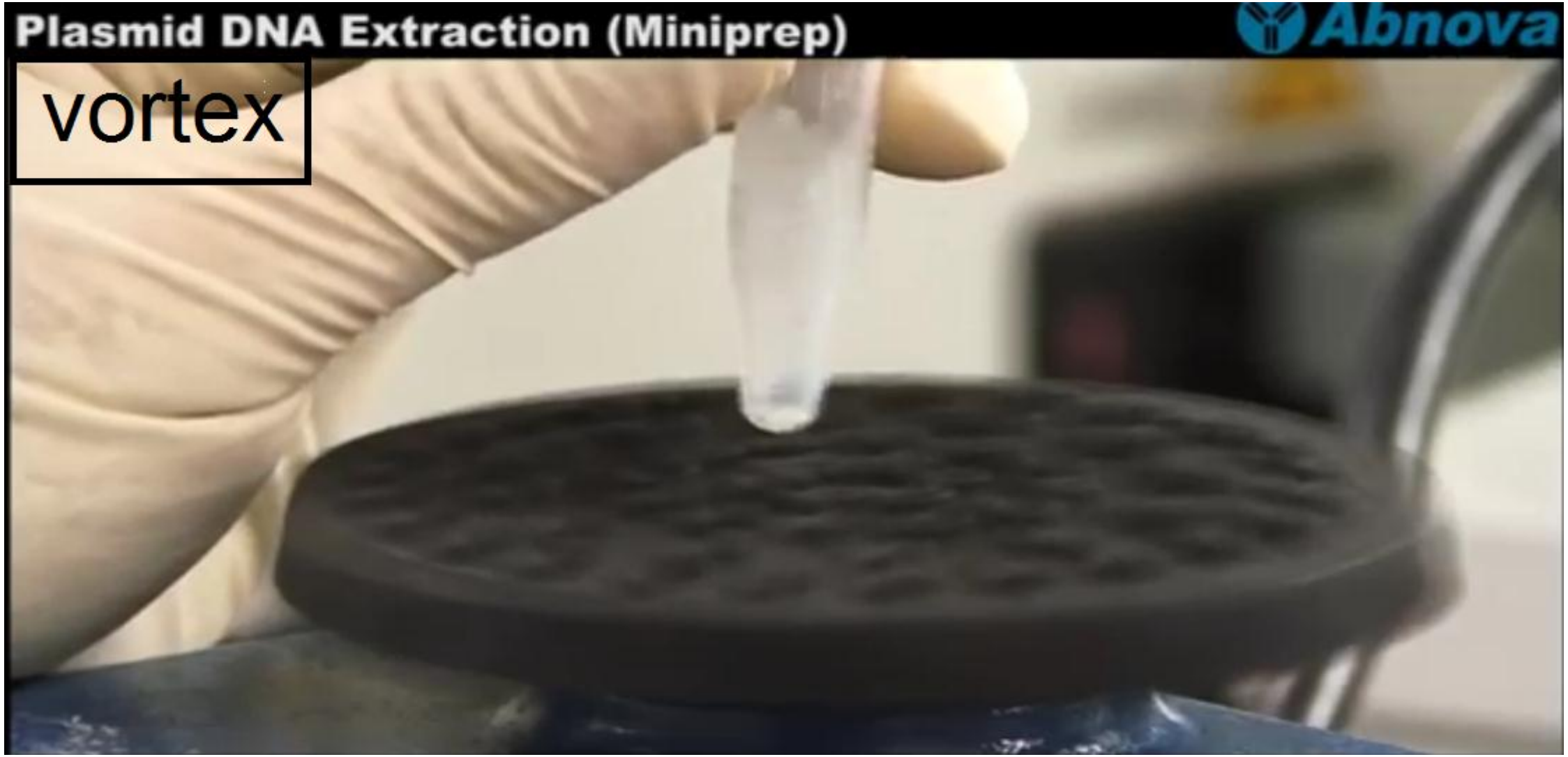
3. Pour off the supernatant and allow to drip for just a few seconds on some paper towels. At this step the pellet can be frozen and stored at -20°C. Resuspend the pellet in 10mls of Solution 1 (located at 4°C.)

4. Add Solution 1

Solution 1

- 50mM Glucose
- 10mM EDTA
- 25mM Tris pH 8.0

5. Vortex this solution

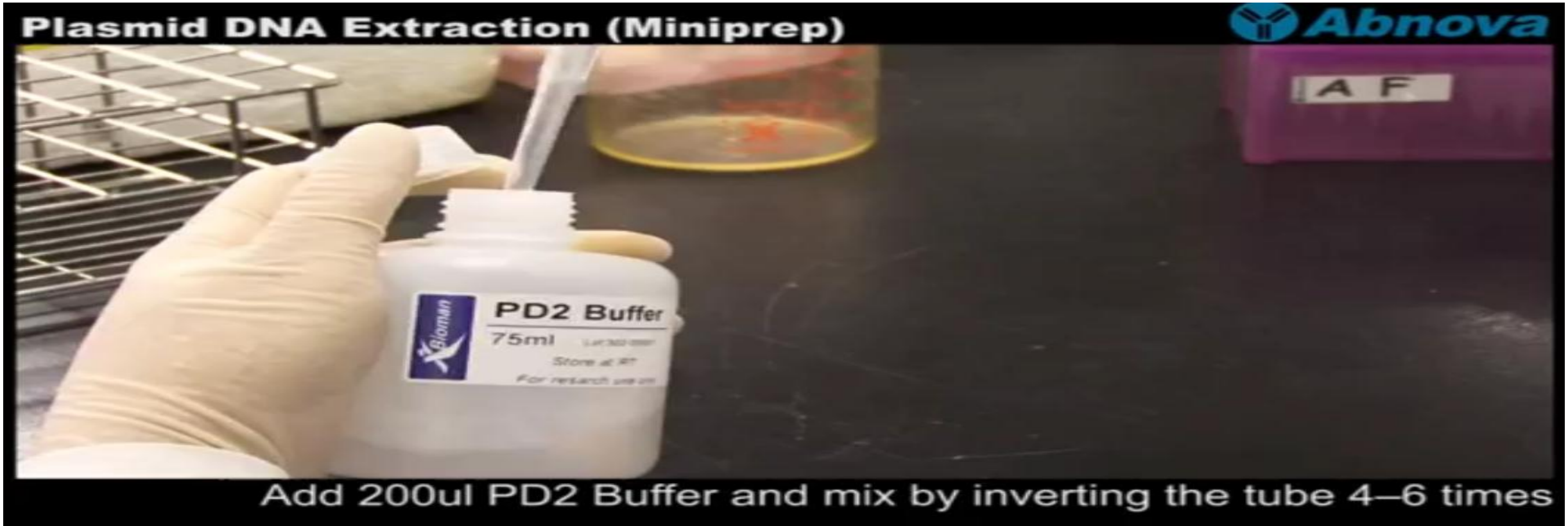


6. Add Solution 2

Solution 2

- 2N NaOH (2mls of 10N NaOH/ 100ml solution)
- 1% SDS (10mls from 10% stock SDS/100ml solution)

After incubation add 20mls of Solution 2. It is very important to mix the samples very well. *Don’t swirl. Use light flicking, like dropping solution of air.



7. Mix by inverting the tube 4-6 time

8. Add Solution 3

Add 300 µl of Solution 3, 3M KOAc

- Gently mix the solution. Don’t shake, but swirl this time.
- When mixed well the yellow should disappear and white stringy pieces should appear. Allow to sit on ice for 15 minutes.

9. Centerifuge at 4 C◦, 13200 rpm for 15 minutes

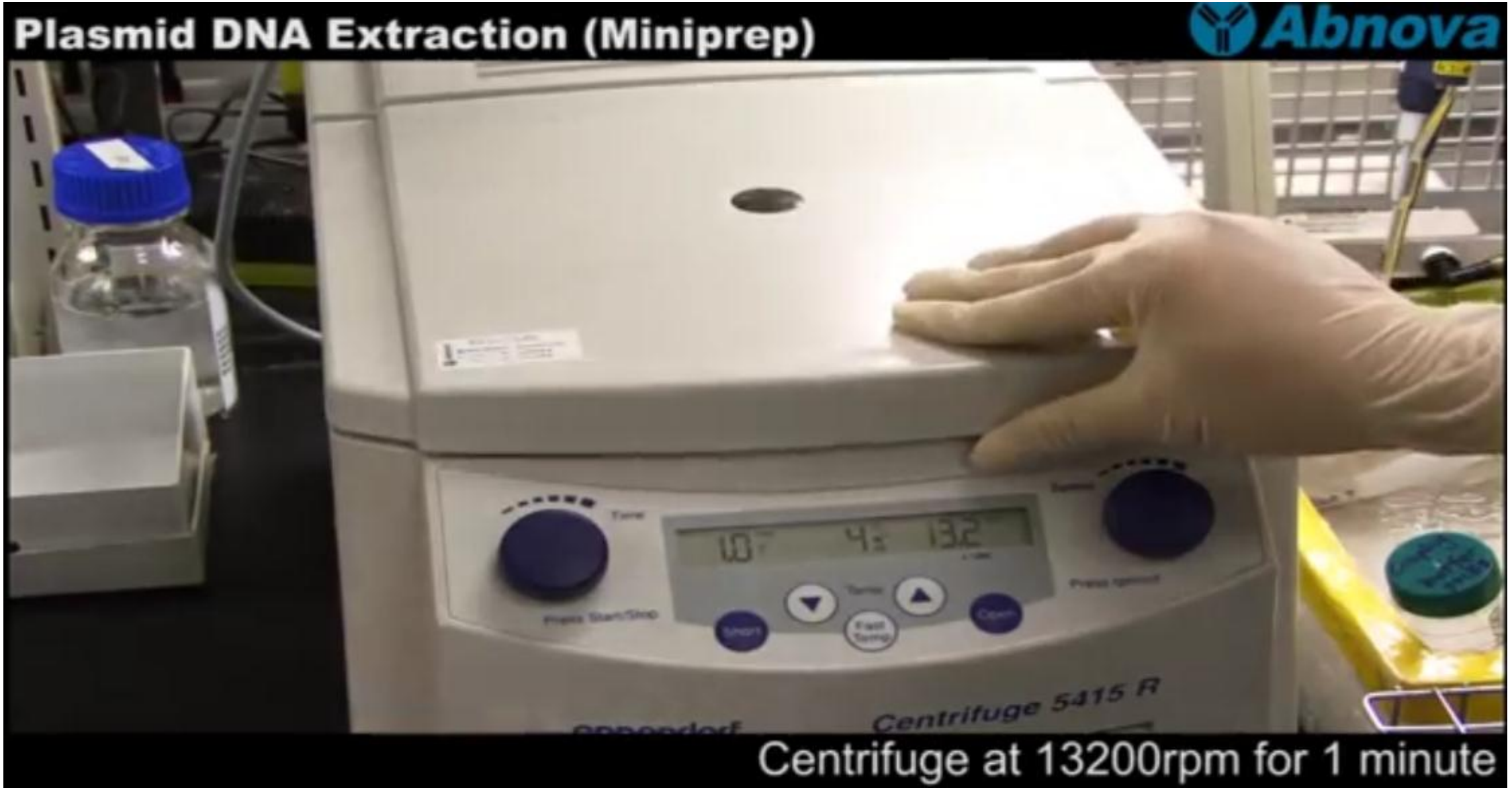
10. Spin the sample in the Sorvall Centrifuge for 1 minutes at 800 rpm. When spun down transfer the supernatant to a 50ml centrifuge tube by pouring the solution through a funnel covered with gauze. Do not allow the white pieces to enter the flask.

11. Centrifuge at 8000 rpm for 1 minute

12. Discard the flow-through

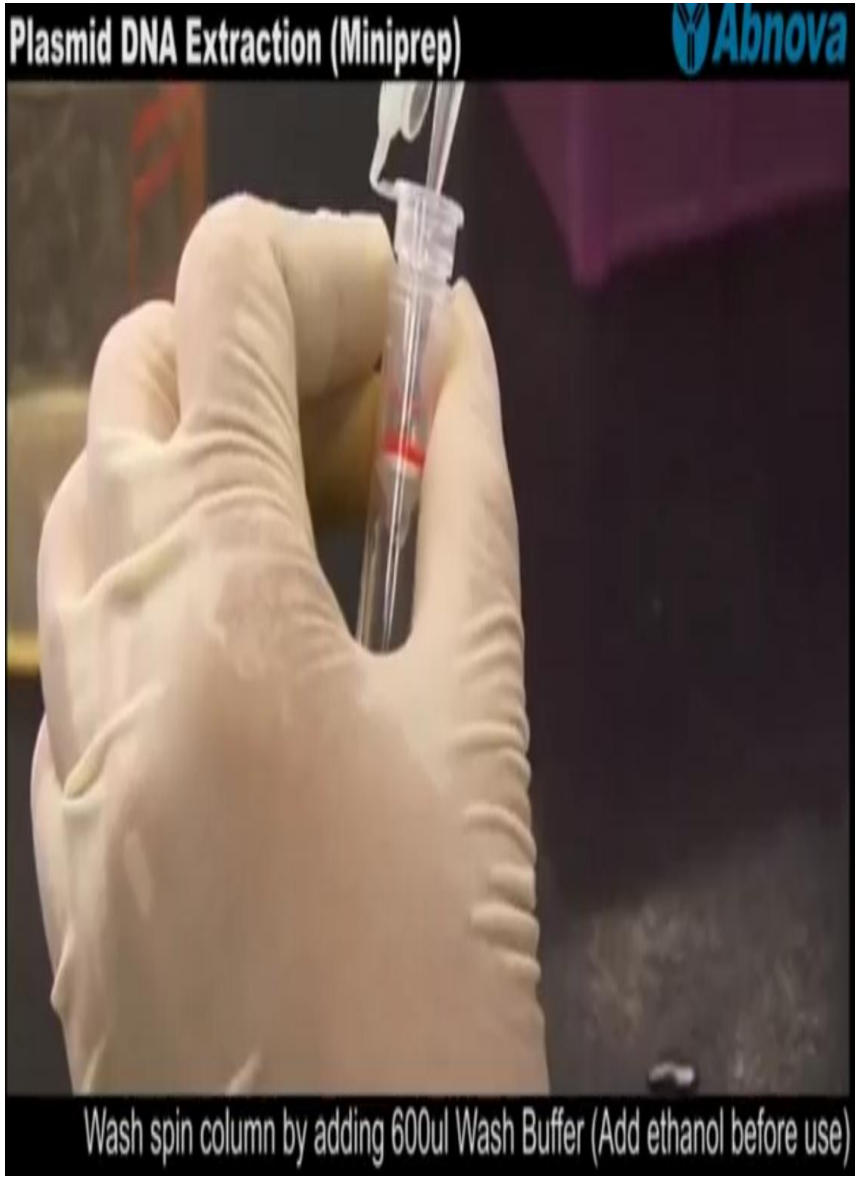
13. Wash spin column by adding 400 µl W1 buffer

14. Centrifuge at 13200 rpm for 1 minute

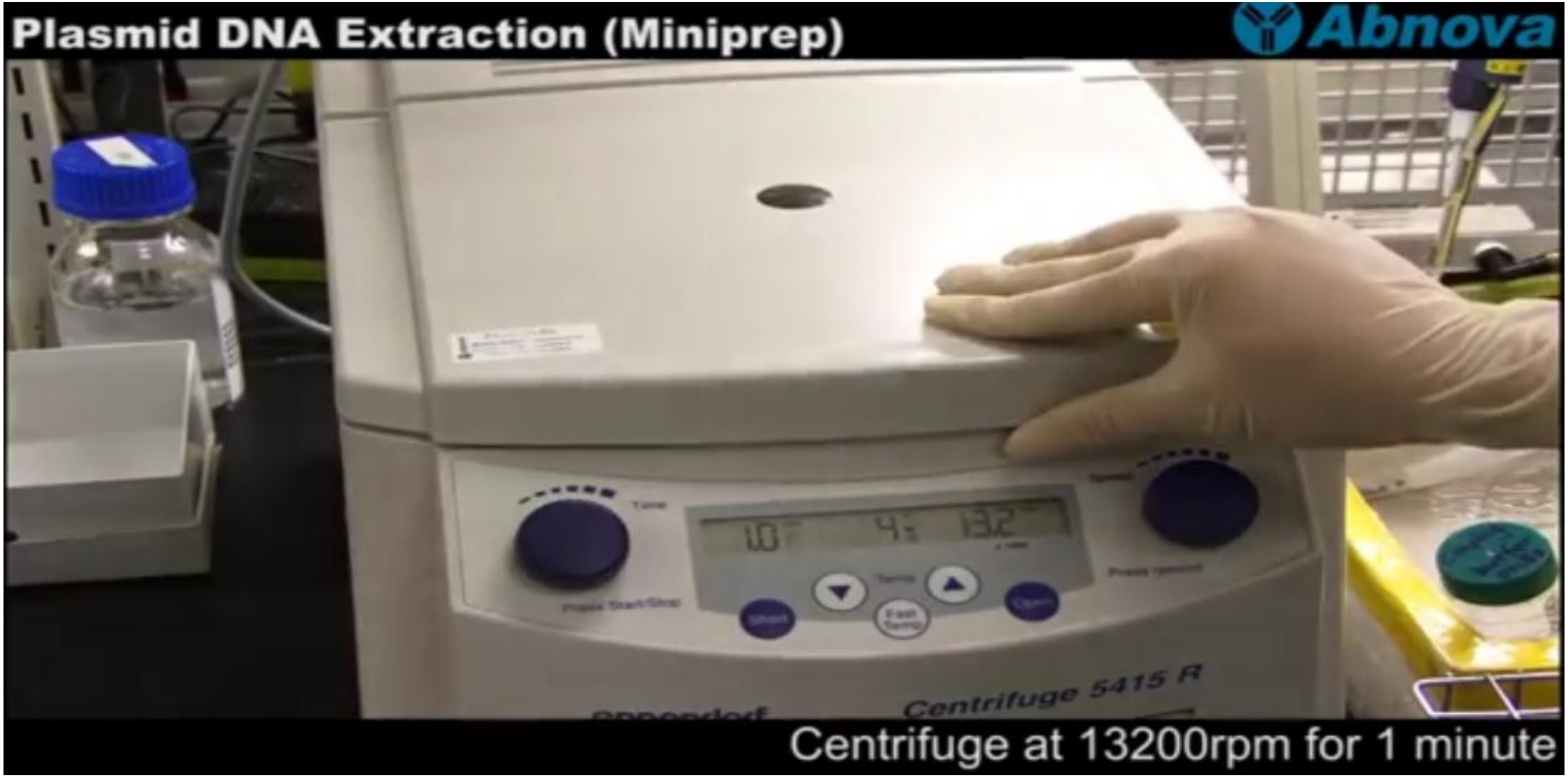


15. Discard the flow-theough

16. Wash spin column by 600 µl wash buffer (Add ethanol before use)



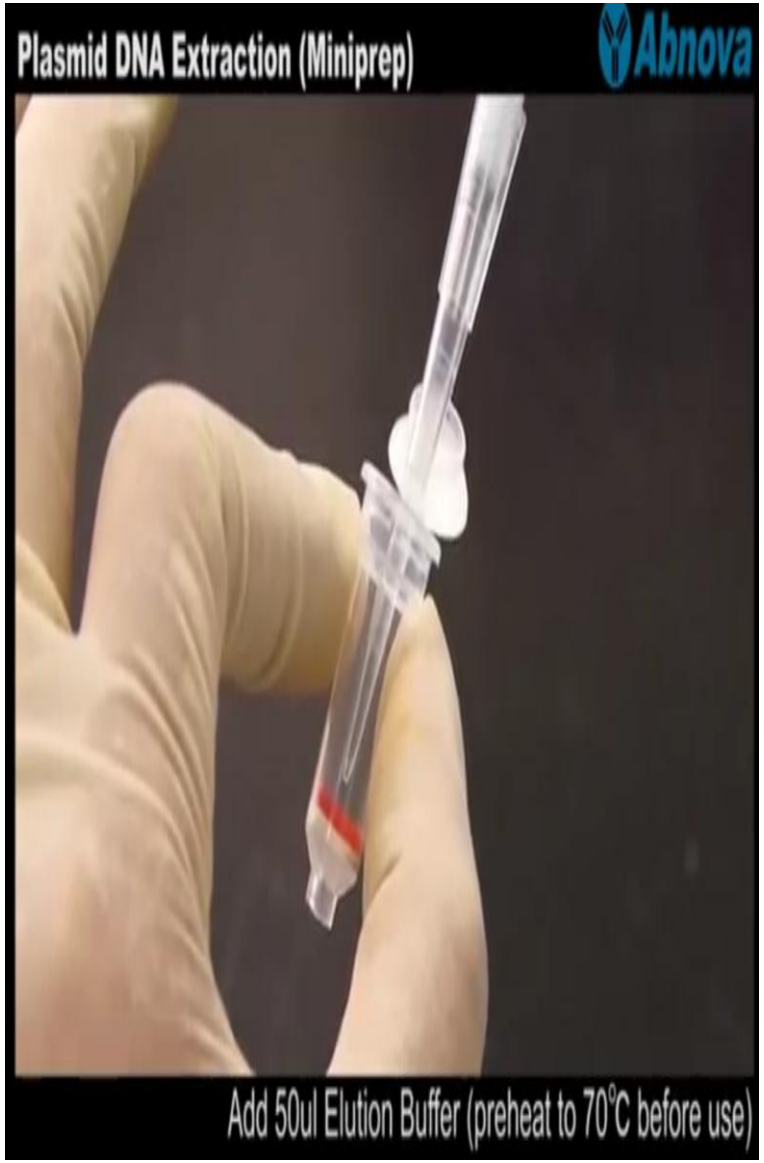
17. Centrifuge at 13200 rpm for 1 minute



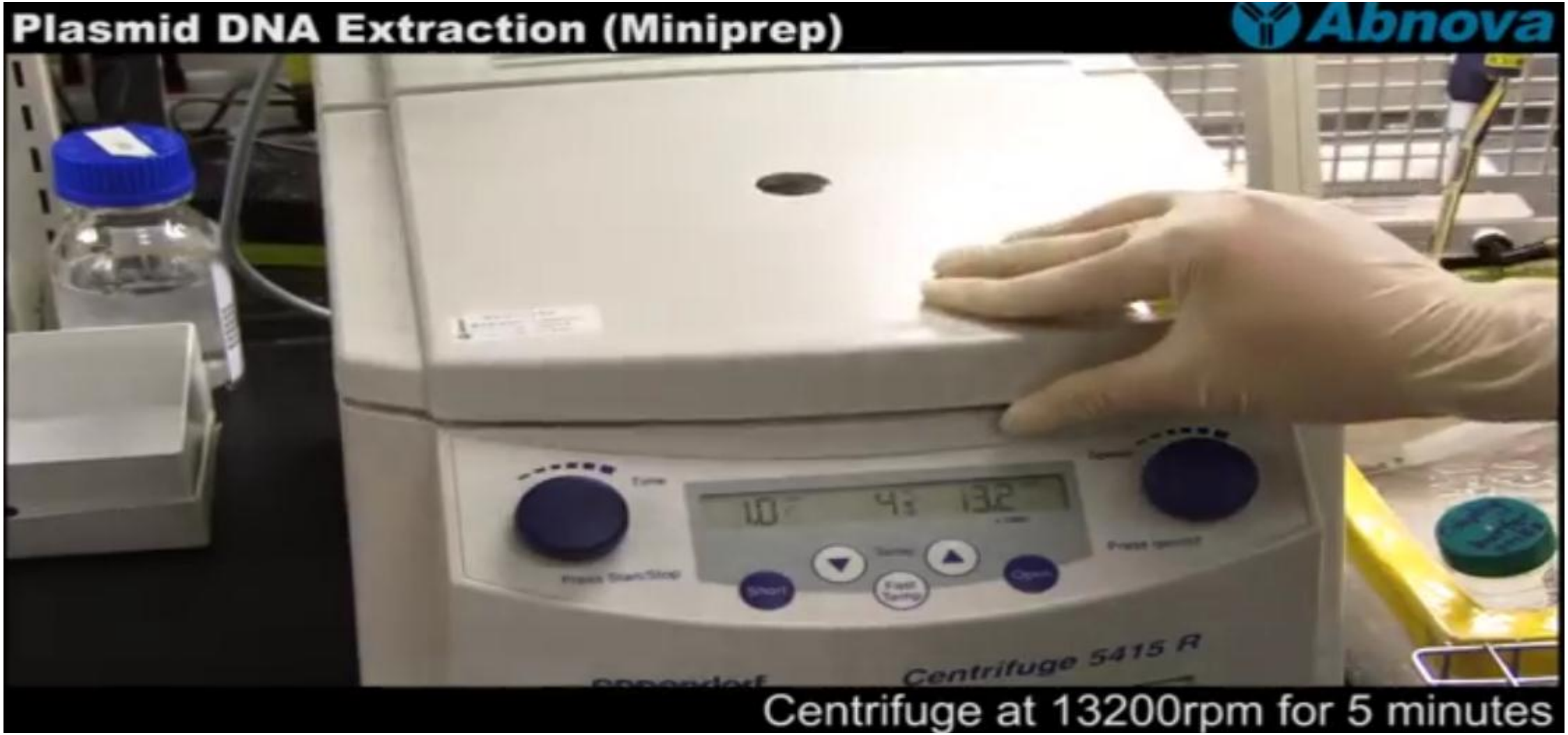
18. Discard the filow-through

19. Centrifuge for an additional 1 minute or place at room tem. For 10 minutes to remove residual ethanol.

20. Add 50µl Elution Buffer (preheat to 70 C◦ before use)



21. Centrifuge at 13200 rpm for 5 minutes



22. Store plasmid DNA at-20 C◦