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minipreps kit (BS413), 50 preps V.3

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Apr 04, 2018

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1 *Works for me*

dx.doi.org/10.17504/protocols.io.n8tdhwn

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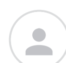 Joshua Lucate
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BEFORE STARTING

1) Add RNAse A solution to the bottle containing Solution 1 and mix well. Once RNAse A solution is added to solution 1, the resulting solution is stable for 6 months at 4°C. If being used infrequenly the solution can be stored at -20 ^{o}C for longer periods as long as freeze thaw cycles are minimized.

2) If solution 2 contains a precipitate, dissolve the precipitate before use by gently warming the solution at 37 ^{o}C.

3) Before using the wash solution, add 80 mL of 96-100% Ethanol to the 20 mL wash solution. Anhydrous Ethanol from Bio Basic Inc. (D0193) is suitable for use with this protocol.

1 Add 1.5 mL of overnight culture to a microcentrifuge tube and centrifuge at 12,000rpm for 2 minutes. Drain the clarified supernatant completely leaving only the cell pellet.

Optional: Depending on ease of plasmid replication add a further 1.5 mL of overnight culture to the microcentrifuge tube containing the previous pellet and repeat until a maximum of 5 mL of overnight culture has been added.

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Steps Abstract Guidelines Materials Forks Metadata Metrics

00:02:00

2 Add 100uL of **Solution 1** to the pellet, mix well, ensuring that no clumps are present and let sit for 1 min

00:01:00

3 Add 1µL of VisualLyse to the solution from step 2 (optional)

4 Add 200µl of **Solution II** to the mixture, and mix gently by inverting the tube 4-6 times and then keep at room temperature for 1 minute. To prevent contamination from genomic DNA, do not vortex.

00:01:00

If VisualLyse has been added, the solution will turn blue after addition of Solution II. A homogenously blue suspension should then be observed. If the suspension contains uneven blue color, or white/brownish cell clumps, continue mixing carefully.

5 Add 350µl of **Solution III**, and mix gently. Incubate at room temperature for 1 minute. A fluffy white material forms and lysate should become less viscous. If VisualLyse has been added in step 3, the suspension should be mixed until all traces of blue has gone and lysate becomes colorless.

00:01:00

6 Centrifuge at 12,000rpm for 5 minutes

00:05:00

7 Transfer the above supernatant (step 6) to the EZ-10 column. Centrifuge at 10,000rpm for 2 minutes

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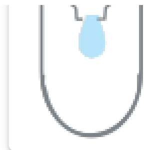

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8 Discard the flow-through in the tube. Add 750µl of Wash Solution to the column, and centrifuge at 10,000rpm for 2 minutes

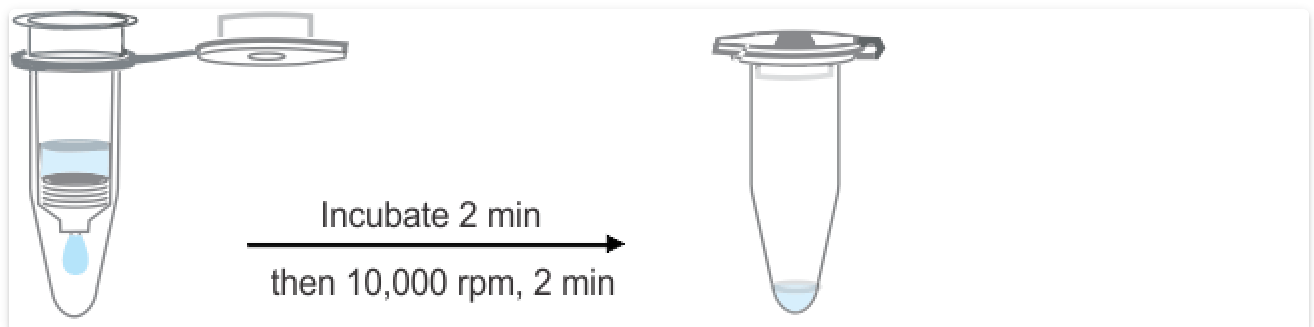
00:02:00

9 Repeat wash procedure in step 8

10 Discard the flow-through in the collection tube. Centrifuge at 10,000rpm for an additional minute to remove any residual Wash Solution

00:01:00

11 Transfer the column to a clean 1.5ml microfuge tube (BT620-NS). Add 50µl of Elution Buffer into the center part of the column and incubate at room temperature for 2 minutes.



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1.5ml Tube, Natural, Sterile, 1000/Bag
by Bio Basic Inc.
Catalog #: BT620-NS.SIZE.1PK

12 Centrifuge at 10,000 rpm for 2 minutes

00:02:00

13 Store purified DNA at -20°C

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