Protocol:

1) Count the number of mini-preps that you intend to do. Read these instructions and label all the tubes/columns/collection tubes that you will need. If you have a guide written down (A=Day1, Timepoint 1, B = Day 1, Timepoint 2, etc…), then you can have the labels be very simple (A, B, C, D...etc).
2) For each mini-prep **(Plus one extra)** make a master mix of *Buffer1-Z*, each miniprep requires:
    a) 400 uL of Buffer1 w/RNAse A
    b) 4 uL of Zymolyase (5U/uL)

    E.g. Buffer 1-Z mastermix for 2 minipreps contains 1200uL of Buffer 1 w/RNAase A+12ul of Zymolyase
3) Add 400 uL of *Buffer1-Z* each pellet and resuspend by pipetting.
4) Incubate this sample at 37ºC for **90 minutes**
5) After the incubation take 225 uL of the resuspended pellet and pipette it into a new labeled tube. Save the original tube/sample at -20ºC.
6) For the new labeled tubes, add 300 uL of Buffer 2 to each sample and mix by inversion 6-8 times. Incubate this mixture at room temperature for **5 minutes**.
7) Add 350 uL of Buffer 3 to each sample. Immediately after the addition of buffer 3, mix the sample by inversion 6-8 times. It is important to **mix the sample as soon as possible**.
8) Pellet the lysate by spinning them in a benchtop centrifuge for 10 minutes at MAX RPM (don't forget the lid or it will be loud!).
9) As soon as possible after the spin has finished, decant the supernatant liquid onto a **pre-labeled** silica column sitting in a waste collection tube. Remember to label both the silica column and the waste tube that it sits in.
10) Spin the column at MAX RPM for 1 minute and discard the flow through (the liquid in the collection tube).
11) Add 500 uL of HBC Buffer to each column
12) Spin the column at MAX RPM for 1 minute and discard the flow through
13) Add 700 uL of DNA Wash Buffer
14) Spin the column at MAX RPM for 1 minute and discard the flow through
15) Discard the waste collection tube and use a 1.7 mL eppendorf tube as a new waste collection tube. Without adding any more solutions, spin the column again at MAX RPM for **2 minutes** and discard the flow through. **This step removes any remaining ethanol from the column and is very important.**
16) Discard the waste collection microfuge tube and transfer the silica column to a brandspanking **new,** 1.7 mL eppendorf (final collection) tube. Do not reuse the waste collection tube!
17) Add 30 uL of elution buffer to the middle of the column membrane and let the column sit at room temperature for 1 minute.
18) Spin the column at MAX RPM for 1 minute. Your plasmid DNA is now in the flow through.
19) Quantify your [DNA] with the nano-drop dsDNA tool and record your yields for your presentations and quality control steps.