ZR Plasmid Miniprep 5ml Modifications

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Here is a modification of the ZR plasmid miniprep kit that I use when doing **5ml preps**. Please note that these are modification notes to supplement the original protocol (not a stand alone protocol).

I increase all of the buffer amounts. I add RNaseA because I had some RNA coming through the prep when using 5ml of culture (RNAse A is kept in the -20 enzyme freezer door). I do a P3 incubation step on ice. I add a second spin after the P3 incubation because some of the precipitate always dislodges and gets transferred with the supernatant. If your plasmid is large (over 6 kb) warming the elution buffer increases recovery.

Here are the modification steps:

- Use 5ml culture for each prep
- Increase buffer amounts: P1 = 250ul, P2 = 250ul, P3 = 500ul
- After resuspending pellet in P1, add 5 ul of RNase A (10mg/ml) and mix
- After addition of P3, incubate on ice for 5-10 min, mixing several times, until no more red precipitate is visible
- Spin at 12,000 rpm, 4°C for 5 min, then transfer supernatant to a clean 1.5ml tube
- Spin at 12,000 rpm, 4°C for 2 min to remove any remaining precipitate, and then load supernatant onto column
- Use recommended buffer volumes for washes
- Perform wash spins in a room temperature centrifuge (4C can cause precipitation)
- Warm elution buffer EB to 65-70° C to maximize elution efficiency for large plasmids