The TIPS for optimal yields using ZymoPURE™ II Plasmid Prep Kits

- **Lysis step**:
  - Mix the lysis reaction by inversion immediately after adding the ZymoPURE P2 buffer
  - Increase the number of inversions to 10 – 12 times and increase the incubation time up to 5 minutes.
  - After addition of ZymoPURE™ P2, the solution should change from opaque pink to clear viscous purple, indicating complete lysis.

- **Neutralization step**:
  - Invert the tube additionally 5-8 times after the sample turns yellow (following the addition of ZymoPURE™ P3). Neutralization is influenced by the cell density and/or the volume of culture being processed.
  - The solution should not be viscous following neutralization and the yellowish precipitate should appear fluffy and readily float to the surface.

- **Lysate filtration**:
  - At least 33 – 35 ml of cleared lysate needs to be recovered from the ZymoPURE Syringe Filter and used for the binding step.
  - You can perform an initial clarification on the neutralized lysate by centrifuging 10 minutes prior to using the syringe filter.

- **Binding Step**:
  - Incubate the bottle of ZymoPURE Binding Buffer at 30-37 ºC for 10 minutes and mix by inversion prior to use to ensure the contents are completely resuspended
  - After the addition of the ZymoPURE Binding Buffer invert at least 10 times prior to loading it onto the column
  - Allow the mixture of lysate and Binding Buffer to incubate on the column for 2 minutes before applying the vacuum.
  - Do not centrifuge the lysate and binding buffer mixture before loading it onto the column.

- **Vacuum manifold**:
  - When using the vacuum manifold procedure, it is important to turn the vacuum source off when stated to in the protocol using the individual stop cock valve.

- **Wash Steps**:
  - Make sure no wash steps have been omitted and ensure that Wash 1 was used for the first wash step and Wash 2 was used for the next two wash steps.
  - Ensure the correct volume of 95% ethanol has been added to the ZymoPURE™ Wash 2 and ensure that the bottle cap is screwed on tightly after each use to prevent evaporation of the ethanol.

- **DNA Elution**:
  - Make sure to centrifuge the Zymo-Spin™ V-P Column at ≥ 10,000 x g for 1 minute in order to remove any residual wash buffer prior to the elution steps.
  - Pre-warm the ZymoPURE™ Elution Buffer to 50 ºC prior to elution.
  - For large plasmids (> 10 kb), incubate the ZymoPURE™ Elution Buffer for 5-10 minutes on the column before centrifugation.