Presto Mini Plasmid Kit Protocol Procedure (for iGEM use)

prepration	• Warm **MBW** to 60 ^{0}C in dry Bath (*for sequencing use **UPW**) • Bring PD1 buffer from 4 ^{0}C on ice • Make Sure nanodrop is available • Bring 16 hr incubated 50 ml tube (starter) from shaker
Step 1: Harvesting	• Centrifuge The starter for 10 min at 4000 rpm • Discharge the **supernatant** carefully, do not lose the pellet
Step 2: Resuspension	• Brake the pellet by vortex • Add **200 μl of PD1 Buffer (make sure RNase A was added)** and resuspend the pellet by vortex or pipette • Transfer the supernatant to 1.5 ml microcentrifuge tube
Step 3: Lysis	• Add **200 μl of PD2 Buffer** then mix gently by inverting the tube 10 times. **Do not vortex!** to avoid shearing the gDNA. • Let stand at room temperature for 2 min to ensure the lysate is homologous
Step 4: Neutralization	• Add **300 μl of PD3 Buffer** then immediately invert the tube 10 times. **Do not vortex!** to avoid shearing the gDNA. • Centrifuge for 5 min at 16000g
Step 5: DNA binding	• Place a **PD column** in a **2 ml collection tube** then transfer the supernatant to the **PD column** • Centrifuge for 1 min at 16000g • Discard the flow-through and dry on wetman • Place the **PD column** back in a **2 ml collection tube**
Step 6: Wash	• Add **400 μl of W1 Buffer** in to the **PD column.** Centrifuge for 1 min at 16000g. Discard the flow-through and dry on wetman. Place the **PD column** back in a **2 ml collection tube** • Add **600 μl of Wash Buffer (make sure absolute ethanol was added)** in to the **PD column** • Centrifuge for 1 min at 16000g • Discard the flow-through and dry on wetman. Place the **PD column** back in a **2 ml collection tube** • Centrifuge for 3 min at 16000g to dry the column matrix • Transfer the dried **PD column** to **new 1.5 ml microcentrifuge tube**
Step 7: DNA Elution	• Add **32 μl of MBW (*for sequencing UPW)** into the center of **PD column** matrix • Let stand at room temperature for 2 min to allow the MBW to be completely absorbed • Centrifuge for 2 min at 16000g to elute the purified DNA
Cleanup and storage	• Measure the concentration by nanodrop. Write the concentration on the microcentrifuge tube. **Use the same MBW as blank!** • Store the purified DNA in -20 ^{0}C freezer • Store PD1 in 4 ^{0}C • Store all the rest buffer in room temperature