# Gel extraction protocol

## Authorship

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## Notes

* The protocol uses the Wizard SV Gel and PCR Clean-Up System (Promega) with some modifications

## Gel

* 1.5% low melt agarose gel with SybrSafe
* ~35V for 1-1.5 hrs

## Gel clean-up kit modifications

* **5.A., Step 3:** flow melted gel through the column twice
* **5.A., Step 6:** flow water through twice

## Method in detail

1. Use the speed-vac to evaporate the compiled pool of samples from each Sequal plate purification to 100 uL (96 compiled samples per tube).
   * The volumes can be divided among more tubed to decrease speed-vac time.
2. While speed-vac is running, prepare a 1.5% low melt (or normal) agarose gel, with SybrSafe.
   * Make 75 mL of agarose solution
     + Large volume needed to cast a gel with deep wells.
   * 1.12 g of low melt agarose
   * 1.5 ul of SybrSafe
3. Add 10 uL of loading dye to each concentrated 100 uL sample and mix well.
   * Mix thoroughly
4. Load 55 uL of the sample into an individual well until all the sample is loaded.
   * Use 7.5 ul of 100 bp ladder in the 1st lane (+1.5 ul of 6X loading dye).
5. Run the gel at ~35 volts for ~1.5 hours.
6. Pre-weight 2 mL microcentrifuge tubes (1 per sample).
7. Visualize the gel using the UV transilluminator in Room 712
   * (make sure to wear face shield!).
   * Cut out 400-600 bp bands from each lane.
8. Put the cut bands into pre-weighted 2 mL microcentrifuge tubes and weight to find final weight of gel.
9. Use the Wizard SV Gel and PCR Clean-Up System kit protocol to elute DNA from gel slices.
   * Protocol modifications include:
     + running binding solution (w. molten gel) through the column twice
       - increases binding of DNA to column
     + running eluted DNA through column twice in order
       - increases DNA eluted off the column.