EZNA Gel Extraction

Rationale:	
Special Observations:	
Results:	
Interpretation:	

Experiment Date:
Experiment Time

Primary Experimenter (contact):
Other Experimenters:

Source: Caleb Radens, Ali Awan

Assembled: 7/19/2012

DNA details	~ug DNA in gel	Yield (ng/uL)

Procedure:

Critical Steps:

- Minimize UV exposure to DNA and yourself
- Protect your eyes from the UV light
- Use a timer for the waiting steps

☐ **Turn on 60 □ water bath**

☐ **Stain gel with ethidium bromide**

- Approximately 1 hour, or just enough to visualize ladder and DNA of interest

- [ ] **Visualize gel to check DNA band of interest is present and take a picture (See: Weill Gel Pictures)**
    - Be sure to bring or memorize the ladder bands and bring documentation of what DNA is in which lane
    - Minimize UV exposure time
- [ ] **After taking picture, use a razor blade to cut the DNA fragment of interest out of the gel**
    - Minimize UV exposure time
    - Cut as close to the DNA as possible
    - After making four incisions around band(s) turn off the UV then excise your fragment. Flip the fragment on its side, turn on UV, then cut off any extra non-glowing gel
    - You want to have a slice weighing near of less than 200 mg
- [ ] **Put the fragment into a pre-weighed and labeled 2 mL centrifuge tube**
- [ ] **Determine the weight of the fragment**
- [ ] **Add (1.25)x(Gel fragment weight in mg) uL of buffer XP2 to tube**
    - Ex) 0.202 g gel slice → add 252.5 µL buffer XP2
- [ ] **Vortex tube, then incubate in 60 □ water bath for 10 - 20 minutes, vortexing every couple of minutes**
    - At this time, label spin column(s)
- [ ] **Transfer dissolved gel sample to the top of a labeled spin column**
- [ ] **Turn incubator up to 70 □**
- [ ] **Centrifuge @ 13,000 rpm 1 minute, re-load flow through to top of spin column**
- [ ] **Add 300 uL of buffer XP2 to column**
- [ ] **Centrifuge column @ 13,000 rpm 1 minute, discard flow-through**
- [ ] **Add 700 uL buffer SPW to column, wait 5 minutes**
- [ ] **Start pre-warming Elution Buffer to 70 □**
- [ ] **Centrifuge column @ 13,000 rpm 1 minute, discard flow-through**
- [ ] **Add 700 uL buffer SPW to column (Yes, again) wait 5 minutes**
- [ ] **Centrifuge column @ 13,000 rpm 1 minute, discard flow-through**
- [ ] **Add 700 uL buffer SPW to column (Yes seriously, again) wait 5 minutes**
- [ ] **Centrifuge column @ 13,000 rpm 1 minute, discard flow-through**

- Be sure to vigorously shake out the residual liquid in the flow through tube

☐ **Centrifuge column with the lid open for 2 minutes @ 13,000 rpm**

☐ **Transfer spin column to labeled tube**

☐ **Add 30 uL of <u>Elution Buffer</u> directly to center of membrane**

- It's ok to touch the membrane to dispel a residual droplet is on pipette tip

☐ **Wait AT LEAST 5 minutes (with lid closed)**

- May wait longer if you're patient, but don't wait more than 30 or so minutes

☐ **Centrifuge column @ 13,000 rpm 1 minute, if there is any leftover EB visibly on the membrane, rotate the column 180 degrees and centrifuge again**

☐ **Expect very low concentration of DNA (~10 – 30 ng/uL from 1 ug DNA)**