Worksheet for tissue processing (13 to 24 pieces of tissue) NAME:_____

1. Tissue processing basket (This should be done the day before processing.)
    1. Take Tissue processing basket(s) out of the acetone jar and air dry.
    2. Rinse the basket(s) a few times with Na cacodylate buffer (0.1M).

2. Epoxy resin (This can be done the day before processing.)
    - Beakers A and B: Mix for ≥15 min with the beakers covered with aluminum foil.
    - Beaker A+B: Mix A and B for 15 min, then add DMP-30, followed by mixing for another 15 min. If preparing the day before, wait until the next day to mix DMP-30.

	For Day 1			For Day 2		
	A	B	A+B	A	B	A+B
LX112	7.00 g	18.00 g	-	14.00 g	36.00 g	-
DDSA		-	-		-	-
NMA	-		-	-		-
A	-	-	13.5 g	-	-	27 g
B	-	-	31.5 g	-	-	63 g
DMP-30	-	-	0.63 g	-	-	1.26 g

NOTE: The amounts of DDSA and NMA must be adjusted according to the WPE value indicated on the LX-112 bottle. Refer to the table in the full protocol and record their amounts in this table. Also record WPE of LX-112 here: _____

3. Reagents for osmium fixation, dehydration, and *en bloc* UA staining
    - In 50-ml conical tubes, add:
        - ddH_2O: 40 ml for setup + 40 ml for processing
        - Na cacodylate buffer (SCB; 0.2M): 20 ml for setup
        - Na cacodylate buffer (0.1M): ~300 ml total for processing
    - In the labeled vials, prepare the following (Mix well by shaking after adding each reagent):

Reagents (add in this order ↓)	Reduced Osmium*			1% OsO_4 **	EtOH 50%	EtOH + UA				EtOH 100%
	1	2	KFeCN			50%	70%	90%	100%	
ddH_2O	-	-	5 ml	5 ml	10 ml	10 ml	6 ml	2 ml	-	-
KFeCN	-	-	0.3 g	-	-	-	-	-	-	-
0.2M SCB	5 ml	5 ml	-	10 ml	-	-	-	-	-	-
4% OsO_4	5 ml	-	-	5 ml	-	-	-	-	-	-
EtOH	-	-	-	-	10 ml	-	4 ml	8 ml	10 ml	20 ml
2% UA	-	-	-	-	-	10 ml	10 ml	10 ml	10 ml	-

*Reduced osmium:
    1. Add 5 ml of KFeCN solution to vial 2.
    2. Place vials 1 and 2 on ice.
    3. When ready, pour vials 1 and 2 back and forth to mix well before adding to tissue.

**1% OsO_4: Keep vial on ice until use.

4. Reagents for infiltration
    - Prepare ~2 ml per tissue of the following:
        - 1:1 = EtOH : propylene oxide (PO)
        - 1:2 = EtOH : PO
        - 100% PO
        - 1:1 = PO : Resin
        - 1:2 = PO : Resin

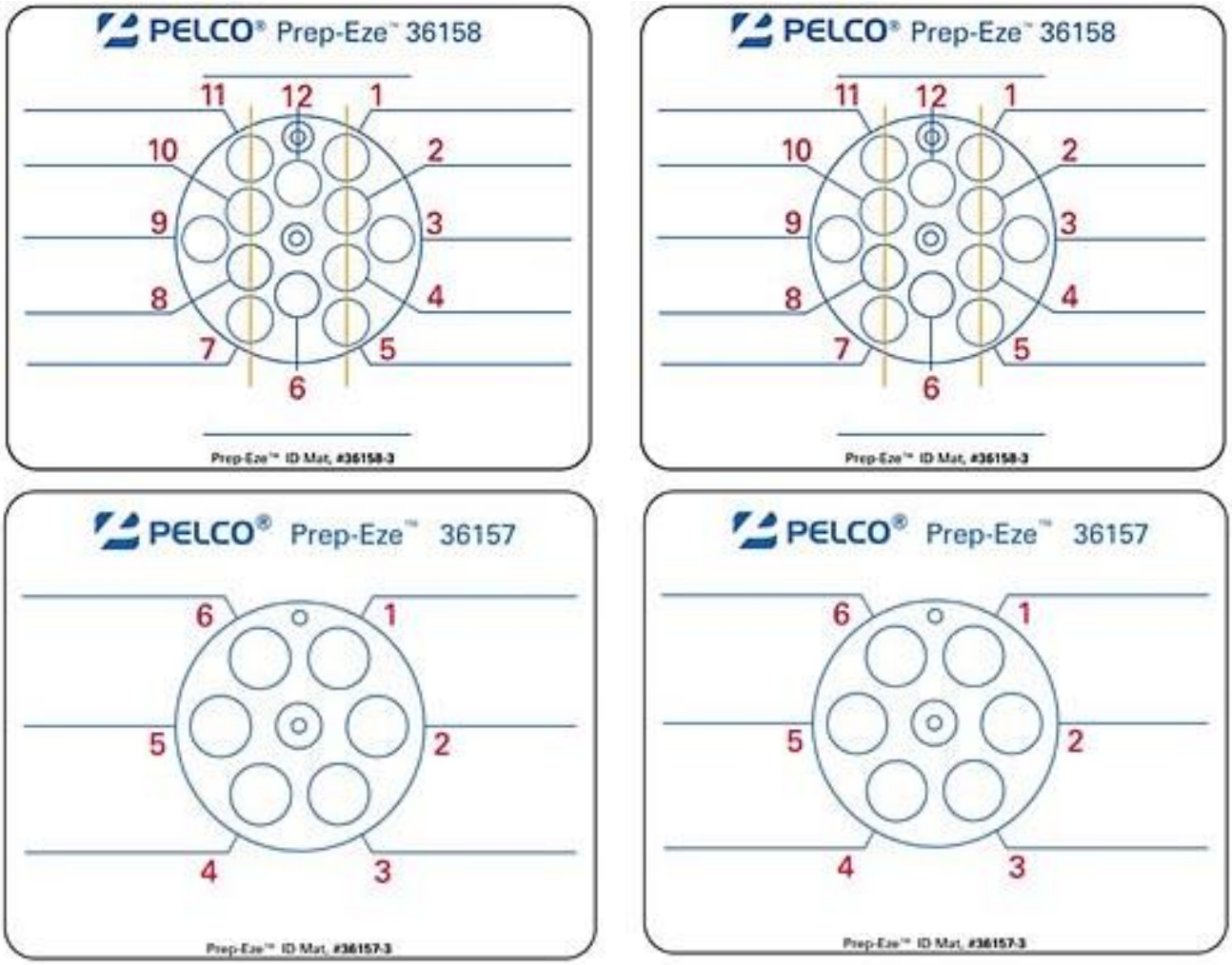
5. Microwave
    1. Fill load cooler with fresh RO water and eliminate air bubbles. Make sure pump is on.
    2. Set power level to 1 (175W).
    3. Run microwave for 2 min to warm up magnetron.

Worksheet for tissue processing (13 to 24 pieces of tissue) NAME:_____

6. Processing (4 days)

DAY 1 (Date:)

- Place a piece of absorbent paper on work surface in the fume hood. Prepare new solid waste bags.
- Embed vibrasliced tissue in 7-9% agarose, and place them into processing basket filled with Na cacodylate buffer (0.1M).



Description of tissue (e.g., animal ID, vibraslice ID, experiment description, genotype, etc.):

Worksheet for tissue processing (13 to 24 pieces of tissue) NAME:_____

Waste: Aldehydes-Caco Osmium-KFeCN

DAY 1 continued (Date:)

- Na cacodylate buffer (0.1M) rinses, 5 min × 5 __ __ __ __ __
- Reduced osmium, 5 min __
- Na cacodylate buffer (0.1M), 5 min × 5 __ __ __ __ __
- Microwave power setting 1 (175W), under vacuum
  - 1% OsO_4, 1 min ON → 1min OFF → 1 min ON __
  - Cool the tissue to about 15°C.
  - 1% OsO_4, 1 min ON → 1min OFF → 1 min ON __
- Na cacodylate buffer (0.1M) rinses, 2 min × 5 __ __ __ __ __
- ddH_2O rinses, 2 min × 2 __ __

UA-EtOH

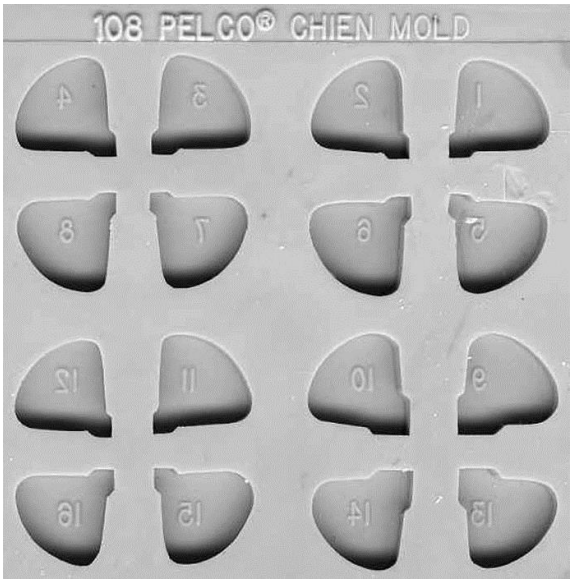
- 50% EtOH, 5 min __
- Microwave power setting 2 (250W), no vacuum
  - 50% EtOH + UA, 40 s __
  - 70% EtOH + UA, 40 s __
  - 90% EtOH + UA, 40 s __
  - 100% EtOH + UA, 40 s __
  - 100% EtOH, 40 s __ [Turn off the pump on microwave.]

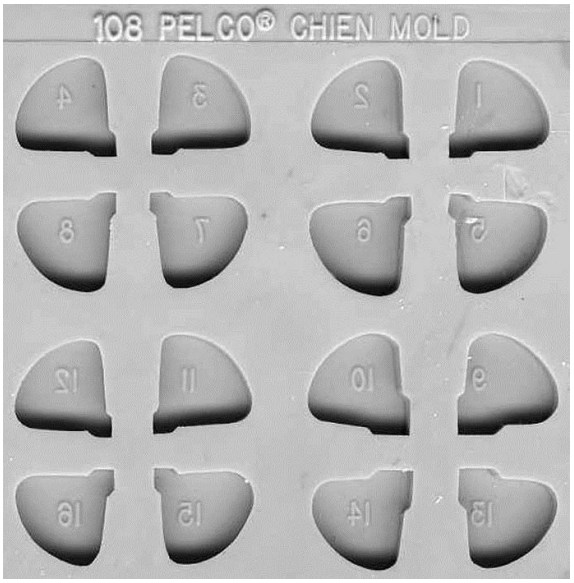
Flammable PO-Resin

- Transfer tissue into shell vials filled with 1:1 = EtOH : PO.
- Infiltration (on rotator)
  - 1:1 = EtOH : PO, 10 min __
  - 1:2 = EtOH : PO, 10 min __
  - 100% PO, 15 min × 2 __ __
  - 1:1 = PO : Resin, 1 hr __
  - 1:2 = PO : Resin, overnight __

DAY 2 (Date:)

- 100% Resin, 1 hr × 3 __ __ __
- Transfer tissue into mold, fill with resin, and place in 60°C oven for 48 hrs. Current time is: ____:_____.





Worksheet for tissue processing (13 to 24 pieces of tissue) NAME:_____

DAY 2 continued (Date:)

- Clean up after yourself.
  - Excess liquid resin: Collect into a disposable beaker along with other resin-contaminated supplies (all vials must be uncapped), then place in 60°C oven for 48 hrs.
  - Solid waste: All vials must be uncapped. Place all UA-contaminated waste (incl. absorbent paper) in a dedicated bag. Other solid waste should be placed in a "No UA" waste bag.
  - 12- or 24-well plates with vibraslices: Discard all liquid into "Aldehydes-Caco" waste bottle. Then place the plates into "No UA" solid waste bag.

DAY 3 (Date:)

- Continue resin polymerization. You may check and adjust the positioning of tissue at this point.

DAY 4 (Date: , Time:)

- Take the resin-embedded tissue blocks out of oven. Remove the blocks out of the mold immediately and store in the cardboard pill boxes labeled with block identification numbers.
- Take solidified resin waste into "Solid Resin" waste drum.
- Examine the blocks under the stereomicroscope and record their images before trimming and cutting.
- Scan this note into a pdf file.