N.B.

It is very important to avoid potential cross-contamination. The following tips should be followed:

- *Clean your bench with bleach (84) every time after you did DNA extraction or fish dissection. Clean your pipettors, rack and bench space with bleach once a while.*
- *Aliquot all reagents into appropriate amount, so not to contaminate the whole tube of reagents.*
- *Make sure you have enough filtered tips, and never use tips without filter.*
- ***Centrifuge the tube every time before you open a lid.*** *Keep you finger off the inside of the lid. If you suspect that your gloves are contaminated, change it.*
- *Leave a space between tubes on rack, if you are not using multichannel pipettors.*
- *When pipetting, do it smoothly, don't pump it too fast. Keep the tip submerged just under the solution, so not push the liquid to overflow.*
- *When adding samples, set the tip against the wall of the tube.*
- *Move your tubes to an up row or switch the orientation of your tubes after adding samples to keep track which one has been added to.*
- *Keep focused; please don't talk to others when they are working on sample prep.*

DNA Clean-Up Using MagNA Beads

(modified from Rohland and Reich, 2012)

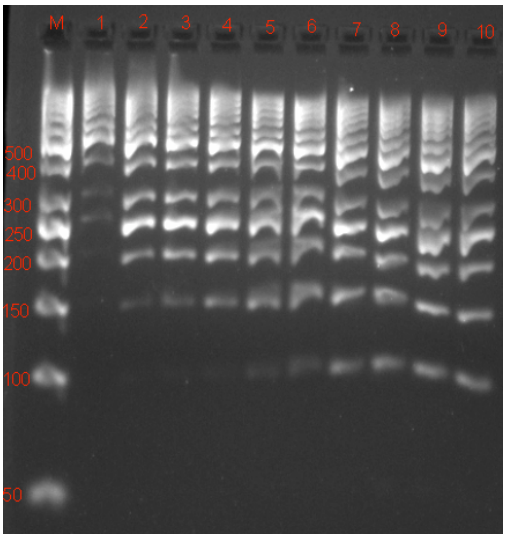

Fig.1. 4% agarose gels showing DNA ladder (GeneRuler 50 bp #SM0373) cleaned up with different amount of MagNA Buffer added (lane descriptions are listed in table 1).

Table 1

Lane	1	2	3	4	5	6	7	8	9	10
Sample(μL)	20	20	20	20	20	20	20	20	20	20
MagNA buffer (μL)	10	15	18	24	30	36	42	48	54	60
MagNA buffer/Sample	0.5x	0.75x	0.9x	1.2x	1.5x	1.8x	2.1x	2.4x	2.7x	3.0x

DNA clean-up procedure

1. First add 30 μL MagNA beads (see next page for preparing MagNA beads mixture) in an empty tube, dry the beads using magnetic plate.

2. Add MagNA buffer and sheared samples to the dried beads (MagNA buffer : samples = 0.9 : 1, e.g., 20uL sample need add 18uL MagNA buffer for cleaning off fragment <100bp; the total volume of the mixture should be less than **200 μL**, so the magnetic plate can be effective). Mix the sample well by pipetting 5-10 times smoothly.

3. Let the tube stand for 5 min at room temperature (**5 min standing is important**). Collect the liquid at the bottom of the tube by briefly centrifuging.

4. Place the tube on a 96-well ring magnetic plate, and let it stand for 5 min or more until the beads separated from the solution. Pipette off and discard the supernatant without touching the beads.

5. Leave the tube on the magnetic rack, and wash the beads by adding 186 μL of freshly prepared 70% ethanol. Let stand for 1 min and remove the supernatant. *Keep the tube on the magnetic rack, do not disturb the beads!!!

6. Repeat Step 5 one more time.

7. Remove residual traces of ethanol. Let the beads air-dry for 5 min at room temperature without caps. **Stop here if "drying beads" with samples is need.**

8. If elution is needed, add 25 μL of TE or elution buffer to the tube. Remove the tube from the magnetic rack, and resuspend the beads by pipetting. Let it stand for 1 min, and then collect the liquid in the bottom of the wells by briefly centrifuging. Occasionally the beads may appear clumpy after vortexing; this does not have a negative effect on DNA recovery.

9. Place the tube back on the magnetic rack, let stand for 1 min, and transfer the supernatant to a new tube. Carryover of small amounts of beads will not inhibit subsequent reactions.

10. If high recovery of DNA is needed, a few more round of elution off the beads using TE buffer may be carried.

1. Recipe for 100 ml MagNA beads (18% PEG8000, 2.5 M NaCl)

Reagent	Amount	Final concentration
5% Sera-Mag Magnetic Speed-beads (FisherSci, cat.#: 09-981-123)	2 mL	0.1%
PEG8000 (cat #: Fisher BP233-1)	18 g	18%
5M NaCl	50 mL	2.5 M
1M Tris-Cl	1 mL	10 nM
0.5M EDTA	200 uL	1 mM
add H_2O to 100 mL		

Note: beads contain sodium azide, wash beads 2x with water or TE before addition.

2. Recipe for 100 ml MagNA Buffer (18% PEG8000, 2.5 M NaCl)

Reagent	Amount	Final concentration
PEG8000 (cat #: Fisher BP233-1)	18g	18%
5M NaCl	50mL	2.5M
1M Tris-Cl	1mL	10nM
0.5M EDTA	200uL	1mM
add H_2O to 100 mL		