Preparation of Buffer Saturated Phenol for DNA Extraction

References:

• Sambrook, J., E. F. Fritch, and T. Maniatis, 1989. Molecular Cloning, a laboratory manual. Cold Spring Harbor Laboratory Press, New York.

Materials:

- Redistilled Phenol, molecular biology grade: Stored in aliquots at -20℃.
- 8-hydroxyquinoline
- 0.5 M Tris•Cl buffer (pH 8.0)
- 0.1 M Tris•Cl buffer (pH 8.0)

NOTES:

Phenol is volatile and caustic.

- PREPARE THE PHENOL IN THE FUME HOOD.
- WEAR GLOVES AND EYE PROTECTION.

Procedure:

1. Heat a water bath to 65°C in the fume hood. Place the bottle of phenol in the fume hood to warm to room temperature.
2. Place the bottle of phenol in the 65°C water bath to melt the crystals.
3. Add 8-hydroxyquinoline to a final concentration of 0.1 % w/v to the phenol. Mix to dissolve the 8-hydroxyquinoline.
4. Add an equal volume of 0.5 M Tris•Cl (pH 8.0) to the phenol. Mix for 15 minutes. Return the bottle to the 65°C water bath. Allow the phases to separate. Siphon off the top layer and discard.
5. Repeat the procedure as in Step 4 twice.
6. Add an equal volume of 0.1 M Tris•Cl (pH 8.0) to the phenol. Repeat the procedure as in Step 4.
7. Repeat the extractions with 0.1 M Tris•Cl (pH 8.0) until the aqueous phase

is ~pH 7.8 (measure with pH paper). Repeat the procedure as in Step 4. Leave a ~1 cm layer of 0.1 M Tris•Cl (pH 8.0) over the phenol. Add 2-mercaptoethanol to a final concentration of 0.2% w/v to the 0.1 M Tris•Cl (pH 8.0).

8. The buffer saturated phenol may be stored at 4°C for 1 month for DNA extraction. Test the pH periodically and do not use if the pH is $\leq$7.5

Note: Phenol will be lost during the preparation of the buffer saturated phenol. Start with at least 2.5 X the final volume of phenol that you will need.