Purification of Genomic DNA from Paraffin Sections for PCR (Alkaline Extraction)

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Deparaffinization and H & E staining:

1) Prepare paraffin-embedded sections (**Note**: we can get nice DNA recovery from one regular slide, i.e. enough DNA for 50-100 PCR reactions).
2) Bake the slides at 55-60°C (in an oven or on a heating block) for 1 hour.
3) Deparaffinize (or dewax) the slides with xylene (immerse the slides with xylene for 5 min) × 3 times.
4) Rehydrate sections by immersing the slides into 100% ethanol for 5 min ×2 >>> 70% ethanol for 5 min ×2 >>> tap water for 5 min. (**Note**: Do not let slides complete dry from this point on).
5) Immerse slides in Harris hematoxylin (Fisher) for 2 min (optimization may be needed).
6) Rinse slides with water for 1 min × twice (or until water looks clean).
7) Immerse slides in 0.3% ammonium hydroxide 10-20 dips.
8) Rinse slides with tap water for 1 min × twice.
9) Immerse slides in Eosin-Y (Fisher) 10-20 dips (optimization may be needed).
10) Wash with water for 1 min × 3 times. (**Note**: You can check the H & E staining under a microscope. Also see the H & E Staining Protocol for other considerations).

Alkaline Extraction of Genomic DNA:

11) Remove unwanted tissue from slides with razor blades.
12) Add 5µl 0.2 M NaOH/1 mM EDTA to the H & E stained samples, then spread over edge of specimen with razor blade, and use the blades to scrape off the tissue you want, and collect tissue from each slide to a screw-cap tube containing 30µl 0.2 M NaOH/1 mM EDTA. (**Note**: For small areas, you can scrape tissue with 200µl pipet tip instead of razor blade.)
13) Vortex the tubes for a few seconds.
14) Heat the tubes in a heating block at 85°C for 30 min (or until most of the tissues are dissolved).
15) Vortex the tubes for a few seconds.
16) Microfuge the tubes at top speed for 2-5 min.
17) Transfer 20µl supernatants to 80µl (or 180µl if the sectioned tissue is large) ddH2O in a new set of tubes. Store DNA at -20°C.

PCR Amplification:

18) For PCR amplifications, use 2-3µl the purified genomic DNA as templates for a 25µl reaction. We usually use the touchdown PCR protocol (followed by 30

cycles of regular cycling amplification). (At the end of this stage, you can check 5µl of the PCR products on an agarose gel).

19) If necessary, the nested PCR amplification can be carried out by using 1µl of the first round PCR products (i.e., outside fragments) as templates (for a 25µl reaction) and a pair of nest (or inside) primers. Either a touchdown or regular cycling protocol can be used.
20) Check 5-10µl of the nested PCR products on agarose gels.