Preparation of genomic DNA from mouse ear punches

1. Obtain one ear punch, place in eppendorf tube.
2. Add 100 μl ear buffer: 10 mM Tris, pH 8.0 + 50 mM KCl + 0.5% NP40 + 0.5% Tween
3. Heat to 95° C for 15 minutes.
4. Add 2 μl proteinase K (stock: 10 mg/ml).
5. Incubate at 56° C for 3-4 hours. Vortex every hour or so.
6. Heat to 95° C for 15-20 minutes.
7. (Freeze samples at -20° C.)

PCR using Invitrogen HotWax beads for "hot-start"

1. Prepare pre-mix of all reaction components except DNA and $MgCL_2$:

   5 μl 5X PCR buffer (Invitrogen; pH 8.5, 9.0, 9.5, or 10.0; see below)
   5 μl dNTP mix (stock: 2 μM each dNTP)
   23-28 μl H_2O (calculated to have final reaction volume reach 50 μl)
   5 μl each oligonucleotide (working stock: 10 μM)
   0.5 μl Taq polymerase
   mix all of these components in multiple needed (e.g., to test 8 samples make 10X pre-mix by mixing 50μl PCR buffer with 50μl dNTPs, etc.)

2. Thaw genomic DNA, heat to 95°C 10 min., place on ice.
3. Aliquot 48 μl pre-mix per geneamp or microamp reaction tube.
4. Add 2 μl genomic DNA to tubes containing pre-mix.
5. Add one Hotwax bead containing $MgCL_2$ (1.5, 2.5, or 3.5 mM $MgCL_2$)
6. Begin PCR cycles:
   typical settings: 95° C 1 min./55° C 1 min./72° C 2 min. 35X (file #18 on 4th floor thermocycler) or 94°C 45 sec./55°C 1:30/72°C 2 min. 35X (file #109 on 5th floor thermocycler).

The hotwax beads will melt and release the $MgCL_2$ needed in the reaction only after reaching 95° C; it is unnecessary to add mineral oil.

For novel reactions, optimize by testing a range of rxn buffers (pH 8.5, 9.0. 9.5 or 10.0) versus a range of concentrations of $MgCl_2$ (1.5, 2.5, or 3.5 mM).

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