

Conklin Lab Tail Tip Digest Protocol

(Modified protocols from Hanahan Laboratory at UCSF)

1. Cut 1/16" to 1/8" of mouse tail from mouse using a razor blade.
2. Place tails in labeled 1.5 ml eppendorf tubes.
3. Prepare digest mixture:
Add 17 μl tail tip buffer and 3 μl proteinase K (20 mg/ml) for a total of 20 μl
4. Add 20 μl of digest mixture into tubes containing tail tip.
5. Incubate tubes in a 55°C water bath for 1-2 hours.
6. Remove tubes from water bath. Spin briefly for 10 seconds.
7. Add 500 μl of milli-Q water to each tube.
8. Boil samples for 5 minutes. Place weight on top of tubes to prevent caps from popping.
9. Spin samples briefly. Store at 4°C until PCR.

Tail tip buffer:

50 mM Tris, pH 8.0

20 mM NaCl

1 mM EDTA, pH 8.0

1% SDS

(adjust to proper volume using milli-Q water)

Conklin Lab PCR Tail Tip Analysis Protocol

1. Label PCR tubes.
2. Add 2.0 μl of digested mouse tail tip to the appropriate PCR tube.
3. Prepare PCR reaction mixture:

39.8 μl Milli-Q water

5.0 μl 10 \times PCR buffer +Mg

1.0 μl 10mM dNTP

1.0 μl 5' primer (25 μM)

1.0 μl 3' primer (25 μM)

0.2 μl Taq (added last)

Total = 48.0 μl

(Taq and 10 \times PCR buffer +Mg are from Boehringer Mannheim)

4. Add 48.0 μl of PCR reaction mixture to each PCR tube.

5. Place tubes in a thermal cycler using the following conditions:

1. 94 °C for 3 minutes
2. 94 °C for 30 seconds
3. 60 °C for 30 seconds
4. 72 °C for 1 minute
5. go to step 2, 34 times
6. 4 °C forever

6. Analyze PCR product on a 2% agarose gel.