肉班

（１）DNA 抽出・精製

Mammalian Tissue and Mouse/Rat Tail Lysate Protocol

1. Set a water bath or heat block at 55°C.
2. Place up to 25 mg of minced mammalian tissue (up to 10 mg minced spleen tissue), or tail clip (1 cm mouse or 0.5 cm rat tail clips) into a sterile microcentrifuge tube:
3. Add 180 µl PureLink™ Genomic Digestion Buffer and 20 µl Proteinase K to the tube. Ensure the tissue is completely immersed in the buffer mix.
4. Incubate at 55°C with occasional vortexing until lysis is complete (1-4 hours). For mouse tails or larger tissue pieces, you may perform overnight digestion.
5. To remove any particulate materials, centrifuge the lysate at maximum speed for 3 minutes at room temperature. Transfer supernatant to a new, sterile microcentrifuge tube.
6. Add 20 µl RNase A to lysate, mix well by brief vortexing, and incubate at room temperature for 2 minutes.
7. Add 200 µl PureLink™ Genomic Lysis/Binding Buffer and mix well by vortexing to yield a homogenous solution.
8. Add 200 µl 96-100% ethanol to the lysate. Mix well by vortexing to yield a homogenous solution.
9. Proceed immediately to **Purification Protocol**, next page.

※カミソリとつまようじを使ってサンプリングする。つまようじとカミソリはサンプルごとに交換すること。

※PCR は微量の DNA でも検出できるので、他のサンプルがわずかに混入するだけで正確に判定することができなくなる。ご注意。

Purification Protocol

The purification procedure is designed for purifying genomic DNA using a spin column-based centrifugation procedure in a total time of **10-15 minutes**.

1. Remove a PureLink™ Spin Column in a Collection Tube from the package.
2. Add the lysate (~640 µl) prepared with PureLink™ Genomic Lysis/Binding Buffer and ethanol to the spin column.
3. Centrifuge the column at 10,000 × g for 1 minute at room temperature.
   Note: If you are processing >200 µl starting material such as blood, you need to perform multiple loading of the lysate by transferring any remaining lysate to the same PureLink™ Spin Column (above) and centrifuge at 10,000 x g for 1 minute.
4. Discard the collection tube and place the spin column into a clean PureLink™ Collection Tube supplied with the kit.
5. Add 500 µl Wash Buffer 1 prepared with ethanol (page 2) to the column.
6. Centrifuge column at 10,000 × g for 1 minute at room temperature.
7. Discard the collection tube and place the spin column into a clean PureLink™ collection tube supplied with the kit.
8. Add 500 µl Wash Buffer 2 prepared with ethanol (page 2) to the column.
9. Centrifuge the column at maximum speed for 3 minutes at room temperature. Discard collection tube.
10. Place the spin column in a sterile 1.5-ml microcentrifuge tube.
11. Add 25-200 µl of PureLink™ Genomic Elution Buffer to the column. Choose the suitable elution volume for your needs.
12. Incubate at room temperature for 1 minute. Centrifuge the column at maximum speed for 1 minute at room temperature.
    The tube contains purified genomic DNA.
13. To recover more DNA, perform a second elution step using the same elution buffer volume as first elution.
14. Centrifuge the column at maximum speed for 1.5 minutes at room temperature.
    The tube contains purified DNA. Remove and discard the column.
15. Use DNA for the desired downstream application or store the purified DNA at 4°C (short-term) or -20°C (long-term).

食肉検査用の PCR

H_2O	13.8 μl	
10x Ex Taq Buffer	2 μl	
2.5 mM dNTPs	1.6 μl	
Primer Beef-F (20 μM)	0.2 μl	
Primer Beef-R (20 μM)	0.2 μl	プレミックス液 18 μl
Primer Pork-F (20 μM)	0.2 μl	
Primer Pork-R (20 μM)	0.2 μl	
Primer Chicken-F (20 μM)	0.2 μl	
Primer Chicken-R (20 μM)	0.2 μl	
Ex Taq polymerase	0.2 μl	
DNA	2 μl	
	(Total 20 μl)	

94℃, 4 min → (94℃, 30 sec → 60℃, 30 sec → 72℃, 30 sec) x 40 cycles
→ 72℃, 7 min → 4℃