Spin Doctor Genomic DNA Isolation Kit Solution Set (SS)
PROTOCOL

Description: The Spin Doctor Genomic DNA Isolation Kit provides for fast and consistent isolation of genomic DNA from rodent tail and ear punch samples. DNA is ready for any application including PCR and Southern Blotting. This kit is a solution set only and does not include protienase K or rapid digest enzymes.

Important: This document contains two different versions of the protocol. Please use the one that best suits your downstream application.

For PCR: Please use the rapid 15 minute version located on page 2.

For Southern Blotting: Please use the 30 minute protocol starting on page 3.

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SPIN DOCTOR GENOMIC DNA PREP KIT SS
RAPID PROTOCOL (WITHOUT PK)

Rapid version suitable for PCR with robust primers (15MIN.)

Important
The rapid version of the protocol produces DNA suitable for PCR analysis with most primers, taking 15 minutes after sample digestion. It is not recommended for Southern Blot analysis or PCR with less robust primers - in this case please use the standard protocol.

Larger sample sizes, up to 1.2cm mouse tails or .5cm rat tails, can be used to obtain higher concentrations.

Protocol

1. Add tail or ear punch sample to a clean 2.0ml tube containing 500ul of Genomic Resuspension buffer

The largest sample size that can be used is 1.2cm from a mouse and .5cm from a rat – these sample sizes will give increased concentrations

3. Add 15ul of Protienase K (20mg/ml) to each sample/tube
4. Incubate at 57°C overnight
   A. Periodic vortexing will help speed the digestion. The digest is complete once the tissue has been dissolved (bone and hair might remain)
4. Add 800 uL of Genomic DNA Wash Buffer and mix by inverting 30 times or by vortexing
5. Add 600 uL of Isopropanol and mix by inverting 30 times or by vortexing

Tail hair is NOT a problem – hair will pellet down with DNA

6. Spin at 14,000 g for 5 minutes
   A. Decant supernatant by pouring it off and then pipetting off remains
   B. Allow samples to air dry for 30 seconds
7. Resuspend in 250 uL of Final Resuspension Buffer (10mM Tris pH 8.0)
   A. Vortex to re-suspend DNA

The supernatant contains clean genomic DNA – hair and solids can be discarded

8. Spin at 14,000-16,000 g for 5 minutes
   A. Transfer 200 uL of the supernatant into a clean tube, use care not to transfer any hair or solids
   B. DNA is ready for PCR

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SPIN DOCTOR GENOMIC DNA PREP KIT SS
STANDARD PROTOCOL (WITHOUT PK)

Standard version suitable for Southern Blot anaylsis or PCR with less robust primers (30 MIN.)

Important
The standard version is recommended for Southern Blot analysis or PCR with less robust primers. The protocol takes approximately 30 minutes after samples have been digested.

Recommended The largest sample size, up to 1.2cm for mouse tails and .5cm for rat tails, should be used to obtain optimal concentrations. Large samples may need to be cut into smaller portions so that they are entirely immersed in the solution.

Please use 1 – 1.2cm mouse tail or .3-.5cm rat tail; Ensure that the entire sample is submerged in PK

The largest sample size that can be used is 1.2cm from a mouse and .5cm from a rat – these sample sizes will require longer digestion times

Protocol

1. Add tail or ear punch sample to a clean 2.0ml tube containing 500ul of Genomic Re-suspension buffer (see note for sample size)
2. Add 15ul of Proteinase K (20mg/ml) to each sample/tube
3. Incubate at 57°C overnight
   A. Periodic vortexing will help speed the digestion. The digest is complete once the tissue has been dissolved (bone and hair might remain)
4. Add 800 uL of Genomic DNA Wash Buffer
   A. Mix by inverting 30 times
      - The wash buffer will retain the contaminating proteins, nucleases, and degraded nucleic acids
5. Add 600 uL of Isopropanol
   A. Mix by inverting 30 times
      - The Isopropanol will precipitate genomic DNA while contaminants will be retained in the wash solution

-Please Continue-

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6. Spin at 14,000 g for 10 minutes
    A. Decant supernatant by pouring it off
        - Pelleting the DNA will allow you to remove the contaminants by simply discarding the supernatant

Tail hair is NOT a problem – hair will pellet down with DNA

7. 70% Ethanol wash
    1. Add 1 mL of 70% Ethanol to each tube
    2. Mix by inverting 20 times
    3. Microcentrifuge at maximum speed for 2 minutes
    4. Discard supernatant by pouring it off
    5. Repeat steps 7.a-7.d once
        - Ethanol wash steps eliminate contaminating salts

8. Pipette off remaining Ethanol, and air dry for 10 minutes
    - Air drying removes contaminating Ethanol

If wide bore pipette tips are not available improvise by cutting off the very end of a standard tip

9. Resuspend in 250 uL of Final Resuspension Buffer (10mM Tris pH 8.0)
    A. Incubate at 57°C for 10 minutes
    B. Pipette midway through the incubation to help loosen the DNA pellet, and once more after incubation. Use wide bore tips in order to prevent shearing of the DNA

The supernatant contains clean genomic DNA – hair and solids can be discarded

10. Spin at 14,000-16,000 g for 5 minutes
    A. Transfer 200uL of the supernatant into a clean tube, use care not to transfer any hair or solids
    B. DNA is ready for any downstream application
        - This final spin is crucial because it removes any DNA which has not re-suspended as well as any remaining solids