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# CST Protocol for Extracting gDNA from Bone Samples

## Protocol

### Introduction

The purification of genomic DNA from fresh and old bone samples has been tested with the following protocol. The extraction is based on the protocol for the ChargeSwitch® gDNA Plant Kit (Invitrogen, Cat. no. CS18000).

Samples were digested at 37°C overnight, resulting in a gDNA yield of 1–2 µg from an initial amount of 100 mg of bone material (including marrow tissue). The OD ratio was between 1.6–1.8, and the purified DNA was successfully used in downstream PCR reactions.

### Materials Needed

- Fresh or old bone sample
- ChargeSwitch® gDNA Plant Kit (Invitrogen, Cat. no. CS18000)
- Drill and/or pestal and mortar
- 10% SDS
- Magnetic Separator (e.g., MagnaRack™ Invitrogen, Cat. no. CS15000)
- *Optional*: Proteinase K (100 mg/mL)

### Before Starting

- Pre-chill the ChargeSwitch® Precipitation Buffer (N5). The precipitation reaction works better at lower temperatures.
- Vortex the ChargeSwitch® Magnetic Beads so that they are fully resuspend before use.

**Optional**:

- If processing multiple samples, prepare a master mix consisting of 100 µL of ChargeSwitch® 10% Detergent and 20 µL of ChargeSwitch® Magnetic Beads per reaction.

### Preparing Bone Samples

1. Wash the bone thoroughly and sterilize the surface with 1% Virkon®.
2. Grind approximately 100 mg of bone using a 1/8$^{th}$-inch drill bit. Pass the drill bit through the bone material on one side of the sample, through the marrow cavity, and into the bone at the other side. Extract the bit, and remove ground bone material from the bit head. Place the ground bone sample in a clean microcentrifuge tube.

   **Note**: Drill the bone at low speed to avoid heating the sample.
3. Add 1 mL ChargeSwitch® Lysis buffer (L18) and 100 µL of 10% SDS to the sample and incubate at 37°C overnight.
4. Allow the sample to cool to room temperature, and add 5 µL of RNase A. Incubate at room temperature for 1 minute.
5. Centrifuge the lysate at maximum speed for 5 minutes to remove the bone and tissue debris.
6. Transfer the cleared lysate to a fresh tube.
7. Add 400 µL of pre-chilled ChargeSwitch® Precipitation Buffer (N5) to the lysate and mix by inversion.
8. Centrifuge for 5 minutes at maximum speed to precipitate the SDS/proteins and any remaining cell debris.
9. Transfer the cleared lysate to a fresh tube.

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## Preparing Bone Samples, Continued

**Optional**:

- Use a pestal and mortar to further homogenised the bone after collecting a sample using a drill bit (Step 2).
- Add 30 µL of Proteinase K (100 mg/mL) to the Lysis reaction (Step 3).
- If overnight incubation is not desirable, perform digestion at 65ºC for 1–3 hours (Step 3).

## Binding of gDNA

1. Add 100 µL of ChargeSwitch® 10% Detergent (D1) to the cleared lysate.
2. Add 20 µL of fully resuspended ChargeSwitch® Magnetic Beads to the lysate, and mix 5 times with the pipette tip to ensure that the beads are evenly suspended.
3. Incubate the lysate at room temperature for 1 minute to bind the gDNA to the beads.
   **Note**: High molecular weight DNA may clump at this stage.
4. Place the tube in the MagnaRack™ magnetic separator for 1 minute, or until the beads form a tight pellet.
5. Remove and discard the supernatant.

**Optional**:

- If a master mix was prepared, add 120 µL of the master mix to each sample (Steps 1–2). Make sure that the beads are fully resuspended before use.

## Washing the Beads

1. Remove the tube from the MagnaRack™ magnetic separator, and fully resuspend the bead pellet in 1 mL of ChargeSwitch® Wash Buffer (W12) by mixing with a pipette tip 5 times.
2. Place the tube back on the MagnaRack™ for 1 minute or until a tight pellet forms.
3. Remove and discard the supernatant.
4. Repeat Steps 1–3 once.

## Eluting the DNA

1. Remove the tube from the MagnaRack™ magnetic separator, and resuspend the bead pellet in 50–100 µL of ChargeSwitch® Elution Buffer (E6).
2. Incubate the tube at room temperature for 1 minute.
   *Optional*: Longer incubation times can increase the final yield of DNA. Heated elutions can also increase the yield if the DNA is of high molecular weight.
3. Place the tube in the MagnaRack™ magnetic separator for 1 minute or until the beads form a tight pellet.
4. Transfer the eluate containing the purified gDNA to a fresh tube.

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## Expected Results

### DNA Yield

The protocol was used to extract gDNA from fresh bone (chemically stripped of flesh) and old bone (naturally stripped of flesh). Table 1 shows the data from several experiments on both fresh and old bone. Genomic DNA yields were determined using Picogreen dsDNA assay (Invitrogen, Cat. no. P-7589).

**Table 1**

| Sample Type | Sample Weight (mg) | Yield (ng) | | OD ratio |
|---|---|---|---|---|
| Fresh bone with Proteinase K | 95.3 | 1593.4 | 1694.2 | 1.69 |
| | 103.4 | 1615.3 | 1672.9 | 1.68 |
| | 89.6 | 1069.6 | 1068.1 | 1.81 |
| | 86.5 | 1302.8 | 1300.8 | 1.82 |
| | 102.4 | 1850.7 | 1881.3 | 1.75 |
| | 128 | 1976.2 | 1970.8 | 1.76 |
| Fresh bone without Proteinase K | 108.8 | 2028.5 | 2087.4 | 1.68 |
| | 89.5 | 1681.6 | 1845.9 | 1.71 |
| Old bone with Proteinase K | 110.5 | 38.0 | 38.1 | 1.29 |
| | 121.3 | 85.5 | 84.6 | 1.26 |
| | 116.4 | 121.7 | 120.5 | 1.20 |
| | 97.7 | 109.5 | 106.8 | 1.17 |

**Table 1**: gDNA Yield and OD ratio of samples taken from fresh and old bone, digested in the presence or absence of Proteinase K.

Typical yields from fresh bone in the presence or absence of Proteinase K, were between 1 and 2 µg gDNA with OD ratios between 1.6 and 1.8. The yield of gDNA and OD ratio was not affected by Proteinase K. Typical yields from old bone were around 100 ng with an OD ratio of 1.2.

### DNA Quality

Figure 1 shows an agarose gel analysis of gDNA purified from fresh and old bone. A high molecular weight band of DNA can be seen above the 40 kb mark with some smearing within the lanes, characteristic of degraded DNA.

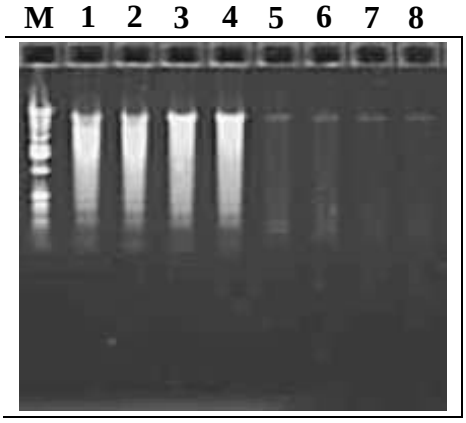


**Figure 1**: 1% agarose gel performed with gDNA from old and fresh bone samples. M = 1Kb DNA Extension Ladder (Invitrogen, Cat. no. 10511012); Lanes 1–4 = Fresh bone; Lanes 5–8 = Old bone.

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## PCR Results

PCR was performed on the eluted gDNA to determine if any inhibitory factors had been co-purified. PCR reactions were performed using 4 µL of eluted gDNA sample and the following primers at a final concentration of 200 nM unless otherwise stated.

| Primer Name | Sequence |
|---|---|
| NR3C1 325 F | 5′ CTCTTGGATTCTATGCATGAA 3′ |
| NR3C1 325 R | 5′ TATAAACCACATGTAGTGCG 3′ |
| GAPDH F | 5′ GGTGAAGGTCGGAGTCAACG 3′ |
| GAPDH R | 5′ CAAAGTTGTCATGGATGACC 3′ |

The temperature cycle used was:

| Temperature (°C) | Time | Cycles |
|---|---|---|
| 94 | 5 minutes | ×1 |
| 94<br>56<br>72 | 30 seconds<br>45 seconds<br>40 seconds | ×30 |
| 72 | 7 minutes | |
| 4 | ∞ | ×1 |

Figure 2 shows the PCR amplification using gDNA purified from 4 individual samples of fresh bone. A single PCR product with the expected size can be seen, indicating that no inhibitors were co-purified with the DNA. All samples resulted in successful amplification in at least one of two duplicates reactions. Only two PCR reactions failed to significantly amplify any DNA. This result shows that PCR can be performed on DNA eluted from fresh bone using the extraction protocol described.

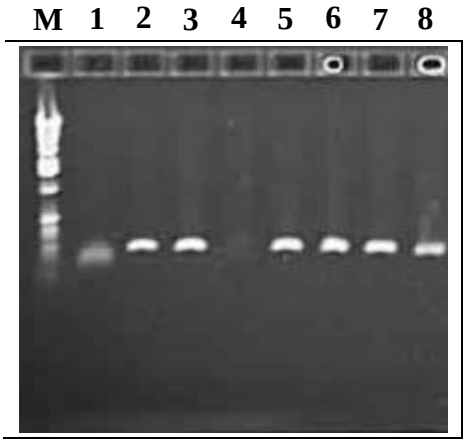


**Figure 2**: PCR amplification of the NR3C1 fragment from genomic DNA extracted from fresh bone.
M = 1Kb DNA Extension Ladder (Invitrogen, Cat. no. 10511012); Lane 1–2 = sample 1; Lane 3– 4 = sample 2; Lane 5–6 = sample 3; Lane 7–8 = sample 4.

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## PCR Results, Continued

Figure 3 shows PCR amplification using gDNA purified from old bone. Reactions were performed with increasing amounts of template DNA (1–10 µL from left to right). A corresponding increase in signal strength to DNA quantity can be seen in the single PCR product. This suggests that no PCR inhibitors are co-purified with the DNA.


**Figure 3**: PCR amplification of GAPDH fragment from genomic DNA extracted from old bone. M = Invitrogen 1kb extension ladder; Lane 1 – 10 = Increasing template DNA from 1 µl to 10 µl respectively.

## Conclusion

The data present here shows that the protocol described for the extraction of genomic DNA is functional for both fresh bone and old bone. Figure 1 shows that significant quantities of gDNA are extracted from both fresh and old bone, as confirmed by the high molecular weight bands visible on the agarose gel. Table 1 shows that 1–2 µg of genomic DNA can be extracted for fresh bone, and approximately 100 ng of genomic DNA for old bone. The OD ratio for fresh bone (1.6–1.8) shows a high level of DNA purity with low protein content. The OD ratio of old bone (1.2) suggests there may be carry over of some proteins with the DNA. Further development is required on the protocol to increase the yield and purity of gDNA purified from old bone, however the work described here is a noteworthy start.

PCR amplification has successfully shown that no inhibitors are present within the eluted gDNA from both fresh and old bone.