Three easy methods for high quality nucleic acid isolations from a variety of veterinary and aquaculture samples

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Introduction

- Molecular testing of animals has become increasingly important for modern veterinary and agricultural applications. Diagnostic tests using PCR (e.g., for Johne's disease in cattle or herpes virus in dogs and cats) are replacing traditional ELISA or culture-based methods due to higher sensitivity and speed. Genotyping provides a valuable tool for selective breeding, from scrapie resistance in sheep to coat colour in horses, dogs, and cats.
- An easy and standardized method for nucleic acid isolation that gives high quality nucleic acids is required. The major challenge when working with such a diverse material is to develop optimized pre-treatments for all sample types. Dependent on the sample type, content of inhibitors and content of nucleic acids, the samples demand different pre-treatment conditions such as mechanical disruption, enzymatical digestion and incubation times.
- One manual (QIAGEN DNeasy Mini), one semi-automated (QIAGEN BioSprint 15) and one automated (QIAGEN EZ1) system were tested for DNA and RNA isolation from a variety of veterinary samples. This presentation compiles data from fresh and dried pig ears, horse hair, chicken blood, fish fins and fish embryo.

Results – DNA from pig ears

- DNA was isolated from Typifix eartags*, frozen tissue (ca 20 mg), lyophilized tissue (ca 6-10 mg) and dried tissue (ca 25 mg) with either DNeasy spin columns or BioSprint 15 DNA Tissue Protocol.

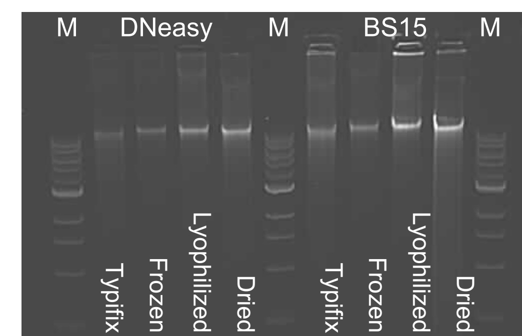

Figure 1: DNA was purified from pig ear samples and eluted in 200 µl elution buffer. Eluates (2 µl from all but dried ears; 0.5 µl) were visualized on a 0.8% agarose gel. The marker (M) was 50 ng 1 kb ladder.

*The resulting punch is directly pressed and sealed into a vial containing a desiccant/conserving reagent. The complete contents of the tube; tissue and white powder was used for DNA isolation.

- Purified DNA gave good results in endpoint PCR analysis of the GAPDH gene.

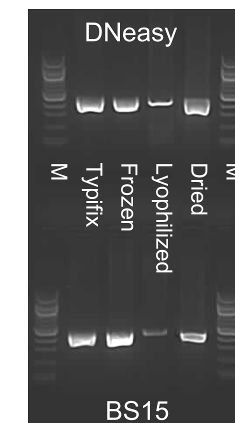

Figure 2: Amplification (30 µl reaction volume) was performed using the GAPDH PCR system with 5 µl eluted DNA from each sample type and each isolation system. PCR products (5 µl) were visualized on a 1.5% agarose gel. The marker (M) was 150 ng 100 bp std.

Results – DNA from horse hair

- DNA was isolated from 1, 4 and 10 horse hairs with the DNeasy, BioSprint 15 and EZ1 systems.
- Ca 1cm length of the root end was used for DNA isolation.
- All samples were incubated at 56ºC with 1M DTT and Proteinase K as pretreatment.

Figure 3: DNA was purified from horse hair samples and eluted in 200 µl elution buffer. Eluates (13 µl) were visualized on a 0.8% agarose gel. The marker was 40 ng 1 kb ladder.

*PCR system from: Schweiz Arch Tierheilkd. 2002 Aug;144(8):405-12. X is allele not covered by this specific test.

- Horse colour coating PCR*: Horse 1; Brown (EeAa – red and black carrier), Horse 2: Brown (EE Aa – black carrier), Horse 3: Brown (Ee Ax* – red carrier), Control DNA: Black (Ee aa – red carrier).

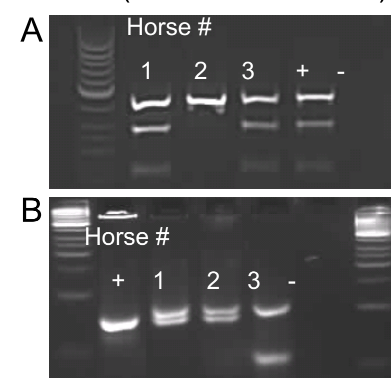

Figure 4 : Amplifications (25 µl reaction volume) were performed using the MSH and ASIP PCR systems with 2 µl eluted DNA from 3 different horses and 1 positive control (+, DNA isolated from horse blood). A: Taq I restriction digest products (3 µl) of MSH PCR products visualized on a 1.5% agarose gel (No restriction; "E", restriction; "e"). B: ASIP PCR products (10 µl) visualized on a 4% agarose gel ("a" is 90 pb and "A" is 100 bp). The marker was 60 ng 50 bp std. "-" is negative control.

Results – DNA from chicken blood

- DNA was isolated from 0.2, 2, 5, 10 and 20 µl chicken blood with the BioSprint 15 system.
- Samples were diluted to 200 µl with PBS.

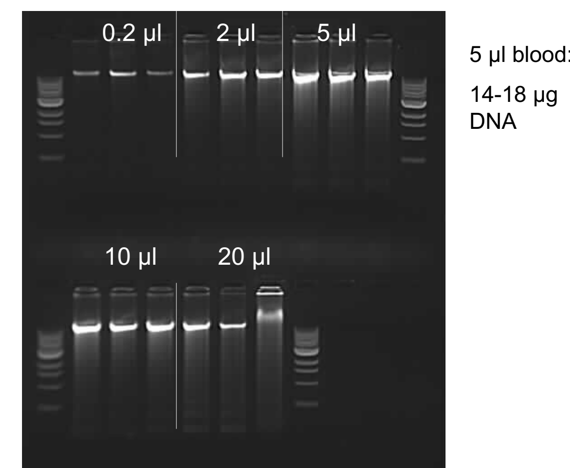

Figure 5: DNA was purified from chicken blood samples and eluted in 200 µl elution buffer. Eluates (2 µl) were visualized on a 0.8% agarose gel. The marker was 60 ng 1 kb ladder.

- Birds are difficult to sex. Nestlings rarely show sex-linked morphology and adult females are estimated to appear identical to males in over 50% of the world's bird species*.
- Using the avian CHD PCR for sexing, there is a single CHD-Z band in males but females have a second, distinctive CHD-W band*.

Figure 6 : Amplifications (25 µl reaction volume) were performed using the avian CHD PCR system* with 2 µl eluted DNA (from 5 µl blood) from 3 different chicken and systems. PCR products (3.5 µl) was visualized on a 3% agarose gel. The marker was 30 ng 50 bp std. "-" is negative control.

*Griffiths et al., 1998. Mol Ec, 7: 1071 – 1075

Results – RNA from zebrafish embryo and DNA from fish fins

- DNA was isolated from 51 zebrafish embryo (24 h) with the EZ1 Universal Tissue Protocol.
- Samples were homogenized with 23G syringe in 750 µl QIAzol.

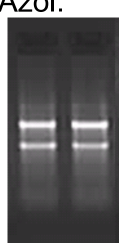

Figure 8: RNA (Universal Tissue Protocol) was purified from zebrafish embryo and eluted in 100 µl. Eluates (ca 300 ng) were visualized on a 1.5% agarose gel. Samples kindly provided by Alestrom Zebra fish Lab; Norwegian School of Veterinary Science.

- DNA was isolated from 20 mg (DNeasy) and 10 mg (EZ1) salmon fin tissue with the DNeasy and EZ1 systems.
- Samples were either incubated at 56°C with Proteinase K (PK) for 3 hours, or disrupted with a TissueLyser (TL) and steel beads in combination with 1 h incubation at 56°C with Proteinase K.

Figure 7: DNA was purified from salmon fin tissue and eluted in 200 µl elution buffer. Eluates (2 µl) were visualized on a 0.8% agarose gel. The marker was 60 ng 1 kb ladder.

- Up to 5 µl DNA eluate was used as template in a 18S qPCR inhibition study.
- When the Ct values correlate with the amount of input DNA as shown in figure 9, the DNA is pure with no inhibitors.

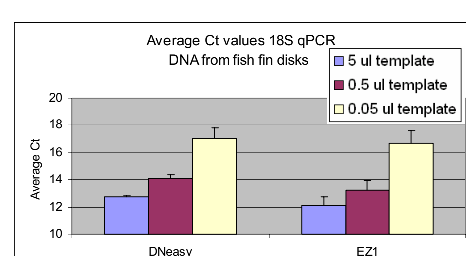

Figure 9: Amplification (25 µl reaction volume) were performed using the general 18S qPCR with 0.05 µl, 0.5 µl and 5 µl eluted DNA as template in an inhibition study.

Conclusions

All three isolation systems gave good and corresponding yields from the various veterinary samples. All samples were suitable for PCR analysis and showed no inhibition.

Mammals:

- DNA from all types of pig ear samples was successfully isolated and gave good results in PCR analysis.
- DNA isolations from increasing numbers of horse hair showed corresponding increasing yields and gave good results in colour coating PCR.

Bird :

- DNA from chicken blood can be isolated from as little as 0.2 µl blood, due to the presence of nucleated erythrocytes. To achive optimal yields and PCR performance, 2-5 µl blood is recommended. The DNA performed successfully in the sexing PCR, identifying all chicken as females.

Fish:

- RNA from zebrafish embryo were successfully isolated on the EZ1 system.
- DNA isolations from fish fins resulted in clear DNA bands on agarose gel, no sign of PCR inhibition was observed, even with high amounts of DNA.