

GENOME BANK OF CHIANINA IN UMBRIA**E. Lasagna, F. M. Sarti, S. Sorbolini, F. Filippini, F. Panella***The antecedent*

A Chianina DNA genome bank must be obtained from 5100 (4500 cows and 600 bulls) heads in four years.

The constraint

Each DNA sample must be divided in three aliquots and stored in three different places after elution in TE (tris[hydroxymetyl]aminomethane - 10 Mm and etylendi-aminetetracetil acid - 1 Mm, Ph 7.0-8.0) and freezing at -80 °C; therefore 30-40 µg of DNA are needed from each animal.

The aim

Find the most suitable method to extract DNA from blood.

The work

Three different kits were tested according to the cost, the DNA yield and the time required for the extraction.

1st kit: low cost kit, but poor DNA yield (15-20 µg) if used with 200 µl of whole blood.

Good DNA yield was obtained using only leukocyte pellets, but due to column saturation the repeatability was very low.

To solve this problem a double extraction with two different columns was performed. The cost was still cheaper than that of other kits suitable to process large quantity of blood, the extraction procedure was too time consuming.

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2nd kit: It was large scale. With this method the same DNA yield was obtained as with the double extraction with kit no.1. The results were more consistent, but column saturation still occurred.

3rd kit: It was a non-column based kit; the kit allowed to obtain 30-40 µg of DNA from 1.5 ml of blood, with a good repeatability and a relatively low cost. This method was therefore chosen for the creation of the Chianina genome bank.

BANKA GENOMA CHIANINE U UMBRIJI

Banka DNA genoma Chianine mora se dobiti od 5100/4500 krava i 600 bikova/grla u četiri godine.

Testirana su tri različita paketa prema trošku, dobivenoj količini DNA i vremenu potrebnom za ekstrakciju.

1. paket: paket niskog troška ali manje dobivenog DNA (15 - 20 µg) ako se upotrijebi 200 µl čitave krvi.

Dobra količina DNA dobivena je samo ako se upotrijebe kuglice/pelete leukocita ali zbog zasićenja stupca mogućnost ponavljanja bila je vrlo niska.

Kako bi se riješio ovaj problem obavljena je dvostruka ekstrakcija s dva različita stupca. Trošak je još uvijek bio manji nego onaj iz drugih paketa koji su mogli obraditi velike količine krvi ali je za postupak ekstrakcije trebalo previše vremena.

2. paket: bio je velikog opsega. Ovom metodom dobivena je ista količina DNA kao i kod dvostruke ekstrakcije u paketu 1. Rezultati su bili dosljedniji ali je još uvijek došlo do zasićenja stupca.

3. paket: bio je to paket bez stupca; paket je omogućio dobivanje 30-40 µg DNA iz 1.5 ml krvi, s mogućim dobrim ponavljanjem i uz relativno nizak trošak. Ova je metoda, stoga, izabrana za stvaranje banke genoma Chianine.

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