

Determination of DNA concentration in
preparations obtained by nexttec™ DNA isolation
kits

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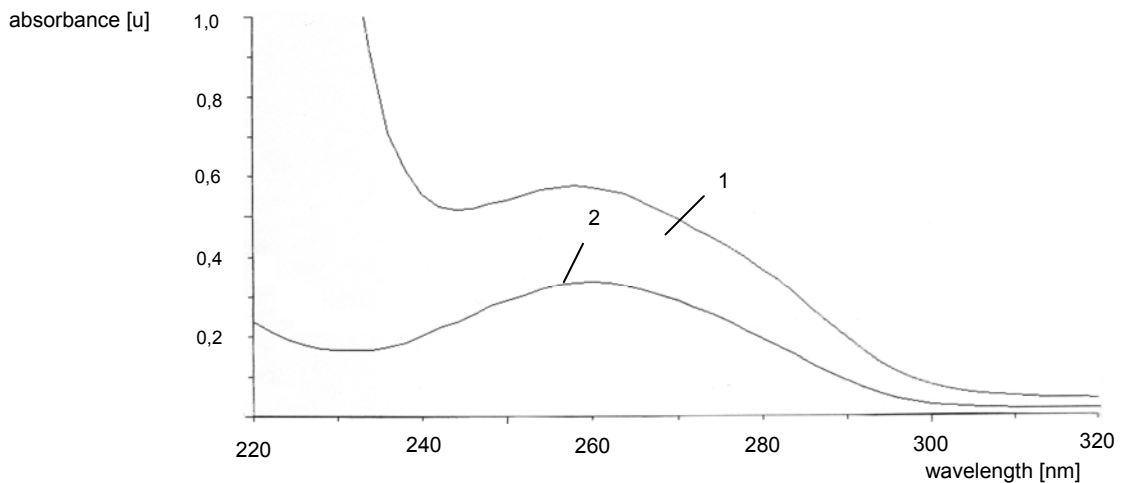
After purification of DNA it is in most cases necessary to determine the exact concentration of nucleic acids in the preparations. This can be achieved in different ways:

1. Calculation of DNA concentration by measuring the UV absorption at 260 nm and the ratio A_{260}/A_{280}
2. Comparing the fluorescence intensity of DNA bands of unknown concentration with that of standards in agarose gels stained with ethidium bromide
3. Using intercalating fluorescent dyes like Picogreen™ (Molecular Probes)

For users of nexttec kits we recommend to determine the DNA concentration by method 2 (see above). It is the simplest way, which does not require expensive equipment, and it gives sufficiently accurate results for a proper dilution of DNA before Restriction or PCR. Method 3 using the fluorescent dye Picogreen™ is the most sensitive and accurate method, but it is expensive and requires a fluorescence reader.

Absorption measurement can't be used for DNA preparations obtained by nexttec kits. This is due to our technology, which differs completely from that of competitors. The DNA is NOT bound to a sorbent's surface, but proteins and other bio-molecules are bound and low molecular weight substances (buffer salts, metabolites etc.) are retained. This enables a very fast one step DNA

purification. On the other hand, it is impossible to bind each kind of molecule to the same high extent. Thus, we concentrated our effort on binding molecules that inhibit enzymes in downstream applications (Taq Polymerase, restriction enzymes). Some substances, e.g. buffer components, which are used for lysis of tissue, remain in the DNA eluate. They cause a higher UV absorption at 260 and at 280 nm and this, in turn, leads to an overestimation of DNA concentration (up to ten-fold) and to a low ratio of A_{260}/A_{280} . In fig. 1 it is demonstrated, that the low ratio of A_{260}/A_{280} is not due to the presence of proteins, which absorb at 280 nm.



purified DNA	A 260nm	A 280 nm	A 300 nm	A260nm/A280nm
before precipitation	0.573	0.369	0.078	1.55
after precipitation	0.338	0.193	0.027	1.75

Fig. 1: UV-spectra in the range of λ from 220 to 320 nm obtained after purification of DNA using nexttec™ clean-columns. The same DNA preparation was measured before (1) and after (2) precipitation with ethanol.

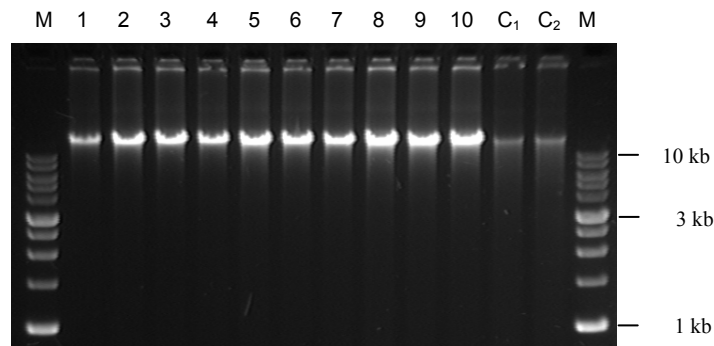


Fig. 2: Electrophoretic separation of genomic DNA from sheep (muscle tissue). DNA was purified using the nexttec kit "Genomic DNA from Tissue and Cells" (lanes 1-10) or, for comparison, a competitor's kit on the basis of columns containing silica membranes (lanes C₁ and C₂). On the average 20 mg of fresh tissue was used for purification. From the resulting DNA preparation 5% were applied per lane.

1 - 10 - DNA purified using a nexttec kit
 C₁&C₂ - DNA purified using a competitors' kit on the basis of silica technology
 M - DNA length standard

A spectrum was taken from a DNA preparation purified using a nexttec™ clean-column before and after ethanol precipitation. Before precipitation the ratio A_{260}/A_{280}

is 1.55 . If this was due to proteins present in the DNA preparation, then after precipitation the spectrum will be nearly the same, because proteins precipitate in ethanol as well as DNA. But this is not the case. After precipitation the spectrum is typical for nucleic acids and the ratio A_{260}/A_{280} is nearly 1.8 as for pure DNA. This shows, that the lower ratio A_{260}/A_{280} is due to remaining buffer components from the lysis mixture or to some metabolites originating from the sample, but not to proteins, which adhere to the DNA.

Usually a low ratio A_{260}/A_{280} indicates that a nucleic acid preparation is not sufficiently clean for enzymatic applications. However, this is not true for nexttec DNA preparations. In fig. 2 DNA preparations from sheep muscle tissue are shown after electrophoretic separation. The DNA has a high molecular weight. It is present in a high amount, which is higher than in preparations obtained with a silica based kit of a competitor (see lanes C₁ and C₂). Table 1 shows the corresponding ratios A_{260}/A_{280} and DNA concentrations for all preparations. Comparing the DNA concentration calculated from the A_{260} measurement with that determined using Picogreen™ it is obvious, that the DNA concentration

sample	A 260	A 280	A 260 / A 280	c DNA (A260)	c DNA (Picogreen)
1	2,07	1,49	1,39	103,6 ng/μl	15,5 ng/μl
2	2,14	1,51	1,42	107,0 ng/μl	28,1 ng/μl
3	2,13	1,48	1,44	106,5 ng/μl	26,4 ng/μl
4	2,14	1,50	1,42	107,0 ng/μl	20,1 ng/μl
5	2,13	1,46	1,46	106,4 ng/μl	25,6 ng/μl
6	2,08	1,46	1,43	104,1 ng/μl	21,6 ng/μl
7	2,11	1,50	1,41	105,7 ng/μl	21,7 ng/μl
8	2,10	1,49	1,41	104,9 ng/μl	28,3 ng/μl
9	2,07	1,46	1,42	103,7 ng/μl	24,1 ng/μl
10	2,10	1,46	1,43	104,8 ng/μl	27,6 ng/μl
C 1	0,85	0,43	1,98	42,7 ng/μl	10,1 ng/μl
C 2	0,96	0,49	1,95	48,0 ng/μl	11,9 ng/μl

Tab. 1: Determination of DNA concentration by different methods. Genomic DNA from sheep muscle tissue was purified by a nexttec kit (1-10) or by a competitors kit based on silica technology (C1 & C2).

after UV measurement is highly overestimated in all samples. Nevertheless, the DNA concentration in the nexttec preparations is higher than in those of the competitor. The ratio A_{260}/A_{280} for silica-based preparations is as expected in the range of 1.8 to 2.0 whereas for nexttec preparations in the average it is 1.42. Despite these differences (especially the low ratio A_{260}/A_{280} in nexttec DNA preparations) there is no inhibition of demanding PCRs like multiplex microsatellite analyses for paternity testing (see fig. 3). All tested nexttec DNA preparations resulted in signals of at least the same height as with template DNA purified using a silica-based kit. In all cases the microsatellite alleles can be detected.

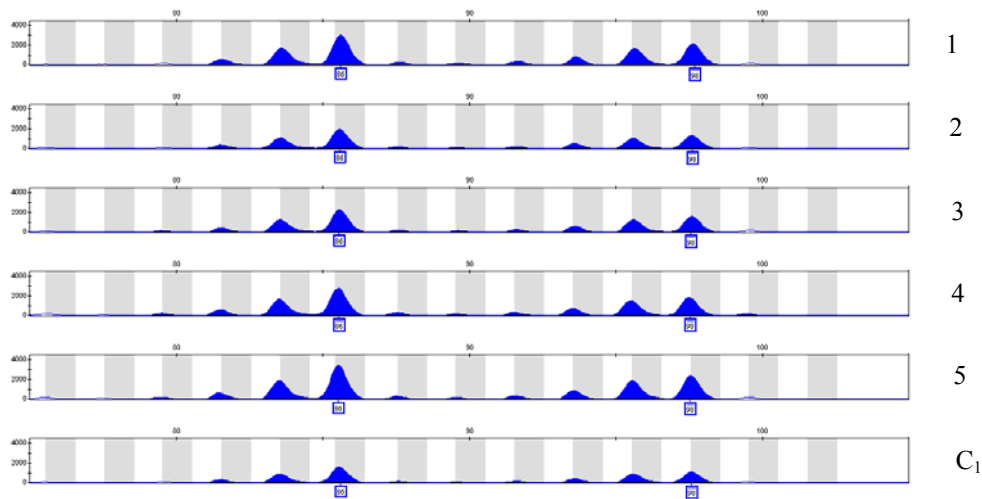


Fig. 3: Microsatellite analysis of genomic sheep DNA for paternity testing (ISAG panel). Five samples of genomic DNA purified using nexttecTM clean-columns (1-5) and 1 purified using a competitor's silica-based kit (C_1) were analysed. 86 and 98 are the detected microsatellite alleles (highest peaks). All DNA samples originate from the same animal and are therefore identical.

Conclusions:

1. The nexttec technology enables a very fast one-step purification of high molecular weight DNA with a high yield.
2. The nexttec DNA preparations are of a very good quality for demanding PCR analyses and give comparable results to template DNA prepared using silica based kits.
3. DNA concentration in nexttec preparations can't be exactly measured spectrophotometrically. The concentration is in all cases overestimated.
4. This and a low ratio A_{260}/A_{280} are due to lysis buffer, which remain in the eluate. These components are not proteins or other enzyme inhibiting substances.