6 secrets to optimize your gel extraction results

Karin Schulte, Regine Stoll, Uritza von Groll
QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, Germany

Introduction

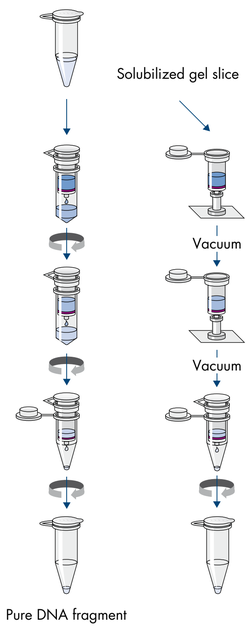
Here, we focus on gel extraction in molecular biology workflows and take a closer look at different parameters that influence performance. We examine agarose gel and related extraction parameters in more detail.

Bind-wash-elute principle

The QIAquick® system combines the convenience of spincolumn technology with the selective binding properties of a uniquely designed silica membrane. DNA adsorbs to the silica membrane in the presence of high concentrations of salt, while contaminants pass through the column. Impurities are efficiently washed away, and pure DNA is eluted with Tris buffer or water.

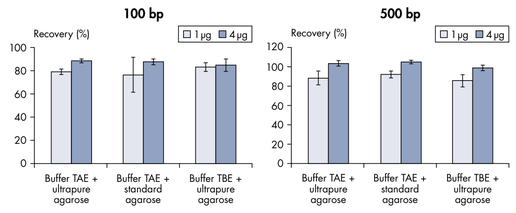
Procedure

After solubilizing the gel slice, isopropanol and binding buffer are added. Adding the solution to the QIAquick column results in binding of DNA fragments to the silica matrix. After washing away impurities and residual salts, the DNA fragments are eluted from the column.



QIAquick gel extraction procedure.

Buffer TAE ensures optimal recovery rate



Influence of agarose quality and running buffer on DNA recovery.

Standard agarose and ultrapure agarose were compared, as were running buffers TAE or TBE.

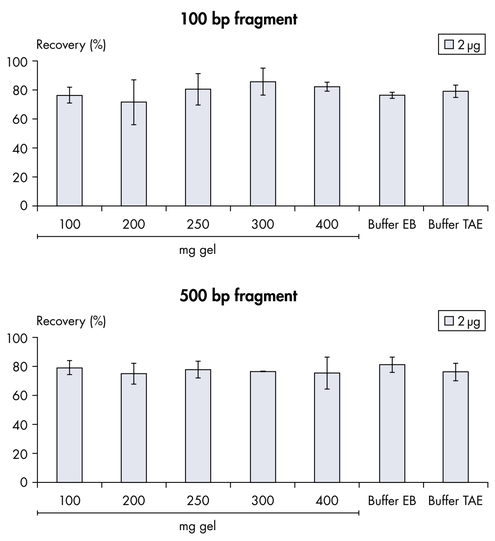
- The recovery rate is slightly increased with the higher amount of DNA.
- Buffer TBE results in a slightly lower recovery rate than Buffer TAE.
- Standard agarose is sufficient for high recovery rates of DNA.

Influence of running buffer age on DNA recovery.

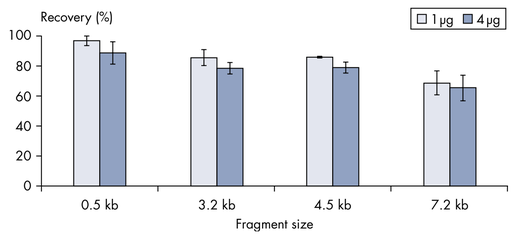
Running buffers were compared using fresh and 4-weekold TAE buffer. The freshness of the running buffer was shown to have no effect on recovery rate.

Larger fragments are more challenging to recover

DNA fragment size may cause variation in the recovery rate. A slight decrease in DNA recovery was observed for larger fragments.



Effect of Buffer QG volume on DNA recovery.



Effect of Buffer QG volume on DNA recovery.

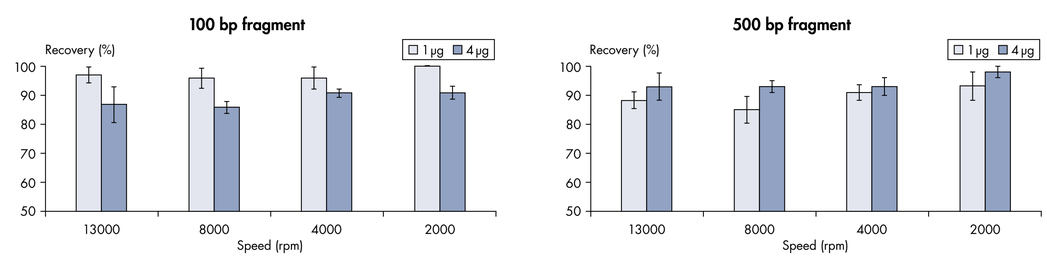
An important parameter in the gel extraction procedure is the binding buffer — Buffer QG.

The QIAquick gel extraction protocol was tested with a reduced volume of Buffer QG (1.5 instead of 3 volumes Buffer QG).

- In general, reduction of the binding buffer volume is possible without a reduction in the DNA recovery rate.
- Results are similar for both 100 bp and 500 bp fragments.

A reduced centrifugation speed enhances binding

During the binding step, centrifugation speed is an important factor. Centrifugation speeds from 2000 to 13,000 rpm were compared and a correlation between centrifugation speed and recovery rate was apparent.

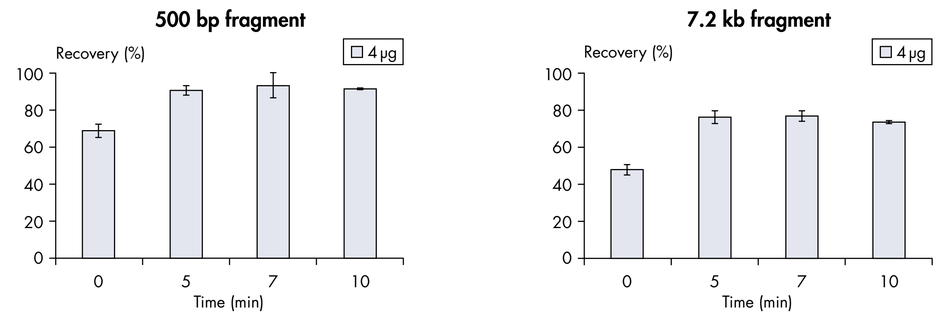


Impact of centrifugation speed on DNA binding.

- A reduced centrifugation speed has an overall positive effect.
- Fragment length and centrifugation speed have no effect on DNA recovery. Recovery rates were shown to be similar for different fragment sizes and centrifugation speeds.

Longer incubation aids recovery of long fragments

Incubation times between 0 and 10 minutes were tested to determine the effect of elution buffer incubation. Two fragment sizes were analyzed. Increasing incubation time of the elution buffer increases DNA recovery. This is especially apparent with larger fragments.



Effect of incubation time with elution buffer.

- Extending incubation time has a positive effect.
- As shown in panel 3, large fragments are particularly challenging to recover and a longer incubation may be beneficial.

Summary: 6 factors for success

- Agarose quality and the age of the running buffer have no effect on DNA recovery rates.
- Using TAE instead of TBE buffer may increase the recovery rate.
- Small DNA fragments have higher recovery rates than larger fragments.
- Using a lower volume of Buffer QG does not negatively influence DNA recovery.

The recovery rate after gel extraction is **positively** influenced by:

- Reduced centrifugation speed during the binding step.
- Extension of the incubation time with elution buffer by up to 5 minutes.

Finally, to improve recovery of larger DNA fragments:

- Use the pre-optimized QIAquick gel extraction protocol — lower centrifugation speed and increased elution buffer incubation.

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