Food & Beverage by Eppendorf

November 27th, 2014

eppendorf

- **Eppendorf – A summary**
- Food & Beverage by Eppendorf
- Importance of consumables in food analysis
- Advantages of automation in food analysis

Eppendorf – A summary

Eppendorf - A Summary

- Founded in 1945 in Hamburg-Eppendorf by Dr. Heinrich Netheler and Dr. Hans Hinz as a workshop for medical instruments, - still today 100% owned by these two families
- The expert partner in life science laboratories, with the highest quality laboratory products and services
- Leading position globally in most market segments served
- 27 direct marketing/sales/service organizations (about 50% of 2850 employees)

Company Mission

Eppendorf shall be a synonym for customer-focused processes, innovative technologies, and premium products and services to improve human living conditions.

Product Milestones in the Company's History

1950	Spectral photometer
1955	Flame photometer
1958	Piston-stroke pipette; Patent in 1960
1962	Microliter centrifuge & first automatic analyzer
1978	Hand-held dispenser Multipette with Combitips
1985	Cell injector / Micromanipulator
1986	Sterile consumables for molecular biology
1997	PCR Cycler with gradient technology
1999	Electronic pipette
2003	Liquid Handling Workstations
2004	Realtime PCR
2007	Fermentors, Shakers, CO2-Incubators & Freezers
2012	Parallel bioreactors and software solutions
2013	Integrated single-use bioreactors

Customers - Life Science Laboratories

- Academic and biomedical research
- Pharmaceuticals, biotech, vaccines and diagnostics
- Agriculture, food, beverages, biofuels
- Healthcare
- Forensics and supervisory authorities

The Eppendorf Competence Areas

Eppendorf products and services simplify the handling of liquids, cells and samples and eliminate cumbersome laboratory work.

> Simply reliable to fulfill highest expectations, nothing less than the safest, most dependable and easy-to-use equipment,
> - to make the lab a better place in every respect

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Nourishing the world in the 21st century requires new ideas — from drought-tolerant crops with high protein yields to streamlined processes in food production and quality control. Nurturing ideas to grown-up solutions requires determination, passion and excellent tools. Eppendorf's tools for the laboratory have been among the finest and best for more than 60 years. Let us sort out the details of your daily lab challenges — so you have the peace of mind to focus on the science of the food of tomorrow.

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Food research
The amount of arable land is not growing with the world population. Thus, applied research has to show ways to increase nutrient levels in crops, optimize crop rotation or make crops more drought tolerant. The seasonal crop samples needed for this research are invaluable.

Food production
Downstream processing of food has to be optimized to minimize nutrient loss. Nutrient levels have to be tested before and after new processing steps. Heating, cooling, packaging—everything can have an influence on the quality of food and beverages.

Food analysis
Food analysis and food quality control require reproducible workflows—in every detail. Only the best lab products give you the confidence in the data you need. Your results can make a big difference.

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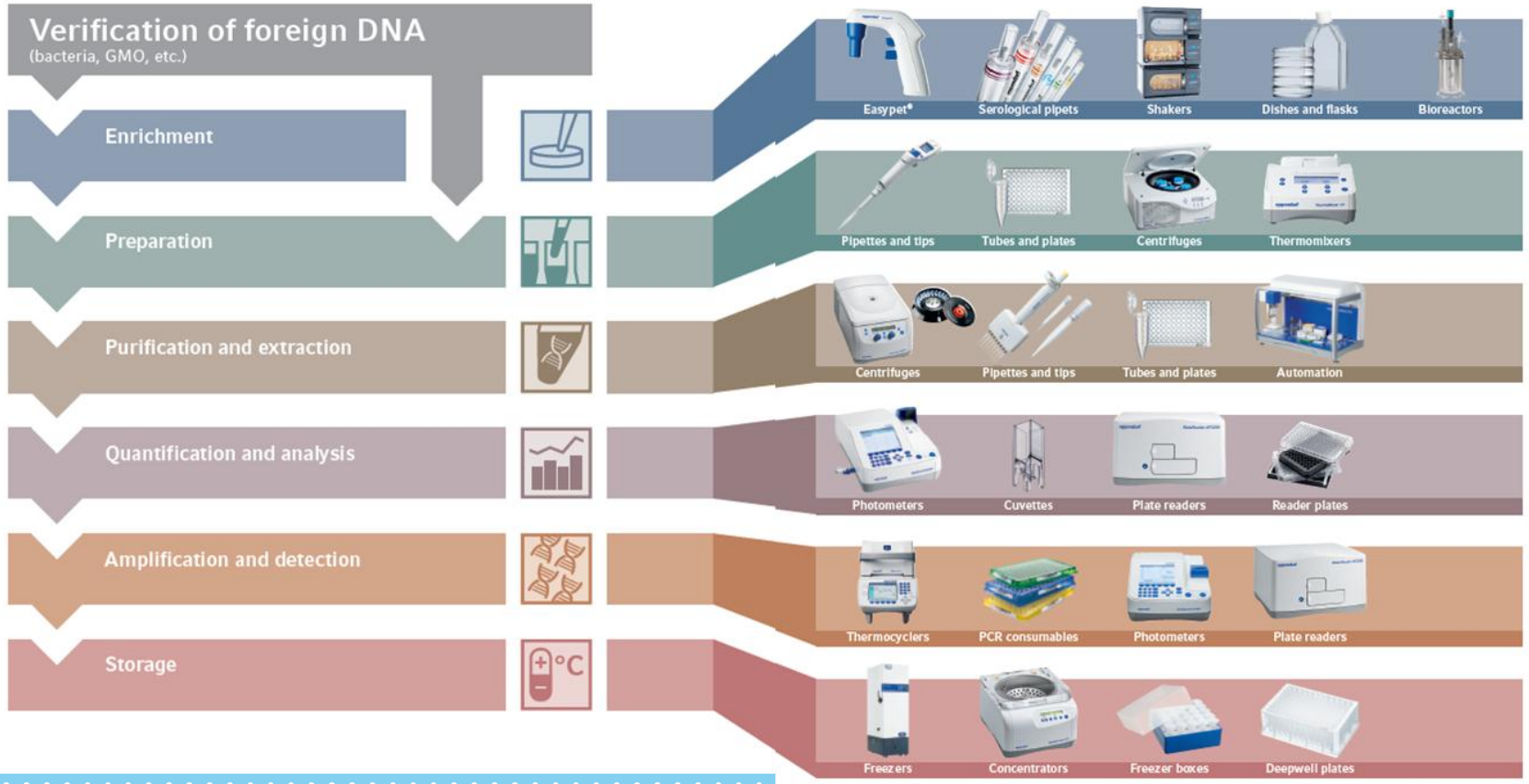
Example: Verification of foreign DNA



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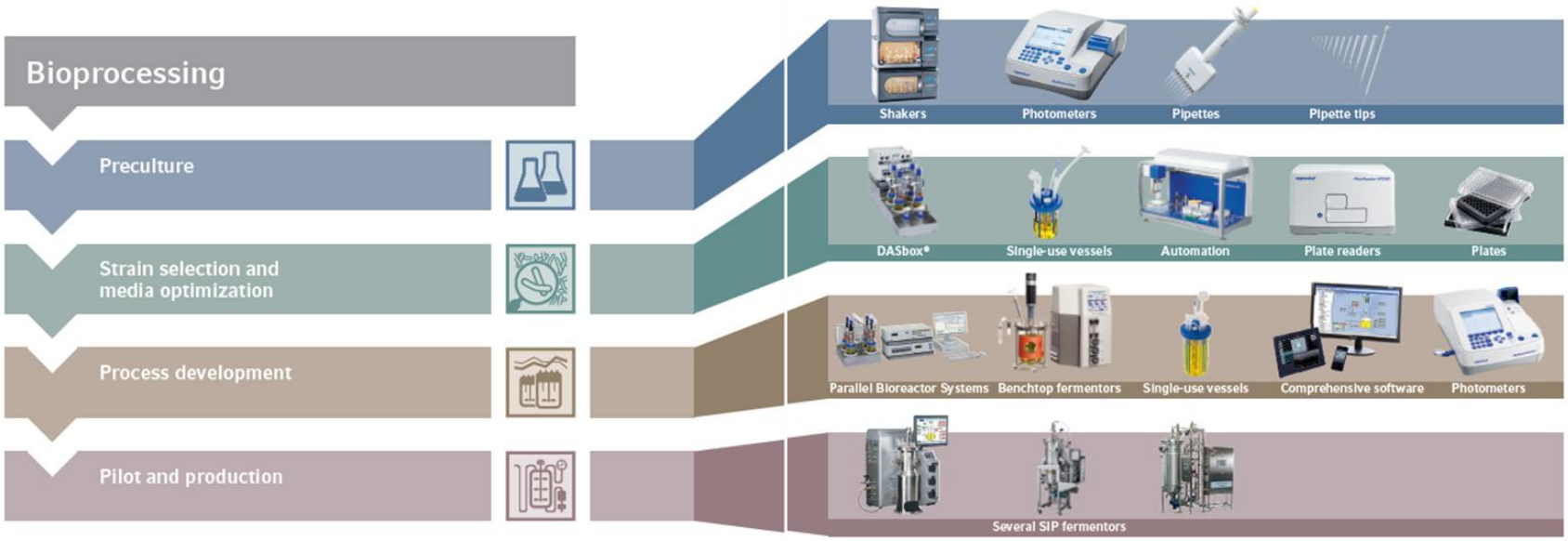
Example: Bioprocessing



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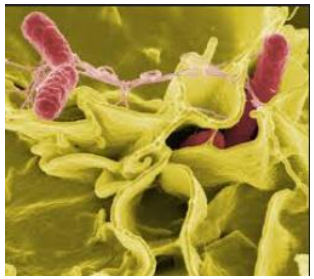
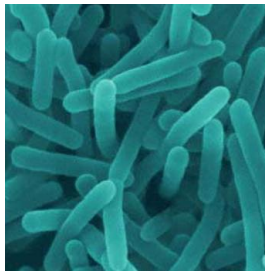
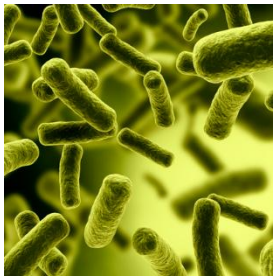
Importance of consumables in food analysis

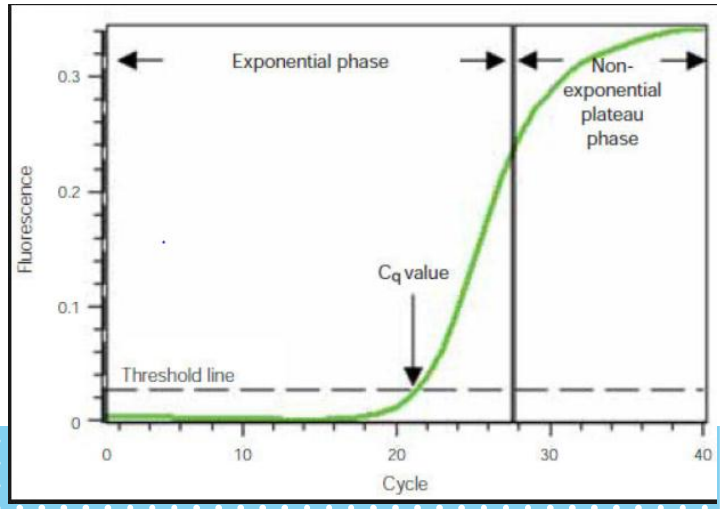
Importance of consumables in food analysis

- Food analysis and food quality control, including research in these areas, have been gaining relevance in the past years.
- The detection and identification methods of several parasites, bacteria, foreign substances, GMO, altering organisms, etc., have been developed for food & beverage applications in microbiological laboratories.
- These typical **real-time PCR** molecular assays are more sensible, quicker and easier to perform, with precise detection of the species and genotype.
- However, these assays are even more expensive than conventional PCR.









Importance of consumables in food analysis

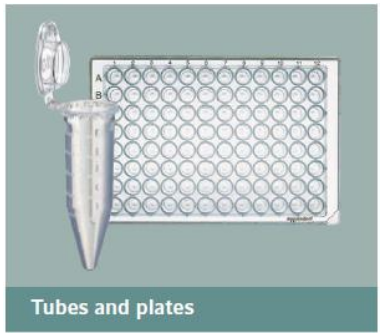
Real-time PCR savings

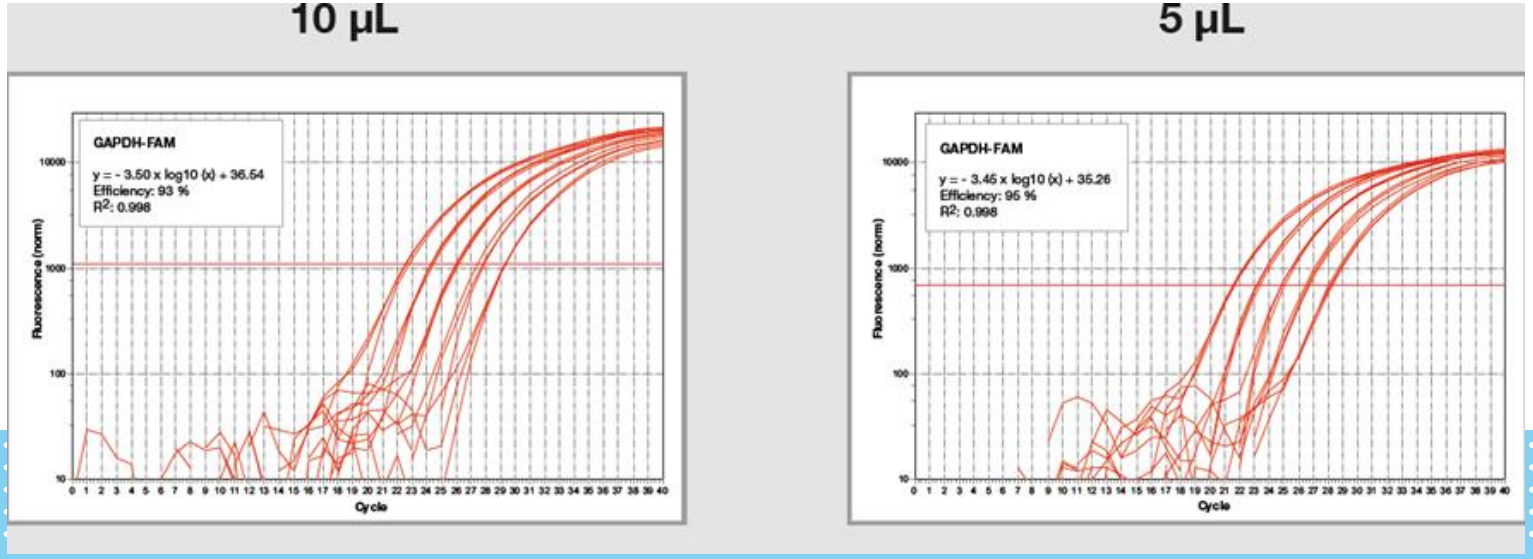
Reducing the reaction volume may considerably decrease the real-time PCR costs.

Volume reducing with plates:

> With white wells, for better signal reflection.

> With thin wall, for better sample-equipment thermal transparence.





Importance of consumables in food analysis

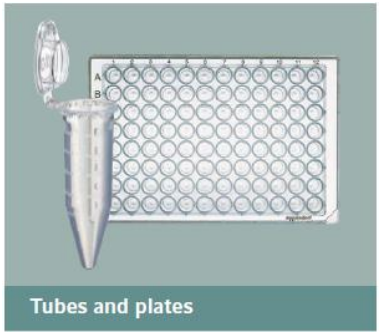
Real-time PCR savings

> The high costs of the required PCR real-time equipment are quickly amortized

> However, consumables and reagents, being countinuously used, require a bigger investment from the lab.

> Volume reduction would save samples and reagents.

Component	Clear or frosted wells 20 µL	White wells	
		10 µL	5 µL
twin.tec PCR Plate	4.20 €	-	-
twin.tec *real-time* PCR Plate	-	6.70 €	6.70 €
SYBR MasterMix	89.00 €	44.50 €	22.30 €
300 nM primer	1.00 €	0.50 €	0.25 €
costs / 96 reactions	94.20 €	51.70 €	29.25 €



Importance of consumables in food analysis

Centrifugation at 30,000 x *g* for obtaining precipitated plasmid DNA achieves higher recovering rates and shorter centrifugation times

Around 90% of the DNA could be recovered with a 5 min. centrifugation at 30.000 *g*

Example: fungi producing mycontoxins can be mainly found on cereals, seeds, fruits and food of which production is based on these materials. To analyze them, most part of their DNA must be recovered.

Importance of consumables in food analysis

Centrifugation at 30,000 x *g* for obtaining precipitated plasmid DNA achieves higher recovering rates and shorter centrifugation times

Plasmid precipitation with 1 v/v isopropanol

Plasmid 5 µg, add water to final volume of 300 µL
3 M sodium acetate, pH 5.2, 30 µL
Isopropanol 300 µL

Plasmid precipitation with 0.6 v/v isopropanol

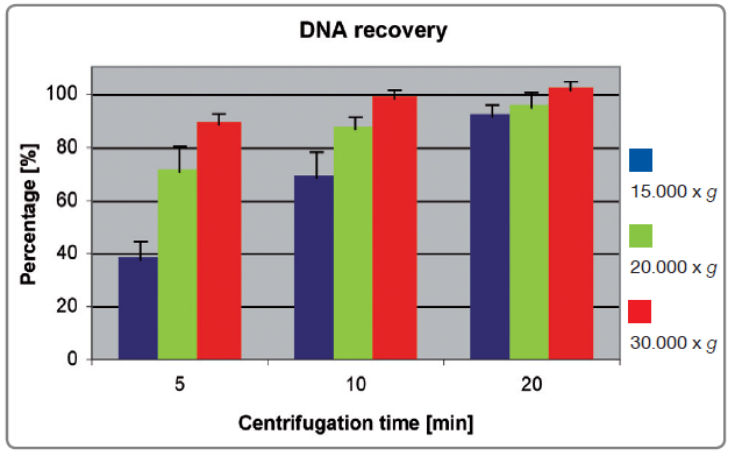
Plasmid 5 µg, add water to final volume of 300 µL
3 M sodium acetate, pH 5.2, 30 µL
Isopropanol 180 µL

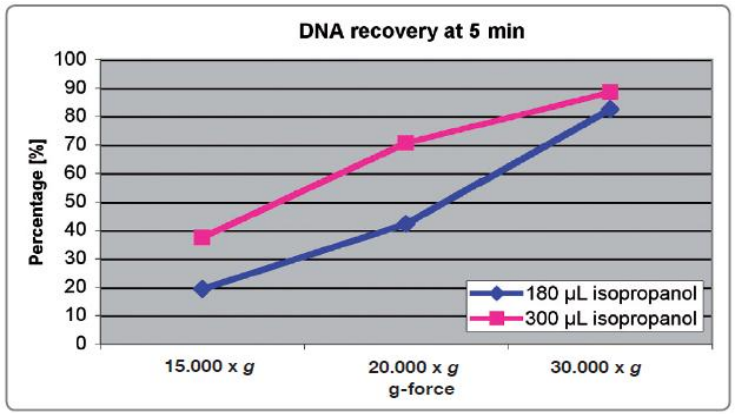
Centrifugation at 4 °C
g-forces: 15,000; 20,000; 30,000 x g
Duration: 5, 10, 20 min

Wash in 250 µL ethanol 70 %, followed by centrifugation as per previous step

Resuspension in 30 µL TE buffer, pH 8.0

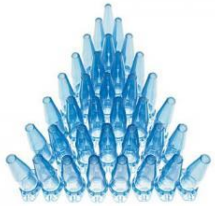
DNA yield measurement with BioPhotometer plus





Importance of consumables in food analysis

Why polypropylene?



Compared to other plastics, polypropylene (PP) has clear advantages:

- More stability and transparency than polyethylene (PE)
- Better chemical resistance and less biological molecule union than Polystyrene (PS) and polycarbonate (PC)
- Wider temperature range than polystyrene
- Comparatively, the low affinity of biological molecules, like nucleic acids and proteins, is especially relevant for molecular applications

	PP	PS	Relevant areas of application
Transparency	Medium	High	Transmission measurements
Temperature stability	ca. 120 °C	up to ca. 60 °C	Incubation, storage, autoclaving
Resistance to organic solvents	High	Low	Nucleic acid purification, protein analytic, compounds, assays
Mechanical strength	High	Low	Centrifugation, automation
Binding of biomolecules	Low	Low to high depending on the type	Applications using nucleic acids and proteins

Comparison of properties of the materials PP and PS

Importance of consumables in food analysis

Why is the manufacturing quality so important?

- The importance of consumables and leachables was underestimated by scientists in the past.
- The growing number of publications on how consumables **affect the experimental assays** are changing this perception, improving the importance of high quality consumables each time more.

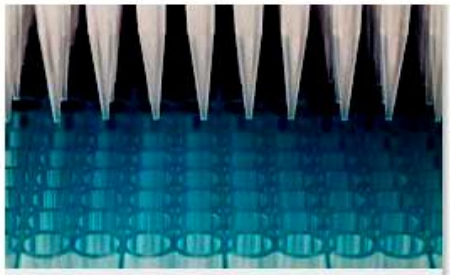
Published online 23 April 2010 | Nature | doi:10.1038/news.2010.200
Updated online: 26 April 2010

News

Plastics hamper DNA assays

Chemicals leaching from lab plastic throw off results.

Alla Katsnelson



Is it DNA or is it just chemicals leaching out of the tube?

dra_schwartz/iStockphoto

Biologists using standard plastic test tubes to gauge the concentration of DNA and proteins in their samples may be getting wildly incorrect readings because chemicals are leaching out of the containers.

An established way of assessing the concentrations and some key properties of DNA and proteins is to measure the

Published online 9 December 2008 | Nature | doi:10.1038/news.2008.1291

News

More biologists report plastic contamination

Chemicals from lab equipment are ruining experiments worldwide.

Daniel Cressey

Andrew Holt describes them as "horror stories".

Ever since Holt published a paper describing how standard pieces of plastic laboratory equipment leached experiment-ruining chemicals into his assays, researchers across the world have been in touch about their own experiences. And manufacturers are starting to take steps that could provide a workaround for the problem.

Reports of chemicals in plastic labware ruining experiments have come in from all over the world.

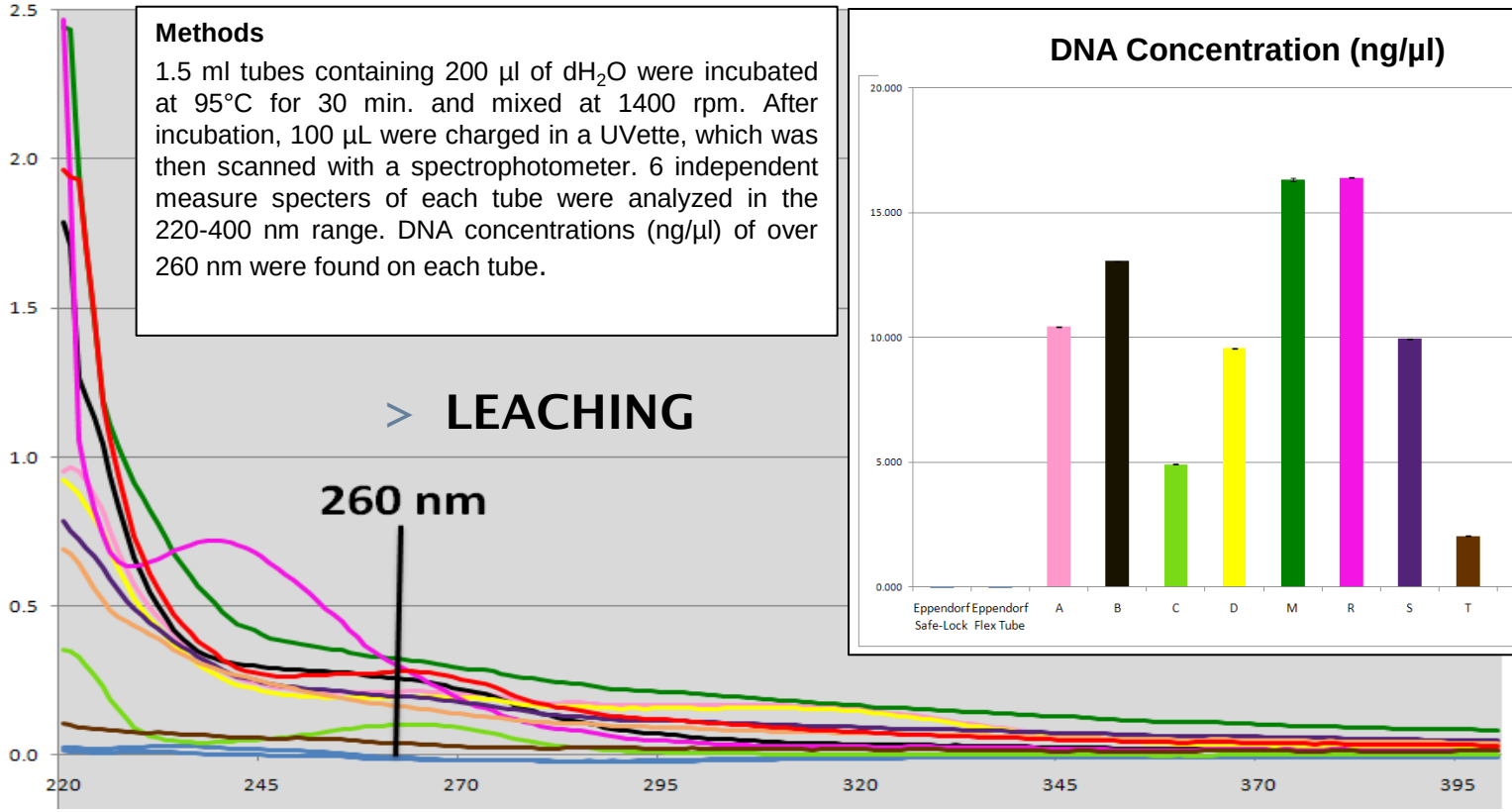
Photodisk

Importance of consumables in food analysis

Why is the manufacturing quality so important?

Methods

1.5 ml tubes containing 200 µl of dH_2O were incubated at 95°C for 30 min. and mixed at 1400 rpm. After incubation, 100 µL were charged in a UVette, which was then scanned with a spectrophotometer. 6 independent measure specters of each tube were analyzed in the 220-400 nm range. DNA concentrations (ng/µl) of over 260 nm were found on each tube.



Reference: Internally tested by Eppendorf North America

Importance of consumables in food analysis

Why is the manufacturing quality so important?

- Consumables must be made of ultra-pure PP, according to the FDA 21 CFR § 177.1520 “Olefin Polymers”, and 21 CFR § 178.2010 “Antioxidants and Stabilizers for Polymers” requirements.
- Additives, like plasticizers, slip agents or biocides must be excluded from the raw materials and manufacturing process of consumables
- Moreover, heavy metals or colorings containing these, must be avoided.
- Certificates to guarantee the quality of the plastics must be available.

Eppendorf Certificate

Certificate of Quality

Eppendorf Tubes®

All Eppendorf Tubes are manufactured in a controlled area and are subject to regular quality inspections within a statistical process control. Tests being performed are e.g. vapor tightness, lid-opening force, wall strength and centrifugal stability.
The Eppendorf Quality Management System is certified according to the international standards DIN EN ISO 9001:2008 and DIN EN ISO 13485:2007.
Eppendorf Tubes are made of virgin Polypropylene of highest purity and quality. Our material suppliers do not use or intentionally incorporate the following agents into the materials Eppendorf is using for the production of Tubes:

- Slip agents (including oleamide, erucamide, stearamide)
- Biocides (including di(2-hydroxyethyl)methyldodecylammonium salts (DiHEMDA))
- Plasticizers (phthalates)

During production of Eppendorf Tubes no slip agents or biocides are used. The used raw materials for Eppendorf Tubes comply with FDA regulations 21CFR § 177.1520 “Olefin Polymers” and 21CFR § 178.2010 “Antioxidants and Stabilizers for Polymers” for food containers. The products are free of substances from animal origin.

Hamburg, March 2010

Qualitätszertifikat/Certificate of Conformity

Eppendorf Safe-Lock Tubes; Typical values for trace metal

The values in the table indicate typical values of trace metal concentrations which are obtained after incubating Eppendorf Safe-Lock Tubes with conc. nitric acid for 1 hour (see: Materials and Methods backside).

As the indicated values were determined in a one-time measurement, they cannot be guaranteed for every lot of Eppendorf Safe-Lock Tubes. Rather, they give the customer an idea to what extent trace elements can be eluted from the Eppendorf Tubes.

Eppendorf Safe-Lock Tubes	Trace metal release [ng/µl]								
	Al	Cd	Cr	Cu	Hg	Mn	Ni	Pb	Zn
Safe-Lock Tube 0.5 ml	0.003	<0.00002	<0.00005	<0.0001	<0.001	<0.00005	<0.00005	<0.00005	<0.001
Safe-Lock Tube 1.5 ml	0.002	<0.00002	<0.00005	<0.0001	<0.001	<0.00005	<0.00005	<0.00005	<0.001
Safe-Lock Tube 2.0 ml	0.002	<0.00002	<0.00005	<0.0001	<0.001	<0.00005	<0.00005	<0.00005	<0.001
Safe-Lock Tube Protein LoBind 0.5 ml	0.006	<0.00002	<0.00005	<0.0001	<0.001	<0.00005	<0.00005	<0.00005	<0.001
Safe-Lock Tube Protein LoBind 1.5 ml	0.004	<0.00002	<0.00005	<0.0001	<0.001	<0.00005	<0.00005	<0.00005	<0.001
Safe-Lock Tube									

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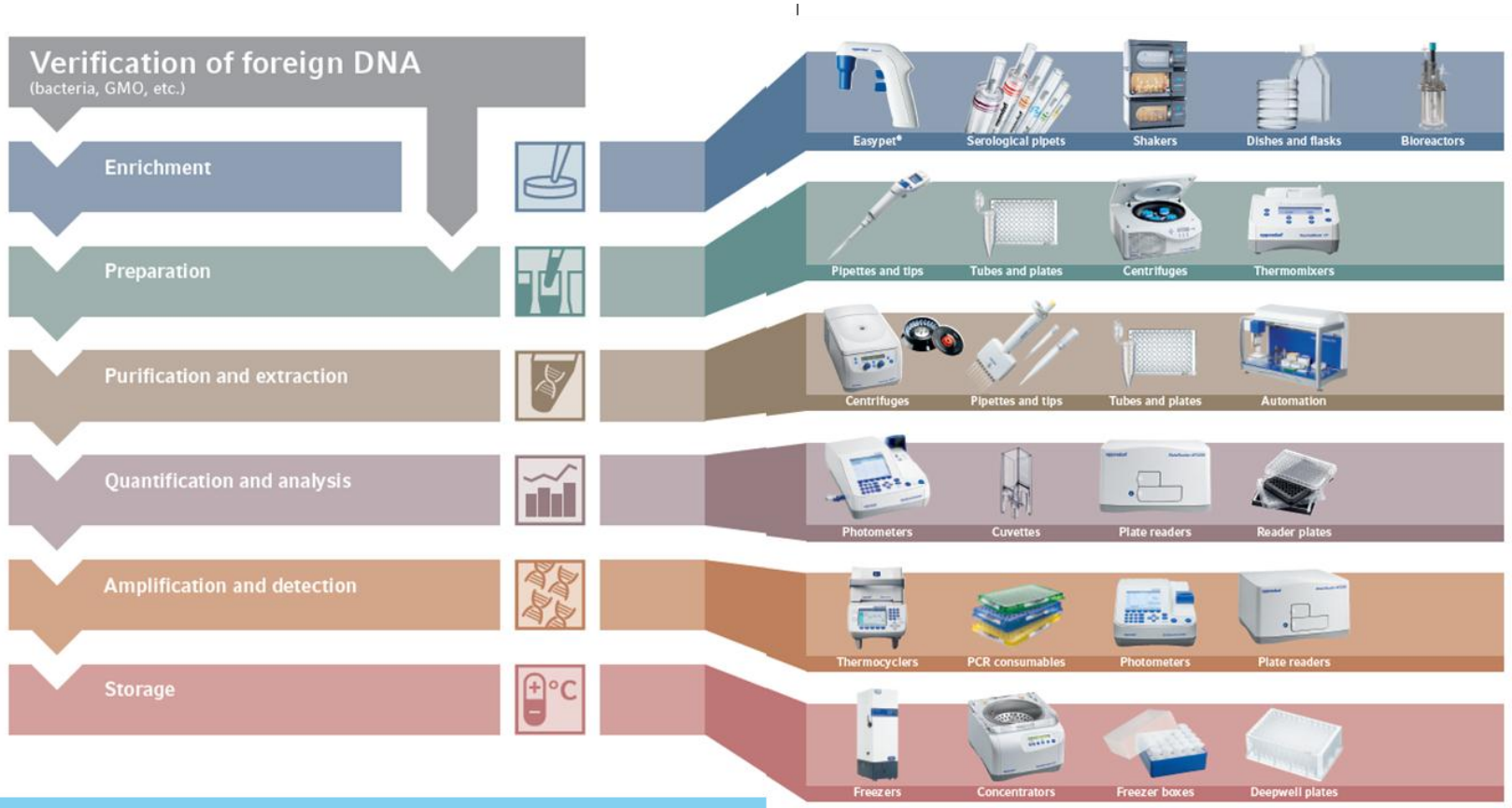
Advantages of automation in food analysis

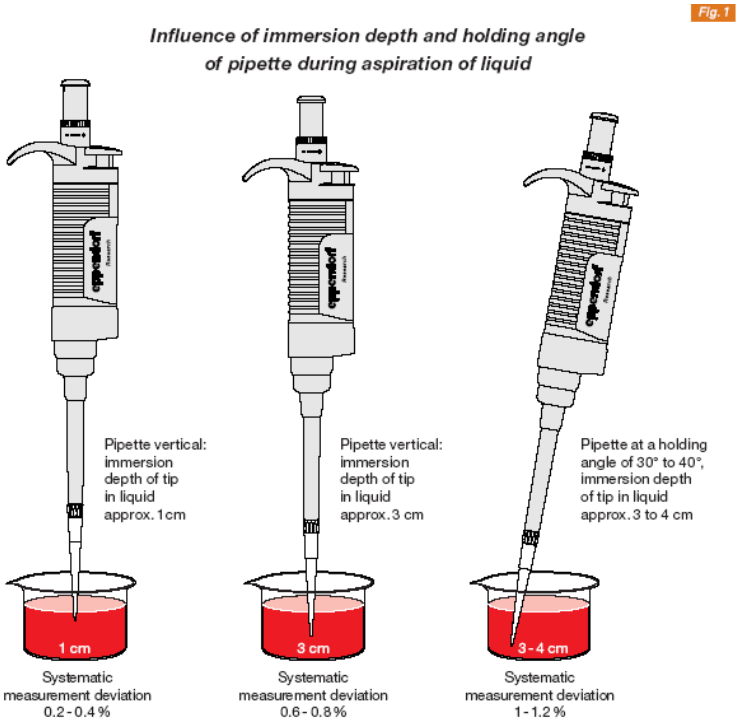
Example: Verification of Foreign DNA (bacteria, GMO, etc.)

> Food analysis and food quality control require precise and reproducible workflows



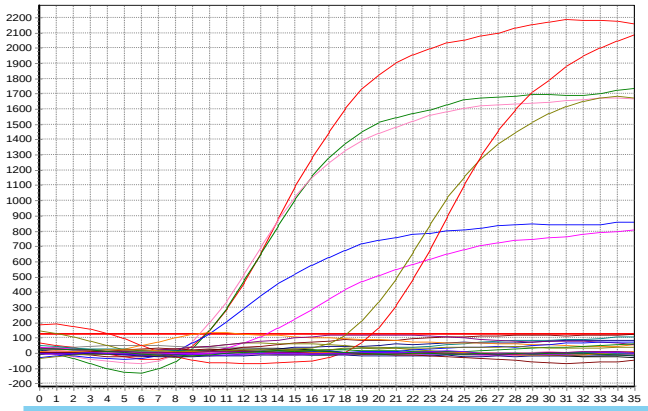
Enrichment and preparation

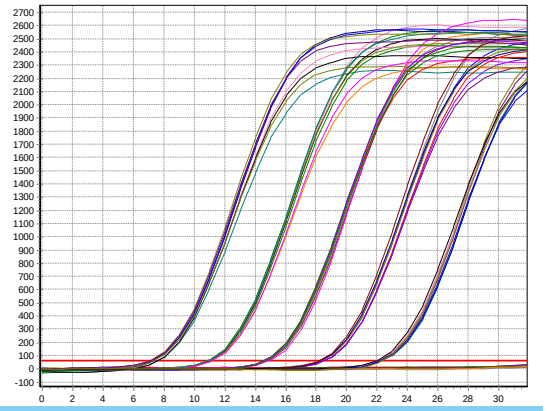
- The reproducibility of your results is improved by using automated systems
- Dispensed volumes are affected by:
  - Pipette immersion depth
  - Immersion angle
  - Temperature variations

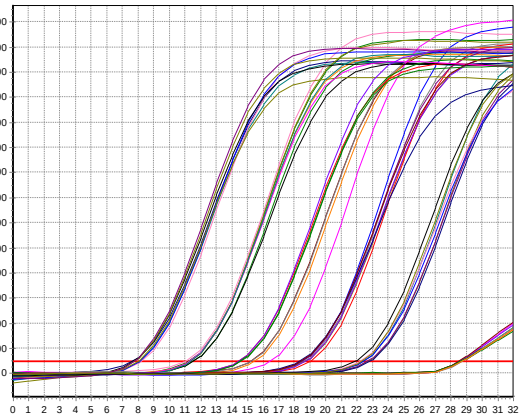


Enrichment and preparation

> Same assay, three different people, same pipettes and reagent, but heterogeneous results

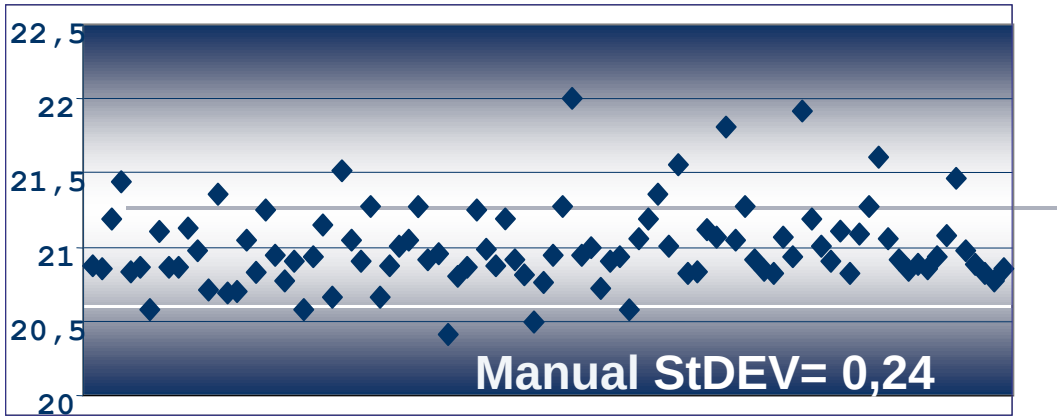


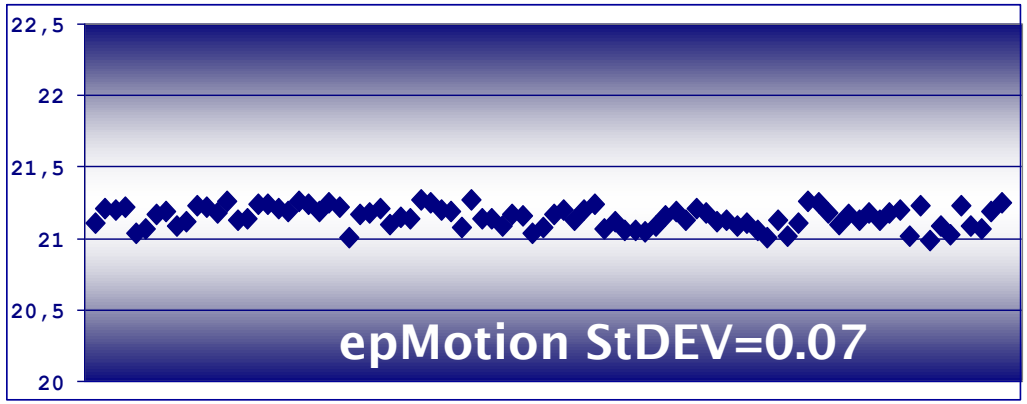




Enrichment and preparation

> Reproducibility: Machine vs. Man





Enrichment and preparation

> Possible alternatives to manual pipettes:

> Electronical pipettes

> Plate dispensers

> Liquid handling automated systems

Enrichment and preparation

What are the advantages of automating certain protocols?

> Less contamination risks

- Autoclavable tools

- Optical probe to detect the liquid levels

- Closed protective screen

> Time to perform other tasks

> Smaller volume handling

> More reproducibility and homogeneous results

Enrichment and preparation

What are the advantages of automating certain systems?

- **Better reproducibility and homogenous results**
- **Accuracy and precision**

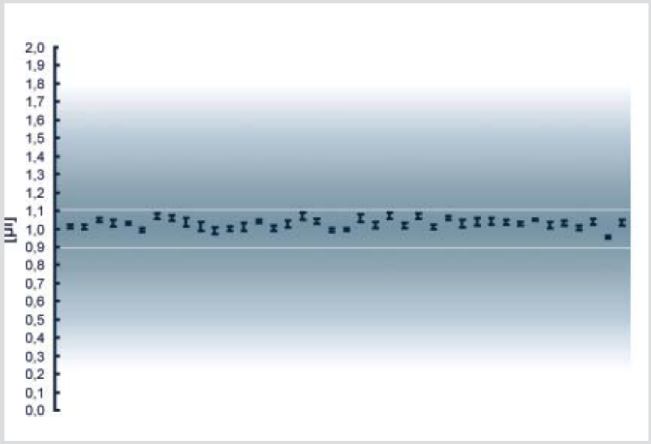


Fig. 2: Accuracy and precision of the epMotion single-channel 50 µl tools. Forty tools from different production lots were tested gravimetrically. Each point represents mean value of 10 single measured values for the given tool (in µl) and the error bars represent their precision. Border values for accuracy (+/- 10%) are indicated. Mean value is 1.025 µl. 82% of the tools have the accuracy of +/- 5%.

Enrichment and preparation

What are the advantages of automating certain systems?

> **No risk of cross-contamination**

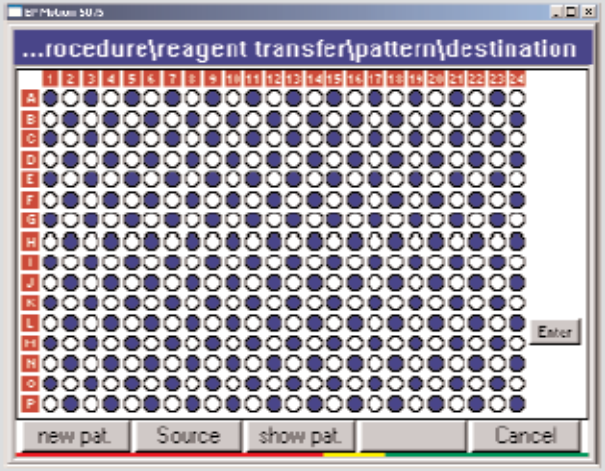


Fig. 2: 384-well Eppendorf twin.tec PCR plate filled in a chess board-like pattern

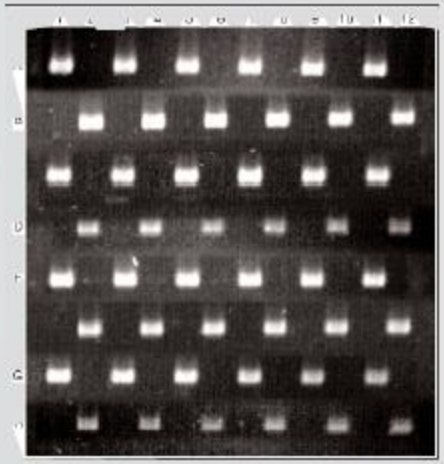


Fig. 3: Amplification of the human ß-globin gene across one fourth of a 384-well PCR plate [2].

Purification and extraction

- DNA must be extracted and purified before analysis. This is a crucial step. If the DNA is lost or forms a slant in certain regions, the results can be no longer amended.
- Moreover, PCR inhibitors must be eliminated to guarantee the amplification of the target DNA and prevent false negative results.
- All these risks may also decrease with automation

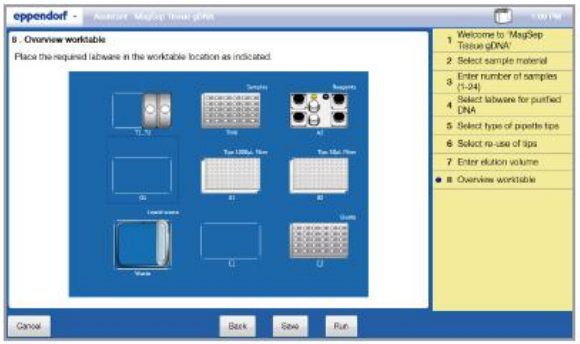


Figure 2: Screenshot from the ep*Motion* Prep-Assistant showing the setup of the ep*Motion* M5073 worktable for the use with the Eppendorf MagSep Tissue gDNA Kit.

Purification and extraction

- Obtaining high quality samples

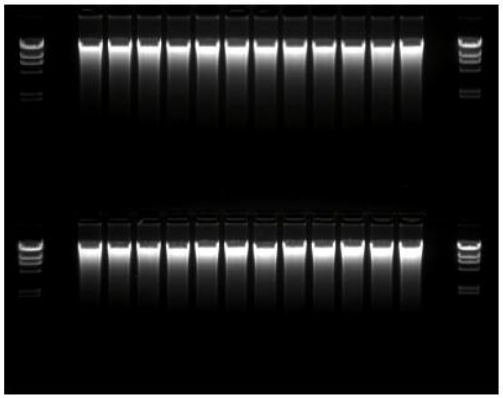


Figure 4b: High quality genomic DNA
On a 1 % agarose gel 7.5 µL out of 75 µL mouse tail gDNA eluates were separated electrophoretically. The obtained DNA displayed a high molecular weight as indicated by the absence of low molecular weight smear. DNA size standard: Lambda *HindIII* (Fermentas).

- Reproducibility

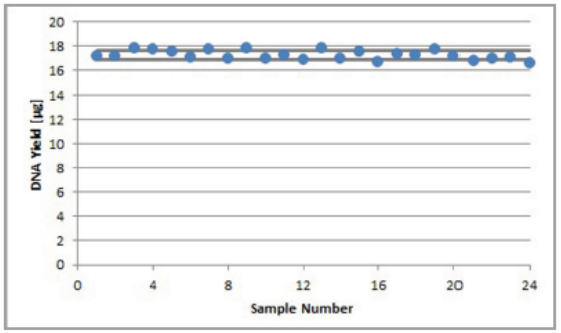


Figure 4a: Reproducibility of DNA purification
Genomic DNA was isolated from 24 mouse tail samples, 10 mg each. The average yield was 17.3 µg gDNA with a CV value of 2.09 %, giving evidence for a high level of consistency over 24 replicates. Each symbol represents the DNA yield of one sample; grey bars denote the average yield +/- 1 SD.

Purification and extraction

- High performance

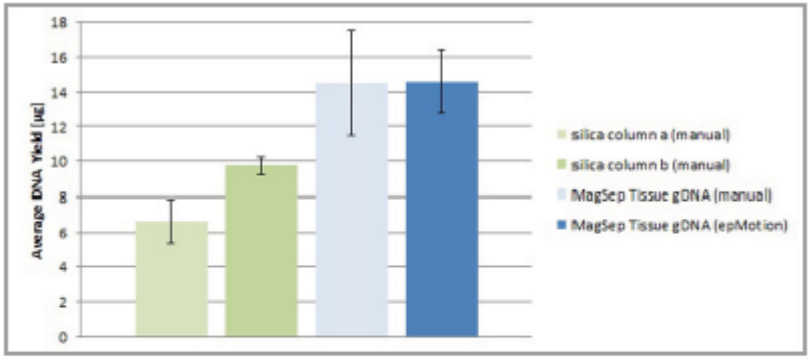


Figure 5: DNA yields achieved with different methods
Genomic DNA was isolated with different methods from 10 mg mouse tail samples. For each method 6 samples were processed in parallel. Other supplier's methods a and b were based on silica column technology. DNA yields obtained by the magnetic bead based manual and automated methods were considerably higher than the yields from silica column based methods.

- No risk of cross-contamination

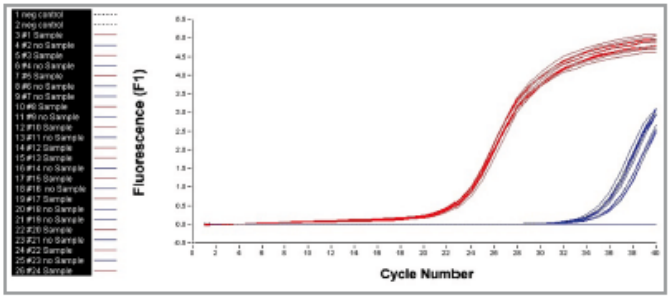


Figure 6: No cross contamination detectable
5 mg tissue samples were processed in a checkerboard pattern along with PBS, re-using the tips. The final eluates were diluted 1:10 and used in a real-time PCR to amplify a 212 bp fragment. Specific amplification was only detectable for positions with tissue lysates (red); from PBS filled positions no specific amplification was obtained (blue). Real-time PCR was performed on a Roche LightCycler system.

Thank you very much for your attention

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