BECKMAN COULTER

Rotor

The
Life Science Magazine for
Genomics, Cellomics, Proteomics, Automation and Centrifugation

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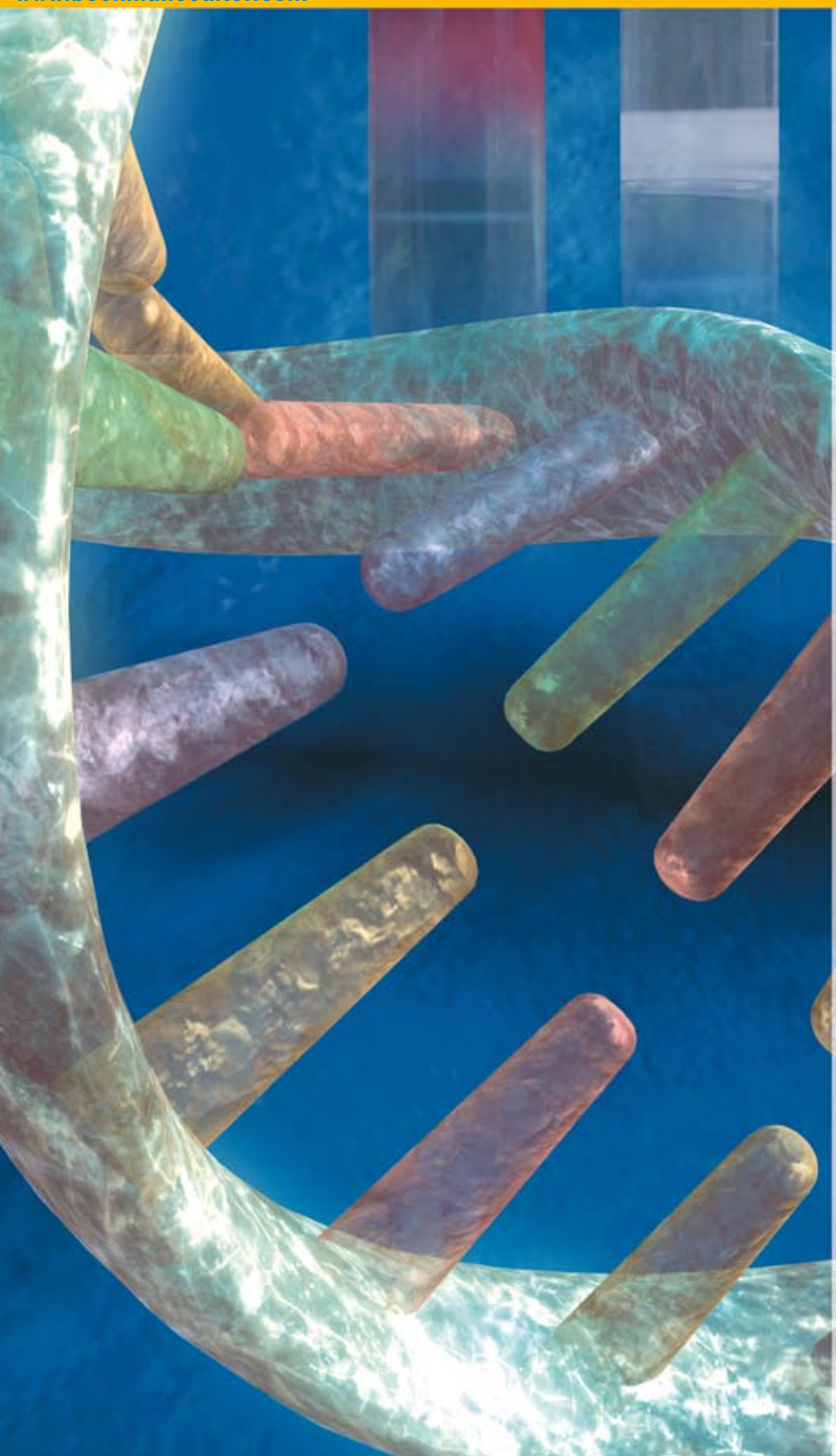
Issue 3 / April 2006



New Trends in Centrifugation

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New Trends in Centrifugation

In recent years, Beckman Coulter has made a series of developments in the field of centrifuges. This has resulted in a large number of new products at all product levels – from labware and rotors to the current centrifuges. New labware and new rotors benefit all existing customers, since Beckman Coulter places great value on continuity. Developments in this field rapidly show up in "the market".
It is a different matter where new centrifuges are concerned – these only become a focus for our customers at specific times, since they are generally acquired only for new facilities or to replace old equipment. Even though not every new development leads to a new "world of centrifuges", a new generation of centrifuges has established itself in recent years, little by little. This brings new options of which we would like to provide an overview by giving some examples.

The classic solution:

We would like to compare the "Classic Four" – the four centrifuges which, thus far, were our guarantee for covering all possible applications within a laboratory:

Until now, all "Classic Four" centrifuges were actually required: The „High-Speed" simply could not hold 6-litre volumes. This required a centrifuge such as the J6 (Beckman Coulter) or RC 3 (Sorvall).

However, these centrifuges were unable to reach the required relative centrifugal force for subcellular separation - so one needed a „High-Speed", such as the J2-21, J2-Series (both Beckman Coulter) or RC 2 or RC 5 (both Sorvall).

The forces required for the frequently occurring classic s-100 preparations, separations at more from 100000 x g, could only be produced with an ultracentrifuge.

And this absolutely required a preparative and a tabletop ultracentrifuge, since the „Tabletop Ultra" did not cover the field of large volumes. The preparative (floorstanding) ultracentrifuge is very inconvenient for small samples, due to the rotor size and the handling of adapters.

In the further text, we will introduce new solutions and trends which formed in past years on the basis of new and further developments.

Current trends:

1. Centrifugation of large volumes:

This class includes two trends with respective new developments: on the one hand, industrial centrifuges with larger rotor capacities (such as the Avanti J-HC with 9 liter rotor volume). And there is an alternative trend towards optimisation of through-put – for which there is now a shift from swinging bucket rotors to fixed angle rotors.

This becomes important when one must, for instance, routinely "harvest" a fermenter. Beckman Coulter's new high-performance fixed angle rotor JLA-8.1000 with its 6-litre capacity carries out separations 5x as fast as the classic swinging bucket rotors – an increase in throughput which no one would previously have thought possible.

However, it is also interesting that in these centrifuges, the classic solutions remain in demand. Low performance did not have to be redefined!

A modern High-Performance centrifuge such as the Avanti J-26XP can handle large-volume separations – but it can also provide a performance gain with a factor of 5! (see Fig. 1)

Using the same unit, the 8 x 50ml rotors (the successors of the classic JA-20 or SS-34, both 20000 rpm rotors) are spun at 26000 rpm today.

Separations go twice as quickly as they used to, and the possible field of gravity is clearly higher, at 82000 x g, than in the classic „High-Speeds" (50000 x g).

2. High Performance Centrifugation:

This brings us to the successors of the High-Speed centrifuges, which we call High-Performance centrifuges today.

The performance limits of the units in themselves have been expanded to higher volumes as well as greater g-forces:
In centrifuges of this class, the above named high-performance fixed angle rotor for a total volume of 6 litres is also functional!

The classic four

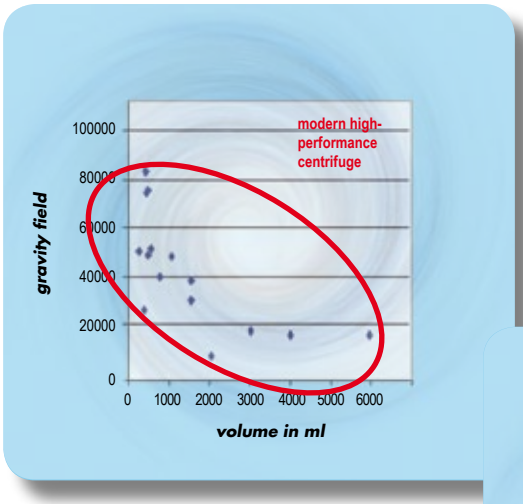
1 **The high-volume centrifuge**
with 6 x 1000ml swinging bucket rotor for cell harvest (forces approx. 5000 x g)
2 **a High-Speed centrifuge**
mainly used with fixed angle rotors of 6 x 500ml to 8 x 50ml for precipitation and preparation of cell- fragments at up to 50000 x g
3 **a preparative ultracentrifuge**
for the preparation of anything smaller than cell membranes. Container sizes are approx. 250ml ranging down to approx. 5ml
4 **a Table-Top ultracentrifuge**
for the low-volume samples from 0.1 ml to 5 ml in the fixed angle rotor and from 0.1 ml to 2ml in the swinging bucket rotor.

New applications become possible: With the 82000 x g, it is still not possible to do a s-100 preparation – but it might already make sense to try the separation.

With a bit of luck, individual separation can also be reached at 82000 x g – of course with a correspondingly longer centrifugation time.

With three rotors, one additionally reaches more than 100000 x g. And this both in pelleting and in gradient separation.

Aside from a fixed angle rotor for 8 x 50ml, there are two swinging bucket rotors - the JS-24.15 and the JS-24.38 – whose volumes and geometry correspond to the preparative ultracentrifuge rotors SW 28 and SW 28.1.



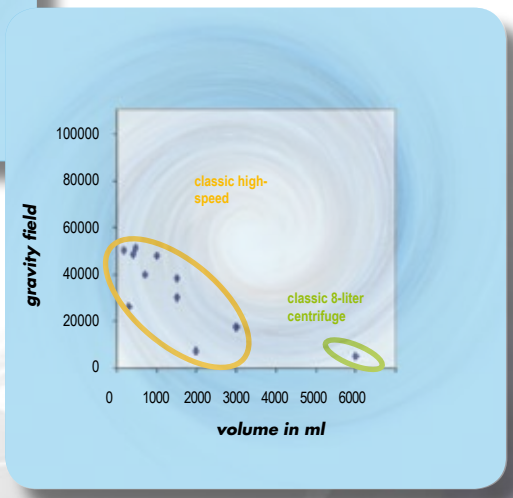


Fig. 1
The performance development of the new High-Performance centrifuges becomes clear when the „g" force and the capacity are shown side by side:
on the left, the "spectrum" of the Allspin-Avanti-26XP, and below, that of the No. 1 and Number 2 of the Classic Four

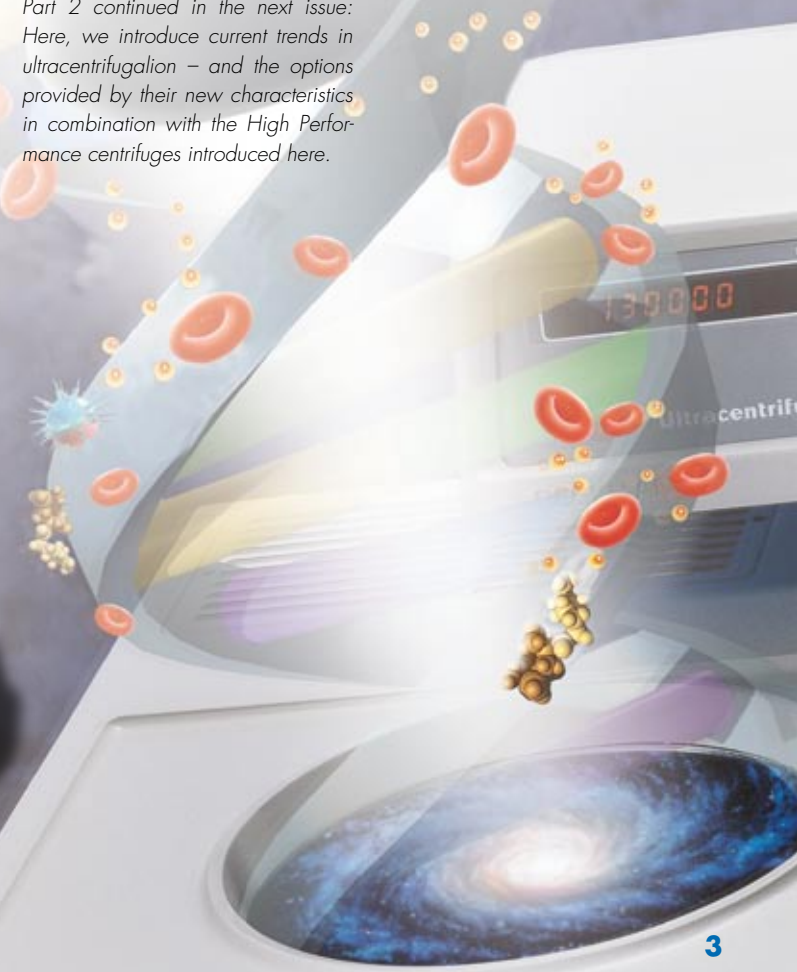
Not to mention the higher „g"-forces and greater volumes. The new Avanti J centrifuges save large amounts of time in all applications with rapid rotor acceleration and deceleration times.

This clearly improves the performance (work vs. time) by comparison to the classic centrifuges.

Also new in this class of centrifuges. **BioSafe centrifuges.**
Aside from biocertified rotors and corresponding labware (see Rotor I), HEPA filters are also used here now, holding back potentially hazardous substances from aerosols.

Therefore, these centrifuges are particularly suitable for use in laboratories with increased safety requirements.

Part 2 continued in the next issue: Here, we introduce current trends in ultracentrifugalion – and the options provided by their new characteristics in combination with the High Performance centrifuges introduced here.



Short protocol on cleansing of adenoviral vectors by means of CsCl gradient centrifugation

Introduction

Adenoviral vectors are efficient gene transfer vehicles in basic research and gene therapy. Derived from human Group C adenoviruses, vectors are today mainly constructed on the basis of serotype 5. These infect numerous cell types in various species.Commonly used so-called first generation vectors provide space for approx. 8 kilobases of foreign DNA. DNA segments crucial for virus replication deleted from the vectors are trans-complemented by production cell lines such asHEK293 or 911 and permit production of several thousand virus particles per infected cell. This leads to preparations with high vector titers (on a laboratory scale, up to 10^{12} particles/ml). The established protocols for purification vectors are largely based on lysis of the infected cells, release of virus particles from the cell nuclei, and separation from contaminants with CsCl density gradient centrifugation. In classic protocols, two CsCl gradient runs are required here. The first is a zonal step gradient, and the second is with commonly is a twenty-hour equilibrium centrifugation in an isopyknic gradient. These long preparation times may negatively affect the titer of infectious viruses. This article will therefore introduce a procedure which permits vector purification applying two 2-hour zonal step CsCl gradient centrifugations.

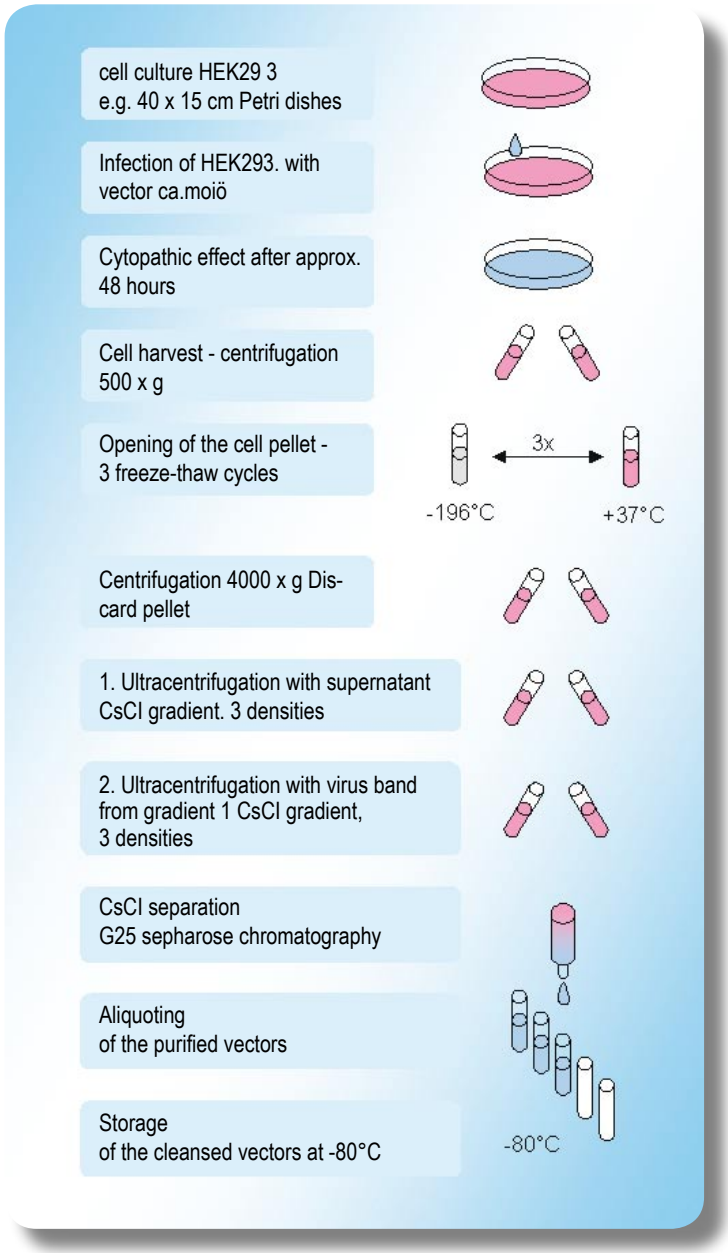


Illustration 1: Flow schema of adenovirus preparation

Procedure

For preparation, HEK293 cells are infected into a culture area of approx. 6000 cm^2 at approx. 80% confluence with the virus stock at a moi of 5. Under these conditions, a marked cytopathic effect becomes visible by rounding of the cells after approx. 48 hours. The cells are then detached by flushing with the medium, and the resulting suspension is centrifuged for 15 minutes at 500 x g and 4°C. The cell pellets are used for further purification, pooled and divided into 6 aliquots. These are resuspended with approx. 5 ml culture medium and frozen in three rounds of freeze-thaw lysis (alternately frozen in liquid nitrogen and thawed in a 37°C water bath) to release viral vector particles from cell nuclei.

Cell fragments are then separated at maximum rotation speed of the 50 ml tubes (approx. 4000 x g) and 4°C, and the supernatant is applied to the following CsCl step gradients: The gradient is built up by means of successive sub-layering of the three solutions in Table 1 in clear SW41 tubes (order no.: 344 059).

On these gradients, one layer of up to 6 ml of the clear cell lysate. Centrifugation in the SW41 rotor for 2 hours at 35.000 rpm and 10°C results in two clearly visible poalescent whitish bands, of which the upper represents empty adenovirus capsids and the lower the DNA-containing adenoviral vector particles.

With a 1000 µl pipette with filter tip, the gradient is successively removed to just above the bands of the empty capsids. With an insulin syringe with a 27 gauge needle, the lower band is picked up in a maximum volume of 1 ml gently driving the needle through the band.

The material of all 6 gradients of a rotor is combined and the second stage gradient is prepared. Up to 1.5 ml material of the first band is now combined with solution 2 (from table 1) to a total volume of 6 ml, and filled up with 6 ml of solution 3 (= 12 ml), and distributed into two SW41 tubes.

CsCl solutions, volumes and densities

Solution	Volume [ml]	CsCl density [g/cm³]
1	1.0	1.2411
2	2.0	1.3393
3	2.0	1.4525

Table 1

SW 41 Ti

6 x 13.2 ml

Separation now takes place for 2 hours at 30 000 rpm and 10 °C. After the run, generally one band has formed by the cleaned DNA-containing adenoviral vector particles, which is collected in a volume of 1ml as in the afore gradient.

No lengthy dialysis is done here to remove the CsCl. Adenoviruses for all *in vitro* and *in vivo* applications are obtained by gel filtration chromatography in a free-flow G25 sepharosis column (such as the NAP25 column, # 17-0852-02, Co. Amersham Biosciences via GE Healthcare).
In this system, the columns are initially brought to equilibrium in adenovirus storage buffer (Ad-L buffer: 10 mM Tris-HCl pH 8.0, 135 mM NaCl, 3 mM KCl, 1 mM MgCl, 10 % [v/v] glycerine, autoclaved).

To the equilibrated column, the collected 1 ml adenovirus band of the second gradient is added. The column is then rinsed with 1.5 ml Ad-L buffer.

The flow-through to this point can be discarded. By the addition of another 2 ml Ad-L buffer, the adenovirus is now eluated, and immediately placed on ice.

This eluate is then aliquoted and stored at -80°C until use.

Aliquot sizes between 10 and 500 µl should be provided, depending on the titre of the preparation and the type of use.

The titre determination of the adenoviruses is carried out after one-time freezing. Further information about storage and stability of adenoviruses is described in Croyle et al (1).

After dilution in the Ad-L buffer to the desired dosage, the adenoviruses prepared in this manner can be used for infections in cell culture, but also in animal experiments for intraperitoneal, intravenous and intratumoral injections (2).

During and after work, it must be noted that work materials and solutions which were contaminated with adenoviral vectors must be decontaminated in accordance with the supplementary regulations and inactivated by autoclaving.

Summary:

The introduced protocol of gentle adenoviral vector purification combines the efficiency of CsCl gradient ultracentrifugation with minimal time requirements resulting in pure adenoviral vector preparations for a broad spectrum of *in vitro* and *in vivo* applications.

Dr. Frank Schnieders

Gene Therapy Group –
Institute for Biochemistry and Molecular Biology II:
Molecular Cell Biology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Gradient 1: The lowest band is formed by DNA containing adenoviral particles. Above it, in the weaker band, there are smaller particles of empty particles, and a diffuse area of cell fragments – coloured red by the culture medium – as well as genomic DNA.

Gradient 2: After this cleansing stage, only a single band of DNA-containing adenoviral particles is still present.

Quoted literature:

(1) Croyle et al., Development of formulations that enhance physical stability ol viral vectors for gene therapy. Gene Therapy 8: 1280-1291, 2001

(2) Waehler R, Ulrich, H, Mueller, L, Krupski G, Ameis, D., Schnieders F. Low-dose adenoviral immunotherapy o! rat hepatocellular Carcinoma using Single chain interleukin-12. Human Gene Therapy 16(3): 307-317,2005.

What to do about moisture in centrifuges ?

Moisture in the rotor chamber of centrifuges must be fundamentally avoided. It can damage mobile parts of the centrifuge (such as the drive and its bearings), slow processes such as suctioning a vacuum, necessitate maintenance (such as an exchange of vacuum oil when moisture has entered it).

There are two possible sources of such undesired moisture:
• Spilled sample, and moisture which has condensed in the rotor chamber.

If the centrifuge is cooled, moisture will always condense from the warm room air on the cold interior wall of the rotor chamber. The condensate will then either flow to the bottom of the rotor chamber or initially freezes onto the rotor chamber wall - and then later flows down when it thaws.
In the following paragraphs, we would like to give you hints and suggestions about what to pay attention to in order to avoid moisture…

If there is moisture in the unit =>

….. it must be removed before it penetrates into areas where it will cause damage!

Sounds simple – and it actually is. Unfortunately, it has been shown in practice that it might not be that simple after all to check for moisture in the chamber. For this reason, our recommendations begin with a few common-sense things:
the rotor should be taken out before every run to check whether there is condensation in the rotor chamber. (water on the rotor chamber floor)
- this is also a good opportunity to look into the rotor holes – water often collects here and either alone or perhaps with sample buffer will begin to corrode the rotor.
- if moisture is present, one should simply remove it with an absorbent soft cloth!
- when the chamber has been wiped dry, one can briefly allow the chamber to air dry while open, with the cooling shut off. While the cooling is switched ON, the rotor chamber should be kept closed - see below.

By the way:
The High-Performance centrifuges of Beckman Coulter continuously pump rotor chamber air to the outside while running.

This helps in the automatic removal of small to moderate quantities of moisture – even though the original purpose is to reduce air pressure in the rotor chamber, and therefore, a reduction in friction heat.

VAC error report – what to do?

If there is moisture in the chamber of an ultracentrifuge, this is continuously removed via the vacuum system. Unlike the High-Performance centrifuges, this is done by an oil diffusion pump. In that case, the moisture first enters the vacuum pump oil, worsening the performance of the vacuum system. This may cause a VAC error report – a run is unable to start, nothing works!

It used to be necessary to change the vacuum oil to correct the problem. Today, with Beckman-Couter's „Ultras", the patented moisture-eliminating vacuum system helps. It is built into all Optima centrifuges – that is, both into the preparative and into the table ultracentrifuges. The oil is heated here to remove the moisture – not all at once, but continuously, over time. If there is a problem, the vacuum system should simply be allowed to run for several hours or even overnight. Then the problem should have rectified itself – so that no service will be needed.

Important:
When a VAC error report has occurred, it must be reset by pressing the CE key before starting a new centrifugation run.

It is always best to avoid moisture in the rotor chamber.

If the moisture is coming from leaky samples, this is simple: Enclose them securely! Here, the selection of the correct labware and its proper handling are important:
- The seals of the centrifugation container and the rotor should be checked regularly.
- The gaskets and the surfaces against which they close must be cleaned regularly (recommendation: after every run!) so that no dirt can collect (such as salts from buffers).
- The rotor gaskets should always be thinly lubricated with vacuum grease.
- Gaskets and the surfaces against which they seal should be dry prior to closing the containers – otherwise, one will have moisture bridges from the start, allowing the sample to wander outside.
- The sample containers, their covers and the gaskets should be checked for cracks, deformations etc. before each run – if applicable, defective parts should be replaced.

Condensing moisture from the room air can be avoided by:
- setting up the unit in a suitable location (e.g. in an air conditioned room)
- only cooling the centrifuge after the rotor chamber cover has been closed
- briefly heating the centrifuge prior to opening or if there is already ice on the rotor chamber wall. The current series, the Optima L-XP series from Beckman Coulter, has a „Precool/heat" function for this purpose. One can obtain the same results with an older ultracentrifuge by turning on the vacuum while selecting a high temperature, such as 25°C.
- The rotor chamber should be kept closed between centrifuge „runs" to keep the rotor chamber dry (and clean). (attention – do not close if there is already moisture in the chamber. See above.)

MLA-55 – a new rotor expands the volume range of the table ultracentrifuges

With a new rotor, Beckman Coulter expands the fields of application of table centrifuges in the Optima series.

The rotor – its name is MLA-55 – provides room for 8 centrifuge containers with 13.5 ml.

These can be centrifuged at 55000 rpm with up to 290000 x g. The rotor is ideally suited to the isolation of subcellular structures and has a wide range of applications.

The rotor utilises the labware of the widespread preparative ultracentrifuge rotors 90Ti, 80Ti, 70.1 Ti (and their predecessors, such as Type 65), which has significant advantages for the user. This labware includes various containers and closure systems, such as the Quick-Seal,

the Opti-Seal, screw-closure and open centrifuge tubes, as well as various adapters and the time-saving g-max system. There are containers for the MLA-55 with a volume range from 2 ml - 13.5 ml.

What is probably of greater importance to users is that the popular polycarbonate bottles with screw covers from the high-performance range can be used, and this in the MLA-55 without restrictions in the maximum rotation speed.

The MLA-55 is already available. It can be used in all centrifu-ges of the Optima Max and Max-E series. The rotor is supplied with a 5-year guarantee without restrictions due to running performance.

Sollte sich in dieser Ausgabe an dieser Stelle keine eingeheftete Faxantwortkarte befinden nutzen Sie bitte diese Seite für Ihre Informationsanforderung oder rufen Sie uns an: 02151 333-724.

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  - ◯ *MLA-55 – a new rotor expands the volume range of table centrifuges*
  - ◯ *Agencourt's SPRP technology automates*
  - ◯ *CELISCA – the competence centre for Life Science Automation*
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Agencourts SPRI® – technology automates

In 2005 Agencourt Bioscience became a part of Beckman Coulter, including the SPRI® technology for isolating and processing nucleic acids.

Agencourt® Bioscience Corporation

The SPRI „kits" were originally developed for commercial use in Agencourt's sequencing service (one of the largest in the USA), where they function daily.

1) Technology

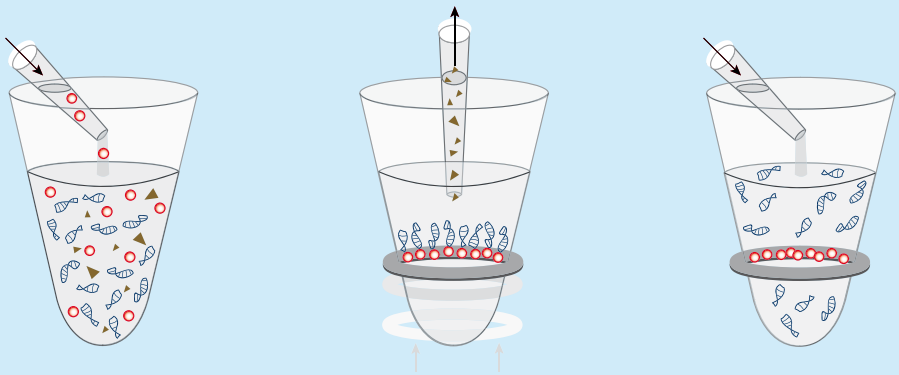
The SPRI® technology (Solid Phase Reversible Immobilisation) was developed in the Whitehead Institute in the USA (Hawkins et al Nucleic Acids Res., 1995; 23:22). The simple principle for isolating and cleansing nucleic acids is very efficient, and produces large yields with high purity (see illustration on right). The technology is also very robust and reproducible – ideally suited for automated sample processing on liquid handlers.

The simple function principle of SPRI®-technology

Step 1:
Immobilization of nucleic acids.
SPRI beads, coated paramagnetic microparticles (see display box), are added directly to the sample. Through specific buffer conditions, the nucleic acids are immobillised on the beads. Contaminants remain free within the solution.

Step 2:
Removal of contaminants.
The SPRI® beads with the nucleic acids immobilised on their surfaces are held by a magnetic field – contaminants are easily removed. In subsequent stages, the sample is washed, wherein the SPRIS® beads with the immobilised nucleic acids remain fixed in the magnetic field.

Step 3:
Elution of the nucleic acids.
While the magnet field continues to hold the paramagnetic SPRI® beads, variation of the buffer conditions detaches the nucleic acids from the bead surface, so that they can be removed in a cleansed condition. The SPRI® beads remain in the sample container.

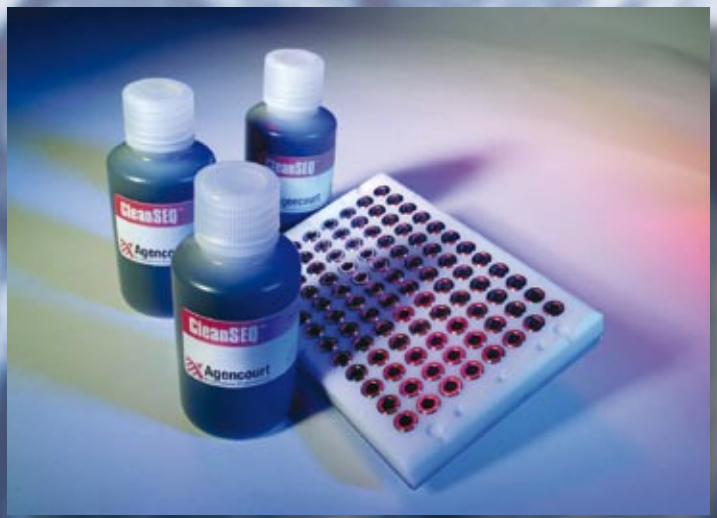


The beads – based upon Seradyn's Sera-Mag microparticles – have a uniform size distribution.

With their size of approx. 1 µm, it is guaranteed that they will not settle during cleansing. Mixing or shaking is therefore unnecessary.

This and the fact that cleaning is done without chaotropic salts or similar substances are the foundation for the high quality of the clean up procedure.

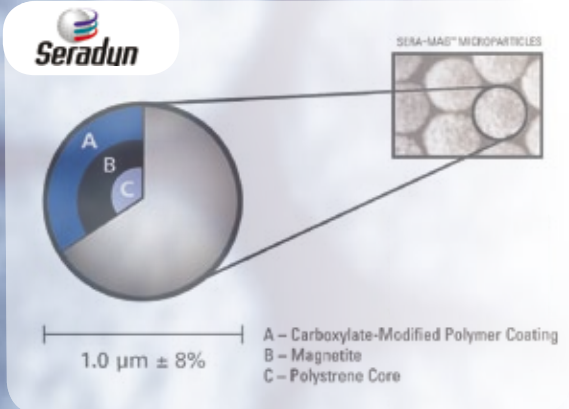
all steps are pipetting stages, the integration of vacuum stations or centrifuges is not required and the technology is particularly simple to automate.



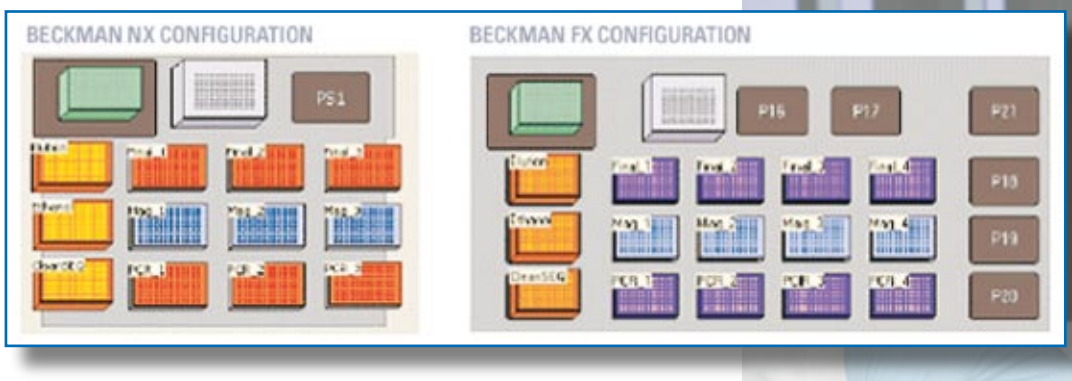
2) Options for cleansing

Cleaning can be done in 1.5 / 2ml-containers, as well as on 96- and 384-well plates. Since

Automation on the Biomek is complete, with no manual intervention required. Complete pipetting protocols for 8, 96, and 384 formats



Examples of Deck Arrangements for Biomek NX and Biomek FX



are available – with a few settings in a dialog box, the application runs as „plug and play".
Since the various Agencourt applications are all based on the same methodology, the requirements for the „Biomeks" are very similar in these applications. In practice, this means that one Biomek can be used to process all of the protocols. This fits laboratory needs because those who wish to cleanse sequencing samples generally already have a need for clean-up of PCR products.
The total pipetting requirement therefore determines the best choice of Biomek. In most cases, the user will choose a 96x pipetting head which is available both for the Biomek FX and for the Biomek NX.
For both units, the alternative use of a 384x pipetting head provides the highest throughput.

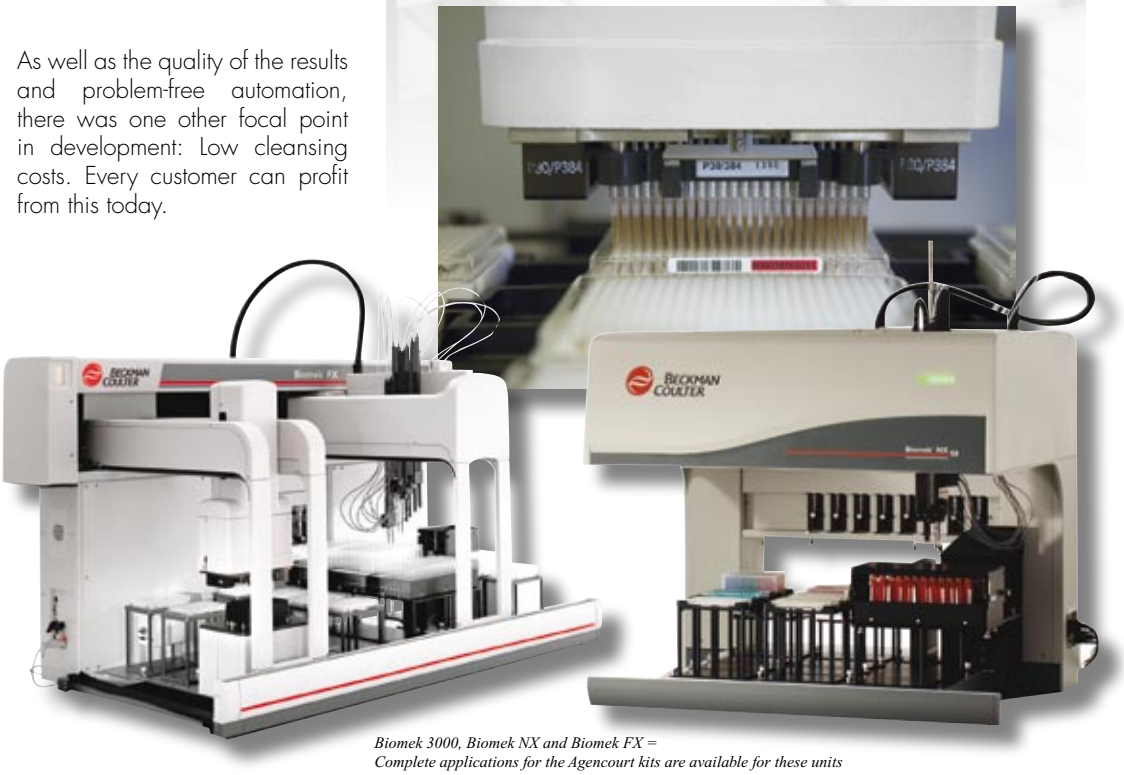
working pipetting heads – which are freely selectable. Two multichannel heads (a 96 and a 384, two 96 or two 384) or a multichannel head and a „Span-8". Maximum sample throughput (up to approx. 20 plates per hour) – that is the „FX".

Current applications which can be cleansed with SPRI technology are:
- Plasmides
- PCR-Amplicons
- Sequencing products
- BACS, Cosmides and Fosmides
- Genomic DNA -RNA

As well as the quality of the results and problem-free automation, there was one other focal point in development: Low cleansing costs. Every customer can profit from this today.

3) Automation on the Biomek NX and Bio-mek FX

The Biomek NX can be configured with either a multi-channel head (96 384 pipette tips) or a „Span-8". In the Span-8, up to 8 samples of one series can be pipetted – the volumes are individually selectable.
The „Span-8" is spreadable, so that formats other than microtiter plates can be served or can serve as a starting base.
For instance, possible throughputs for cleansing PCR products are eight 96-well plates per hour or twelve 96-well plates. The Biomek FX has two independently

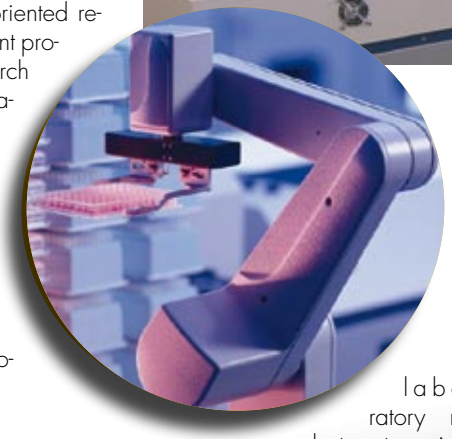


Biomek 3000, Biomek NX and Biomek FX =
Complete applications for the Agencourt kits are available for these units

CELISCA – the competence centre in Life Science Automation

As competent partners in questions of modern laboratory and life science automation as the establishment of new applications, Beckman Coulter and the Centre for Life Science Automation work together closely.

As one of six Germany-wide centres for innovation competence, CELISCA offers an ideal roof for effective, application-oriented research and development projects. Here, top research in the fields of Automation & Engineering, Screening & Analytics, Chemistry & Biotechnology, Automation Assessment and Real Time Systems are combined with the development of user-oriented system solutions.



Research and development center on the design of automated systems for diverse application areas in biology, chemistry and pharmacology. The key focus is on integrating various appliances - readers centrifuges, shakers, cooling reservoirs or even dosing systems in ORCA systems. Among other things, specialties include the integration of analysis systems such as GC and LC in combination with mass spectrometric detectors or self-developed high-parallel reaction systems. Intelligent solutions for the direct inclusion of laboratory robot systems into superior laboratory information management systems, system control, as well as visualisation and transfer of measurement data are likewise part of the scientists' portfolio.

Alongside the realization of hardware solutions, the CELISCA specialists also develop customer-specific applications in the fields of biology, chemistry and pharmacy. These include, for instance, the establishment of a high-performance, fully automated isolation method for viral RNA.

Since the method is not, in principle, limited to the selected cell-free samples, other RNA and DNA species from blood, tissues or cells can also be processed analogously (depending on the utilized kit).

Another classic question in biochemical studies is the quantitative determination of protein concentrations. Enzyme-linked immunesorbic assays (ELISA) are one of the most commonly used procedures and represent a simple, rapid, safe and cost-effective alternative.

The developed automated solution provides a time-efficient method for High Throughput analyses of protein concentrations. As the method is not limited to the protein selected for implementation and validation, it can basically be used for all soluble proteins.

For the implementation and establishment of this type of new applications CELISCA uses a broad range of Beckman devices. Alongside several ORCA-based systems for biological and chemical screening studies, these include laboratory automation workstations (Biomek FX and Biomek NX), plus several individual components such as CO_2 incubators, plate readers and washers, etc. **celisca** was the first European user to work with the new SAMI software, enabling research & development at the highest level. Further information is available at www.celisca.de.

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GenomeLab GeXP: a new system for gene expression analysis

DNA sequencing is used for analysing the order of the DNA building blocks A, C, G and T in a gene. This technique was used to analyse human DNA sequences in the „Human Genome Project" resulting in the publication of the human genome. The genomes of two people are different at some of the building block positions and these may cause genetically determined differences between these individuals. Only identical twins have identical genomes. The tissues from which the human body is built are made up of completely differentiated cell types. At first glance, a white blood corpuscle has little in common with a bone cell or an egg cell. However, interestingly enough, the genome of each person is identical in all its cell types.

So what causes the differences between the white blood corpuscle and the bone cell, keeping in mind that their genes are identical? The answer is found in the gene expression pattern of the cells. Not all genes are active in all cells all the time. In other words, there are tissue-specific genes which trigger the differences between the cell types. Activities of the genes change as a human being passes through each developmental stage of growth and as the cell responds to various environmental influences. What causes the different expression patterns of the each cell type?

The cells of the organism are in close contact with their environment. This provides them with information about their spatial position within the organism and about environmental influences. These influences act on molecular switches which in their turn only activate specific genes from the genome. Copies of these activated genes are produced in the form of messenger molecules (mRNAs) which carry the information to help make proteins. As well as analysis of the DNA sequences which form the genome, it is of great importance for research to analyse the expression patterns of cells, since this is the information about how the cell reacts to its environment. While units of the CEQ family (CEQ 2000, CEQ 8000 and CEQ 8800) have enabled DNA sequencing for many years, the new GeXP (which is based on the CEQ principle of capillary electrophoresis) will now make analysis of gene expression possible as well. For this, starting from the mRNAs of a cell type in a modified PCR (polymerase chain reaction), a series of DNA fragments are produced, electrophoretically separated and processed (for details, see Fig. 1).

Electrophoretic detection of fragments has the advantage that in addition to the signal intensity (peak height and area), the fragment size is also measured.

It is, therefore, possible to determine the expression of up to 30 genes simultaneously in one separation.

This can include housekeeping genes which do not change alongside those genes of interest. Comparison of the test genes with the housekeepers gives a measure of relative gene expression.

As this approach may be used with clinical specimens (eg biopsies) where little material is available, it has the advantage of using up to 30 times less material than current technologies.

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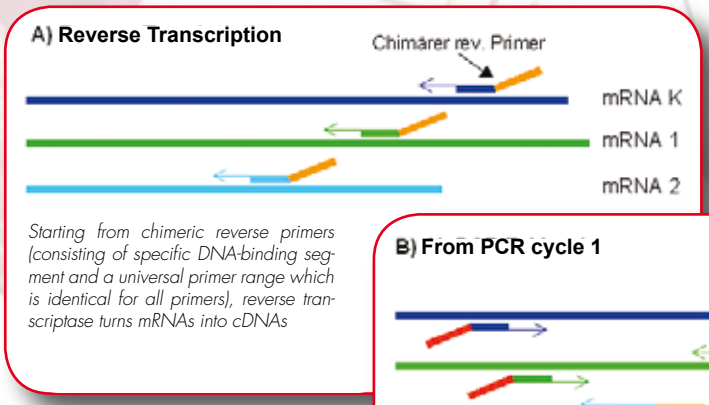
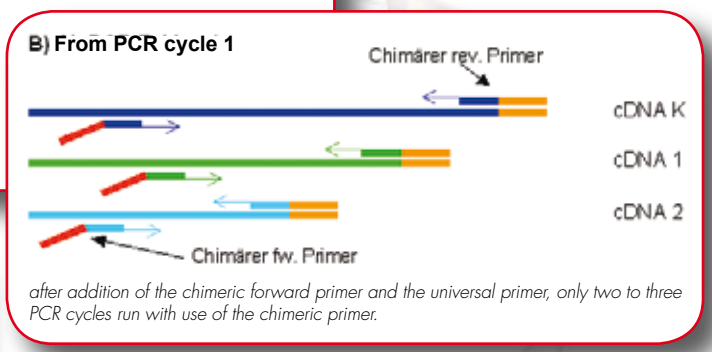
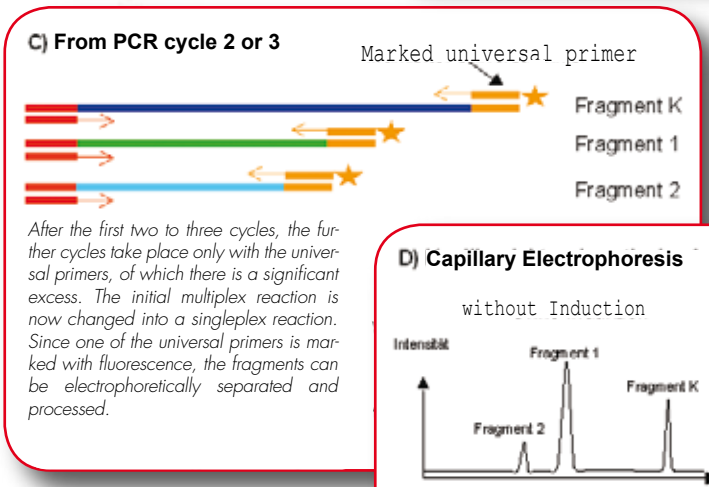






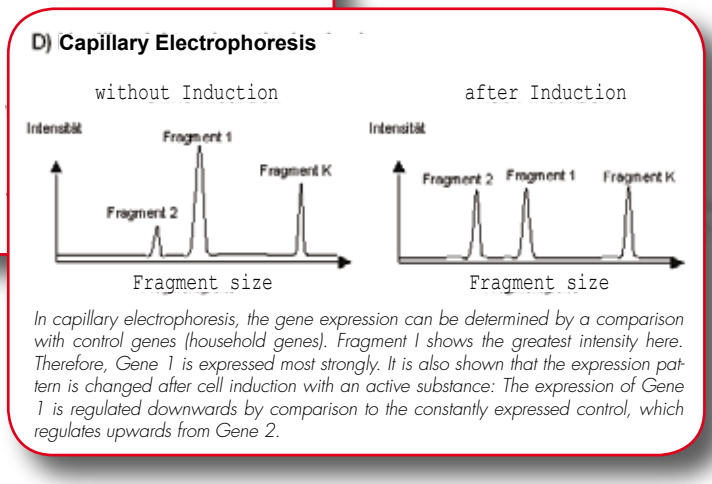
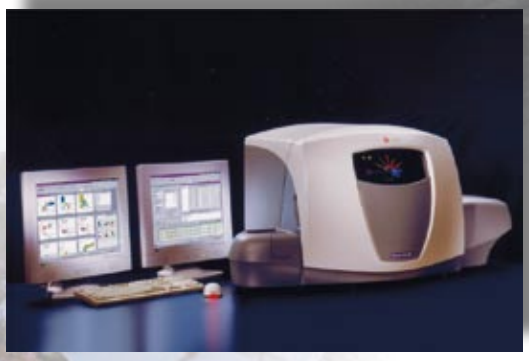


Fig. 1:
Principle of the Express Profiling Method

Flow Cytometry

The Cytomics FC 500 – a flow cytometer at the Olympic Games

The inventive employees of the Lausanne Laboratoire suisse d'Analyse de Dopage and the Cytomics FC500 flow cytometer have been working on blood doping. Particularly in bike sports, some successful athletes have had an infusion of erythrocyte-rich donated blood in order to escape the doping investigators, who were at that time concentrating on the use of EPO for erythrocyte production. Unnoticed, however, the Swiss group had successfully developed an assay of an Australian group in which the presence of donated blood in a person can be proven from the small blood group antigens. For this proof, the researchers around



institute head Dr. Martial Saugy use antibodies against these antigens. For its detection, they use a flow cytometer which is normally used in leukaemia diagnostics and for AIDS monitoring. The flow cytometer being used – the Cyto-mics FC 500 – is eminently suitable for this task, since it has the unique ability of standardising cytometric assays and thereby meeting the high demands placed on doping investigations. The quality of the analyses also convinced the International Olympic Committee (IOC), which consequently mandated the Lausanne laboratory to follow this form of doping with flow cytometry at the Olympic Winter Games 2006 in Turin as well. This was how two Cytomics FC 500 from Beckman Coulter „did their duty" in Turin at the Olympic Games and were able to make their contribution to fairness.

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Masterhead
Issue 3 / 2006

Number of copies
7.500

Publisher
Beckman Coulter GmbH
Europark Fichtenhain B 13
47807 Krefeld
Telefon 02151 333-724

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Design
New Graphic Design
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Printing
Druckerei Thierfelder