JETFLEX Genomic DNA Purification Kit Handbook

Description of the kit

GENOMED's JETFLEX Genomic DNA Purification Kit provides all reagents necessary to prepare high-quality, high molecular weight, RNA-free genomic DNA from a large variety of samples, among them blood, bone marrow, cell cultures, tissues of animal origin, plant tissues, bacteria (gram-positive and gram-negative) and yeast.

After primary lysis of the DNA-containing cells and nuclei the unwanted components are removed by a salt precipitation, thus avoiding any handling of organic solvents like phenol or chloroform.

The kit contains proteinase K to break up solid samples like a multitude of animal tissues, and RNase A to optionally remove cellular RNA. While residual RNA may not interfere with a variety of subsequent applications, its removal is essential for other ones. Both enzymes are provided with the kit.

The purified DNA is an excellent template for PCR amplification, restriction enzyme digestion, Southern blotting, slot blotting and subsequent hybridization.

Kit Components / Storage & Stability

Component	100 prep Kit (Cat.-No. 600 100)	500 prep Kit (Cat.-No. 600 500)
Red Blood Cell Lysis Buffer (RBC)	33 ml	165 ml
Cell Lysis Buffer (CLB)	33 ml	165 ml
Protein Precipitation Buffer (PPT)	17 ml	83 ml
‚Pellet Compactor'	8 ml	30 ml
DNA Dissolving Buffer (TE)	22 ml	110 ml
Proteinase K (20 mg/ml)	2 x 21 mg	10 x 21 mg
RNase A (4 mg/ml)	4.2 mg	5 x 4.2 mg

The amounts of buffers given in the table above are calculated from the following starting amounts per preparation:

A single prep from whole blood and other liquids is based on a volume of **300 µl**, a single prep from tissue is based on the amount of up to **10 mg** of solid matter.

Preparation sizes can be varied and scaled up and down proportionally according to the user's requirements. If the preparation size is scaled down, the number of preparations will increase, if preparation size is scaled up, the number of preparations will decrease proportionally.

Storage:

All components except the enzymes should be stored at room temperature. The enzyme solids should be stored at 4°C. Once dissolved in water, the enzymes should be stored at –20°C to prolong their lifetimes. To avoid repeated freeze-thaw cycles, GENOMED recommends to divide the dissolved enzymes into single-use aliquots. When stored as directed, all components are stable for at least 1 year from the purchase date.

Additional Materials Required

The following components are not provided with the kit and have to be supplied by the user.

--100% isopropanol (2-propanol)
--70-80% ethanol
--1.5 ml microfuge tubes (for mini preps), 15 ml or 50 ml centrifuge tubes (for larger preps)
--Pipet and pipet tips
--Vortex mixer
--Microfuge or clinical centrifuge
--Water bath or thermoblock
–Buffers YS1, YPD/YEPD and lytic enzymes belonging to DNA preparation from yeast
–Cell resuspension buffer with lysozyme for DNA preparation from gram-positive bacteria
–PBS, TBS or another equivalent saline buffer

General recommendation

The different JETFLEX protocols are calling for several steps where vigorous vortexing is required. A **multi-tube vortexer** (different brands are available from nearly each labware vendor) enables you to simultaneously and effectively vortex multiple samples, resulting in significant time savings.

Expected Results

Species & Material	Typical DNA yield	Proteinase K treatment	RNase A treatment
Human Whole Blood (300 µl)	5 – 10 µg	Optional	Optional
Human Whole Blood (2 ml, 10 ml, 50 ml)	**2 ml:** 50-100 µg, **10 ml:** 250-500 µg, **50 ml:** 1.25-2.5 mg	Optional	Optional
Cultured Cells	10-30 µg per 3 x 10^6 cells	Optional	Required
Swabs (Buccal, Pharyngeal, Nasal, Vaginal, etc.)	0.5 – 3.5 µg	Optional	Optional
Body Fluids	2 – 50 µg / ml	Optional	Optional
Solid Animal Tissue	20 – 150 µg per 10 mg	Required	Required
Plant Tissue	10-15 µg per 40 mg	Required	Required
Non-Mammalian Blood	2,5 – 7,5 mg/ml	Optional	Required
Gram-Negative Bacteria	20 µg / ml	Optional	Required
Gram-Positive Bacteria	10 µg / ml	Optional	Required
Yeast	5 – 7 µg / ml	No	Required

Protocols

This protocol booklet is divided into the following sections:

a) Processing of DNA-containing sources / Preparation of nuclear lysates

   a1) Whole blood (300 µl, 2 ml, 10 ml, 50 ml)
   a2) Other body fluids
   a3) Swabs (Buccal, Pharyngeal, Nasal, Vaginal, etc.)
   a4) Eucaryotic cell cultures
   a5) Solid tissues from animal + plant origin
   a6) Gram-positive and gram-negative bacteria
   a7) Yeast

b) Preparation of DNA from nuclear lysates

c) Re-purification protocol for still impure DNA's

a) Processing of DNA-containing sources / Preparation of nuclear lysates

a1) Whole Blood Samples (300 µl / 2 ml / 10 ml / 50 ml)

a11) Sample Collection and Handling

Collect whole blood or buffy coat samples in tubes with a suitable anticoagulant (EDTA, citrate or heparin) to prevent clotting. As EDTA also reduces DNA degradation, GENOMED recommends EDTA for the collection of blood samples.

Store fresh blood and buffy coat samples for not longer than 5 days at 4°C for optimal results. If longer storage times are necessary, the samples should be stored frozen at –80°C. Frozen samples are stable at –80°C for at least two years. Before use, blood samples should be thawed quickly in a 37°C water bath and then kept on ice until use.

Buffy coat is a concentrate of DNA-containing white blood cells. When processing buffy coat, be careful, not to overload the chemistry of the protocol. 300 µl / 2 ml / 10 ml / 50 ml of whole blood correspond to approximately 15-25 µl / 100-170 µl / 500-850 µl / 2.5-4.25 ml of buffy coat. Adjust the respective amounts of buffy coat with PBS (phosphate-buffered saline, not provided with the kit) to the volumes of the corresponding blood samples and handle them as described in the protocol.

a12) Preparation of a DNA-containing lysate from various amounts of whole blood

1.) Add **300 µl** / **2 ml** / **10 ml** / **50 ml** of whole blood to a suitable tube with **300 µl** / **2 ml** / **10 ml** / **50 ml** of **RBC lysis buffer**. Mix well by inverting until the mixture is homogeneous and go ahead immediately to step 2.
If the blood sample is not older than 1 hour after having been drawn from the donor, let stand the mixture at room temperature for 1 min to ensure complete lysis of the erythrocytes.

2.) Centrifuge for 30 sec. at 13.000 x g (for an initial blood volume of 300 µl) or 10 min at 2.000 x g (for larger volumes). Remove the clear red supernatant, leaving behind a visible pellet of DNA-containing white blood cells.
Do not disturb the white cell pellet, which may still appear slightly redish at this stage. It is <u>not</u> necessary to wash the white cell pellet with RBC lysis buffer again as the red stain will be removed completely during the subsequent procedure.

3.) **IMPORTANT:** Bump vortex the tube with the white cell pellet vigorously to loosen the cells from the wall. This will greatly enhance cell lysis in the next step.

4.) Add **300 µl** / **2 ml** / **10 ml** / **50 ml** of **buffer CLB** (Cell Lysis Buffer) to the resuspended cells and pipet up and down to lyse the cells. Usually no incubation is required.
If cell clumps are visible after mixing, incubate at 37°C or room temperature until the lysate is homogeneous and clear. Samples are stable in CLB for several months at room temperature. CLB will disrupt the cellular structure of the white blood cells and the cell nuclei, thus setting free the genomic DNA.

5.) **OPTIONAL RNase treatment:** Add **1.5 µl** / **10 µl** / **50 µl** / **250 µl** of RNase solution (4 mg/ml; provided with the kit) to the lysate of step 4 and incubate at 37°C for 5 min.

6.) Go ahead to procedure b (Preparation of DNA from nuclear lysates).

a2) Other body fluids (50 µl, 1 ml)

a21) Sample Collection and Handling

When body fluid samples are stored at 4°C or on ice, they should be processed quickly. For long-term storage store them at –70 to –80°C. If body fluids with low cell numbers are to be processed, the cells should be concentrated by a centrifugation for 10 min at 2.000 x g. Discard or pipet off the supernatant leaving behind the desired volume of residual liquid to thoroughly resuspend the pellet.

a22) Preparation of a DNA-containing lysate from various amounts of body fluids

1.) Add **50 µl** / **1 ml** of body fluid (i.e. plasma, saliva, serum, milk, urine, cerebrospinal fluid, sputum) into a suitable reaction tube containing **250 µl** (for 50 µl initial sample volume) or **5 ml** (for 1 ml initial sample volume) **buffer CLB**. Mix immediately and thoroughly by pipetting up and down.
If the sample has a high protein content, use 550 µl CLB per 50 µl or 11 ml CLB per 1 ml of original sample volume.

2.) Incubate at 58°C until the mixture becomes fully clear.
OPTIONAL: Add **20 µl** (for 50 µl initial sample volume) or **400 µl** (for 1 ml initial sample volume) proteinase K stock solution (20 mg/ml) and incubate at 58°C until the mixture is fully clear and all cells are lysed.
This shouldn't take longer than 1 hour, but the incubation time can be extended to overnight. CLB will disrupt the structure of the cells and nuclei, thus setting free the genomic DNA.

3.) **OPTIONAL RNase treatment:** Add **10 µl** (for 50 µl initial sample volume) or **200 µl** (for 1 ml initial sample volume) of RNase solution (4 mg/ml; provided with the kit) to the lysate of step 2 and incubate at 37°C for another 5 min.

4.) Go ahead to procedure b (Preparation of DNA from nuclear lysates).

a3) Swabs (Buccal, Pharyngeal, Nasal, Vaginal, etc.)

a31) Sample Collection and Handling

Swabs in general are the least invasive way of collecting DNA specimen from humans and animals for diagnostic purposes. In contrast to blood samples swabs have the additional benefit of minimizing the exposure to blood-borne pathogens.

The collection of swabs (buccal, nasal, pharyngeal, vaginal) are the method of choice when obtaining specimen for DNA testing. Swabs are easy to use and readily available. Cells collected on buccal swabs do not require special storage conditions and the DNA remains usable even after years of storage.

The JETQUICK Blood & Cell Culture Kit provides a fast and very efficient method for isolating genomic DNA from nearly all kind of swabs. The DNA isolated from the swabs is an ideal template for subsequent diagnostic PCR assays.

1.) Collect the swab with a suitable tool. Buccal, nasal, pharyngeal or vaginal swabs can be collected in a similar way.

To collect a sample properly, make sure, that the donor abstains from consuming any foods or drinks at least 30 min before sample collection. To further reduce possible interference from unwanted components, the donor optionally can rinse his mouth twice with water immediately before sample collection.

Suitable tools for the collection of buccal swabs are:

--T-Swab Kit (Isohelix),
--DACRON® swabs (Fitzco),
--C.E.P. Omni swabs (Whatman),
--Cotton swabs (e.g. TriTech, Inc. or Puritan Hardwood Products).

2.) Collect the swab sample by rubbing the buccal swab brush firmly on the inside of the cheek, approximately 6-10 times on each side of the brush. Be sure to move the brush over the entire cheek.

*OPTIONAL: At this point you can dry the swab for **long-term storage**. Air-dry the swab for approximately 2 hours at room temperature. When completely dry, the swab can be stored in a suitable bag, that is usually delivered together with the swabs. Dried swabs can be stored for more than 1 year without affecting the DNA. To process dried swabs, simply transfer them into a capped 2 ml microcentrifuge tube and go ahead beginning with step 3 of this protocol.*

*IMPORTANT NOTE: Any swabs containing cellular material should **ONLY** be treated with gloves. Don't touch the swab with your finger tips or let it come into contact with your skin, as this may lead to contaminations and false-positive or false-negative results*.

3.) Place buccal swab into a capped 2 ml microcentrifuge tube (not provided with the kit). While C.E.P. Omni swabs can be ejected into the microcentrifuge tube by pressing the stem end towards the swab, the handle from other swabs can either be cut off by scissors or snapped off at the break point.
The swab should fit entirely inside the tube allowing the cap to close.

a32) Preparation of a DNA-containing lysate from the collected cells

1.) Pipet **300 µl** of **buffer CLB** into a 1.5 ml reaction tube. Dip the collection brush into the buffer CLB aliquot and rotate the swab 10-20 times to release as many of the collected cells as possible into the liquid. Finally squeeze out the swab at the tube's wall to recover a maximum amount of liquid.

If clumpy material remains visible, incubate at 58°C until the mixture becomes fully clear. Vortex the sample vigorously from time to time to obtain an effective cell lysis.

OPTIONAL: Add **10 µl** proteinase K stock solution (20 mg/ml) and incubate for 30 min to overnight at 58°C until the mixture is fully clear and all cells are lysed.

CLB will disrupt the cellular structure of the collected cells and nuclei, thus setting free the genomic DNA. A treatment with proteinase K will enhance cell lysis, thus improving the DNA yield.

2.) **OPTIONAL RNase treatment:** Add **10 µl** of RNase solution (4 mg/ml; provided with the kit) to the lysate of step 1 and incubate at 37°C for another 5 min.

3.) Go ahead to procedure b (Preparation of DNA from nuclear lysates).

a4) Cultured cells (1-2 x 10^6 cells / 1-2 x 10^7 cells)

a41) Sample Collection + Handling

When preparing DNA from cultured cells, the cells should be placed on ice immediately before processing them. For long-term storage keep the cells at –70 to –80°C. Determine the number of cells using a hemacytometer or other cell counter.

a42) Preparation of a DNA-containing lysate from various amounts of cultured cells

4.) Apply **1-2 x 10^6 / 1-2 x 10^7 cells** in balanced salt solution (e.g. PBS, TBS or another standard saline buffer; not provided with the kit) into a suitable reaction tube.

5.) Centrifuge for 15 sec. at 13.000 x g (for 1-2 x 10^6 cells) or 2 min at 2.000 x g (for 1-2 x 10^7 cells) to pellet the cells.
Remove the supernatant leaving behind a desired volume of residual liquid to thoroughly resuspend the cells.

6.) **IMPORTANT**: Bump vortex the tube with the cell pellet vigorously to loosen the cells from the wall and resuspend them in the residual liquid. This will greatly enhance cell lysis in the next step

7.) Add **300 µl** (for an initial cell volume of 1-2 x 10^6 cells) or **3 ml** (for an initial cell volume of 1-2 x 10^7 cells) **buffer CLB** to the resuspended cells and lyse the cells by pipetting up and down. If cell clumps remain visible, incubate at 58°C until the mixture becomes fully clear.

OPTIONAL: Add **20 µl** (for an initial cell volume of 1-2 x 10^6 cells) or **200 µl** (for an initial cell volume of 1-2 x 10^7 cells) proteinase K stock solution (20 mg/ml) and incubate for 1 h to overnight at 58°C until the mixture is fully clear and all cells are lysed.

CLB will disrupt the cellular structure of the cultured cells and the cell nuclei, thus setting free the genomic DNA.

8.) **OPTIONAL RNase treatment:** Add **10 µl** (for an initial cell volume of 1-2 x 10^6 cells) or **100 µl** (for an initial cell volume of 1-2 x 10^7 cells) of RNase solution (4 mg/ml; provided with the kit) to the lysate of step 4 and incubate at 37°C for another 5 min.

9.) Go ahead to procedure b (Preparation of DNA from nuclear lysates).

a5) Solid Tissues from animal + plant origin (10 mg / 100 mg)

a51) Sample collection and handling

Fresh, frozen, fixed or paraffin-embedded tissue samples as well as mouse tails can be processed with the JETFLEX kit. If tissue samples are to be stored for a longer period of time, they should be stored at –70 to –80°C.

Before entering the JETFLEX procedure, tissue samples are best reduced to small pieces (e.g. by grinding in liquid nitrogen with mortar and pestle). When using fresh tissue samples, keep them on ice until cell lysis buffer (CLB) has been added to minimize any degradation of the DNA by the action of DNases.

a52) Preparation of a DNA-containing lysate from various amounts of solid tissue

1.) Add **up to 10 mg** / **up to 100 mg** fresh or frozen tissue that was optimally reduced to small pieces into a suitable reaction tube. Keep tube on ice.
When working with plant tissue material, it may be necessary to vary the amount of starting material depending upon species, age, tissue type and genome size. If ever possible, plant tissue samples should be finely ground to disrupt cellular structures before the addition of CLB.

2.) Add **300 µl** (for an initial sample volume of up to 10 mg tissue) or **3 ml** (for an initial sample volume of up to 100 mg tissue) **buffer CLB** to the tissue sample.

If the tissue sample wasn't reduced to small pieces, homogenize the sample thoroughly using 30-50 strokes with a suitable tube pestle. Add **20 µl** (for an initial sample volume of up to 10 mg tissue) or **200 µl** (for an initial sample volume of up to 100 mg tissue) proteinase K stock solution (20 mg/ml) and incubate for 1 h to overnight at 58°C until the mixture is fully clear and any tissue structure is fully desintegrated.

CLB will disrupt the cellular structure of the tissue cells and nuclei, thus setting free the genomic DNA.

3.) **OPTIONAL RNase treatment:** Add **10 µl** (for an initial sample volume of up to 10 mg tissue) or **100 µl** (for an initial sample volume of up to 100 mg tissue) of RNase solution (4 mg/ml; provided with the kit) to the lysate of step 2 and incubate at 37°C for another 5 min.

4.) Go ahead to procedure b (Preparation of DNA from nuclear lysates).

a6) Gram-positive and Gram-negative bacteria (500 µl / 5 ml)

a61) Preparation of a DNA-containing lysate from various amounts of bacterial culture

1.) Apply **500 µl** / **5 ml** of bacterial culture (e.g. after overnight growth) into a suitable reaction tube.

2.) Centrifuge for 1 min at 13.000 x g to pellet the bacterial cells. Remove the supernatant quantitatively with a pipet.
For gram-positive bacteria proceed with step 3, for gram-negative bacteria go directly to step 4.

3.) **If gram-positive bacteria are to be processed:** Due to the high murein content of the cell walls of gram-positive bacteria, a much rougher treatment is necessary to disrupt the cells efficiently. Resuspend the cells in **300 µl** (for an initial sample volume of 500 µl bacteria suspension) or **3 ml** (for an initial sample volume of 5 ml bacteria suspension) of a buffer containing 50 mM Tris-Cl (pH 8,0) / 10 mM EDTA with 2 mg/ml lysozyme (not provided with the kit). After having obtained a homogeneous suspension incubate for 30 min at 37°C.
Then centrifuge for 1 min at 13.000 x g (for an initial sample volume of 500 µl bacteria suspension) or 5 min at 2000 x g (for an initial sample volume of 5 ml bacteria suspension) to pellet the cells again. Remove the supernatant.
For most gram-positive species a treatment with lysozyme is sufficient to weaken the cell wall. However, some Staphylococcus species require an additional treatment with 1 mg/ml lysostaphin (not provided with the kit) to get the cell walls efficiently lysed.

4.) Add **300 µl** (for an initial sample volume of 500 µl bacteria suspension) or **3 ml** (for an initial sample volume of 5 ml bacteria suspension) **buffer CLB** to the resuspended cells and lyse the cells by pipetting up and down. If cell clumps remain visible, incubate at 58°C until the mixture becomes fully clear.

OPTIONAL: Add **10 µl** (for an initial sample volume of 500 µl bacteria suspension) or **100 µl** (for an initial sample volume of 5 ml bacteria suspension) proteinase K stock solution (20 mg/ml) and incubate for 1 h to overnight at 58°C until the mixture is fully clear and all cells are lysed.

CLB will disrupt the cellular structure of the bacterial cells, thus setting free the genomic DNA.

5.) **OPTIONAL RNase treatment:** Add **10 µl** (for an initial sample volume of 500 µl bacteria suspension) or **100 µl** (for an initial sample volume of 5 ml bacteria suspension) of RNase solution (4 mg/ml; provided with the kit) to the lysate of step 4 and incubate at 37°C for another 5 min.

6.) Go ahead to procedure b (Preparation of DNA from nuclear lysates).

a7) Yeast (1 ml / 10 ml)

a71) Preparation of a DNA-containing lysate from various amounts of yeast culture

1.) Grow a *Saccharomyces* culture to saturation in YPD or YEPD (= 1-2 x 10^8 cells/ml). Harvest **1 ml**/ **10 ml** of the cells by centrifugation (**1 ml culture:** 13.000 x g for 1 min, **10 ml culture:** 2.000 x g for 3 min) and remove the supernatant quantitatively.

2.) Wash the cells by resuspending them in **300 µl** (for an initial sample volume of 1 ml yeast culture) or **3 ml** (for an initial sample volume of 10 ml yeast culture) **buffer YS1** (0.9 M sorbitol / 0.1 M Na_2EDTA [pH 7.5] / 14 mM 2-mercaptoethanol, not provided with the kit).
This is for the removal of remaining media components and to ensure optimal enzymatic performance with the Zymolyase in step 4.

3.) Spin down the cells at 13.000 x g for 1 min and remove the supernatant quantitatively with a pipet. Resuspend the cells in **300 µl** (for an initial sample volume of 1 ml yeast culture) or **3 ml** (for an initial sample volume of 10 ml yeast culture) **buffer YS1**.
The cell suspension must be homogeneous; no cell clumps must be visible!

4.) Add **30 µl** (for an initial sample volume of 1 ml yeast culture) or **300 µl** (for an initial sample volume of 10 ml yeast culture) of **Zymolyase 60,000** (Miles; 2 mg/ml) and incubate at 37°C for 20 - 30 min.

Zymolyase will break down the cell wall of the yeast cells enzymatically during incubation. Equivalent enzymes to Zymolyase are Lyticase (Sigma, Cat.-No. L8137) or Zymolase (ICI, Cat.-No. 32-093-2). These enzymes should be diluted from their respective stock solutions in distilled water to a final concentration of 1000 U/ml and the incubation with them be performed for at least 30 min at 30°C. Stock solutions of these enzymes should be stored in aliquots at -20°C and each aliquot only used once.
Monitor spheroplast formation by examination to detergent sensitivity: a small sample of cells is diluted into 1% SDS, and spheroplasting is sufficient when greater than 90% of the cells burst when examined under the microscope.

5.) Spin down the spheroplasts at 13.000 x g for 1 min (for an initial volume of 1 ml yeast culture) or 5000 x g for 10 min (for an initial volume of 10 ml yeast culture). Remove supernatant quantitatively with a pipet.

6.) Add **300 µl** (for an initial sample volume of 1 ml yeast culture) or **3 ml** (for an initial sample volume of 10 ml yeast culture) **buffer CLB** to the yeast cells and lyse them by pipetting up and down. If cell clumps remain visible, incubate at 58°C until the mixture becomes fully clear.
CLB will disrupt the structures of the spheroplasts, thus setting free the genomic DNA.

7.) **OPTIONAL RNase treatment:** Add **10 µl** (for an initial sample volume of 1 ml yeast culture) or **100 µl** (for an initial sample volume of 10 ml yeast culture) of RNase solution (4 mg/ml; provided with the kit) to the lysate of step 6 and incubate at 37°C for another 5 min.

8.) Go ahead to procedure b (Preparation of DNA from nuclear lysates).

b) Preparation of DNA from nuclear lysates

1.) Before processing any cellular lysate from one of the procedures under ‚a)' any further, make sure that it has reached **room temperature** again.

2.) For each **300 µl (3 ml)** of cellular lysate from one of the procedures of part ‚a)' add **150 µl (1.5 ml)** of **protein precipitation buffer PPT**. Mix thoroughly by vortexing for 20 seconds to obtain a homogeneous suspension.

3.) Centrifuge at 13.000 – 16.000 x g for 3 min at room temperature.
The precipitated proteins should form a compact pellet. The supernatant should be clear.

3a) OPTIONAL: The JETFLEX system is designed as a general purpose device to isolate DNA from a multitude of sources. So if the resulting protein pellet after treatment with buffer PPT is small or loose and/or shows a tendency to be co-transfered with the supernatant, proceed with one of the following options:

a1) For a volume of **450 µl** of **protein precipitation mix** (= 300 µl of cellular lysate + 150 µl of protein precipitation buffer PPT, see step 2) add **50 µl of ‚Pellet Compactor'** (provided with each kit) and vortex until a homogeneous suspension is obtained.
For higher volumes of protein precipitation mix the amount of ‚Pellet Compactor' has to be increased proportionally!!!
Then centrifuge at 13.000 – 16.000 x g for 3 min at room temperature. Continue with step 4.

a2) Vortex again and chill the mixture on ice for 5 min. Then centrifuge again for 3 min at 13.000 – 16.000 x g **in a chilled centrifuge** at 4°C. Continue with step 4.

4.) Transfer the clear supernatant into a fresh tube. Add 1 vol. of isopropanol (2-propanol).

5.) Mix thoroughly by multiple inverting until a homogeneous mixture is obtained. Avoid vortexing.

6.) Centrifuge at 13.000 – 16.000 x g for 3 min at room temperature. The precipitated DNA should be visible as a white pellet.

7.) Decant the supernatant and let residual liquid drain on a sheet of absorbant paper towel for a few minutes. Add **1 ml** (for an initial volume of 300 µl cellular lysate) or **10 ml** (for an initial volume of 3 ml cellular lysate) of **70-80% ethanol** to the tube and wash the DNA pellet by inverting several times.

8.) Centrifuge at 13.000 – 16.000 x g for 1 min. Carefully decant the ethanolic supernatant.
The DNA pellet can sit very loosely at the wall of the tube at this stage. Be very careful not to discard the DNA.

9.) Let residual liquid drain on a sheet of absorbant paper towel for a few minutes. Evaporate residual ethanol by a 10 min incubation at elevated temperature (50-56°C).
One can also allow the DNA pellet to air-dry at room temperature, but it may take significantly longer for all remaining ethanol to fully evaporate.

10.) Add a suitable volume of DNA Dissolving Buffer (TE) to the DNA pellet.
Dissolve the DNA by standing at room temperature overnight. The dissolution process can be enhanced by incubating at 65 °C for up to 1 h.

11.) Store the DNA at 4 °C.

The dissolved DNA should be quantified spectrophotometrically. Pure DNA has a A_{260}/A_{280} ratio of 1.7 – 1.9. One OD_{260} corresponds to a DNA concentration of 50 µg/ml. Analysis on an analytical agarose gel should reveal the majority of DNA migrating at a size of ≥ 50 – 150 kb.

c) Re-purification protocol for still impure DNA's

Pure DNA should dissolve readily in the provided DNA Dissolving Buffer (TE), giving a clear, colorless solution. If the DNA solution still contains insoluble matter, is stained in some way or the A_{260}/A_{280} ratio is <1,70, the DNA is still contaminated with protein or another impurity. This may occur, when e.g. the chemistry of the purification procedure has been overloaded.

Still contaminated samples may be further purified easily using the following protocol.

1.) For every **100 µl** of contaminated DNA solution add **500 µl** of **buffer CLB** and mix thoroughly by inverting or pipetting up and down.
If necessary, incubate at 37°C until any particulate matter has dissolved.
***IMPORTANT NOTE**: Cool down the sample to room temperature before proceeding to step 2*.

2.) For every **600 µl** of DNA solution/CLB mixture add **300 µl** of **protein precipitation buffer PPT** and vortex vigorously for 20 sec to obtain a homogeneous mixture.

3.) Centrifuge for 3 min at 13.000 – 16.000 x g (or 10 min at 5.000 x g) to pellet any precipitated impurities.

If a pellet is not visible, re-vortex and incubate the sample for 5 min on ice. Then re-centrifuge the sample as described.

*If there is still no stable pellet obtained, mix **900 µl** of protein precipitation mix (= 600 µl of cellular lysate + 300 µl of protein precipitation buffer PPT, see step 2) with **100 µl of ‚Pellet Compactor'** (provided with each kit) and vortex until a homogeneous suspension is obtained. Centrifuge at 13.000 – 16.000 x g for 3 min at room temperature. Continue with step 4.*

4.) Transfer the supernatant into a clean suitable receptacle without transferring any precipitated particles.

5.) Add 1 vol. of isopropanol (2-propanol) to the transferred supernatant and mix thoroughly by multiple inverting.
The precipitated DNA should become visible as a thread-like matter.

6.) Centrifuge for 1-3 min at 12.000 x g to collect the precipitated DNA at the bottom of the tube. Discard the supernatant and let residual liquid drain from the DNA pellet by placing the tube upside-down on a sheet of absorbant paper towel.

7.) Wash the DNA pellet with a suitable volume of 70-80% ethanol. Centrifuge again for 1-3 min at 12.000 x g to collect the DNA at the bottom of the tube. Discard the ethanolic supernatant.
The DNA pellet may sit very loose at the tube's wall at this stage. Be careful not to discard the DNA.

8.) Let residual liquid drain on a sheet of absorbant paper towel for a few minutes. Evaporate residual ethanol by a 10 min incubation at elevated temperature (50-56°C).

One can also allow the DNA pellet to air-dry at room temperature, but it may take significantly longer for all remaining ethanol to fully evaporate.

9.) Add a suitable volume of DNA Dissolving Buffer (TE) to the DNA pellet. Dissolve the DNA by standing at room temperature overnight. The dissolution process can be enhanced by incubating at 65°C for up to 1 h.

10.) Store the DNA at 4°C.

The dissolved DNA should be quantified spectrophotometrically. Pure DNA has a A_{260}/A_{280} ratio of 1.7 – 1.9. One OD_{260} corresponds to a DNA concentration of 50 µg/ml. Analysis on an analytical agarose gel should reveal the majority of DNA migrating at a size of ≥ 50 – 150 kb.

Notes

Notes

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