

Preparation of plasmid DNA with JETSTAR 2.0 MIDI columns with Lysate Filter Unit (LFU)

The standard protocol for preparation of plasmid DNA with JETSTAR 2.0 is calling for a centrifugation step to get the bacterial lysate cleared from the bulk of bacterial debris, proteins and genomic DNA. This requires the presence of a suitable centrifuge, all the handling and equipment needed to get the centrifuge loaded properly and careful pipetting of the cleared lysate from the centrifuge tube into the equilibrated column.

Now plasmids can be purified with JETSTAR 2.0 **without** the centrifugation step to clear the bacterial lysate, thereby gaining much additional convenience, as a centrifuge and all the handling associated with such a device are no longer needed. Additionally, this new protocol saves much time as the time for all the centrifugation work is completely eliminated from the protocol.

The protocol below shows the volumes of bacterial culture and buffers required for JETSTAR 2.0 preparations on the MIDI scale.

This protocol is compatible with bacterial culture volumes **up to 50 ml**. If it is necessary to process larger culture volumes of up to 100 ml (e.g. when working with low-copy plasmids, cosmids or BAC DNA), use higher volumes of buffers E1, E2 and E3 to prepare the cleared lysate as outlined in the protocol below.

The volumes of buffers E1, E2 and E3 in the kits are for the designated number of standard preparations with 8 ml of each buffer. The required volumes of these buffers are higher for elevated culture volumes in order to ensure optimal cellular lysis. So, if more buffer E1, E2 and E3 is required, this additional buffer either has to be purchased separately or prepared by the respective user (buffer compositions see below).

Most protocols are recommending LB broth or a variant thereof plus the appropriate antibiotic as the medium of choice for the propagation of *E. coli* cells. With GENOMEDs JETSTAR 2.0 you can use almost all media that are commonly in use for the propagation of *E. coli* (i.e. LB, Terrific Broth, 2 x YT, special plasmid growth formulations like Qbiogene's PGF series or MacConnell's 'Magnificent Broth').

Protocol:

Plate out *E. coli* cells being transformed with the plasmid construct of choice or streak out *E. coli* cells carrying the desired plasmid from a glycerol stock onto a LB agar plate with the appropriate antibiotic.

After having grown the cells overnight at 37 °C, inoculate a single, well-grown bacterial culture in medium with the appropriate antibiotic and grow the bacteria for 14 – 16 h (usually overnight). If a buffered medium (e.g. Qbiogene's PGF series or MacConnell's 'Magnificent Broth') is used, longer

growth times of the *E. coli* cells are not only possible, but even recommended. With such a medium we recommend growth times for the bacterial culture of 24 – 26 h.

For a standard MIDI prep 25 – 50 ml of bacterial culture can be processed. For volumes of 100 ml culture increase the amounts of buffers E1 / E2 / E3 as outlined below to ensure optimal lysis.

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1.) **The JETSTAR 2.0 Midi columns are delivered with the Lysate Filter Unit (LFU) inserted into the column housing.**

Place the JETSTAR 2.0 column into a suitable rack and equilibrate the column with **14 ml of equilibration buffer E4** by pouring the buffer directly into the filter device.

The equilibration buffer will go through the filtration matrix thereby pre-wetting it. Then the buffer will pass through the JETSTAR resin and equilibrate it. The column will start dripping. While column equilibration is in process, harvest the bacterial cells and prepare the cleared lysate as described below.

Shortly after the column has begun dripping, some drops at the outlet may appear turbid. This is normal and due to the interaction of the equilibration buffer with the matrix. The turbid drops will not affect the preparation in any way.



2.) Spin down the bacterial cells from the *E. coli* culture for **3 min** at **≥12,000 x g**. Remove the supernatant quantitatively with a pipet.



3.) Resuspend the cells in **8 ml buffer E1 (reconstituted with 100 µg/ml RNase)**. No cell clumps must be visible.

*If working with volumes of 50 – 100 ml of bacterial culture, increase the volume of buffer E1 and also of buffers E2 and E3 in the following steps to **10 ml**.*

IMPORTANT: *The volumes of buffers E1, E2 and E3 in the kits are for the designated number of standard preparations with 8 ml of each buffer. The required volumes of these buffers are higher for elevated culture volumes in order to ensure optimal cellular lysis. So, if more buffer E1, E2 and E3 is required, this additional buffer either has to be purchased separately or prepared by the respective user (buffer compositions see below).*



4.) Lyse the bacterial cells with **8 ml buffer E2**. Mix gently but **thoroughly** (e.g. by multiple inverting) until a **homogeneous mixture** is obtained. **DO NOT VORTEX!!!** Due to the

release of genomic DNA the mixture is very viscous at this stage. Incubate at **room temperature** for **5 min.**



- 5.) Neutralize the lysis mix from step 4 with **8 ml buffer E3**. Mix gently but **thoroughly** (e.g. by multiple inverting) until a **homogeneous mixture** is obtained. **DO NOT VORTEX!!!** The liquid of the neutralized lysate must be **completely non-viscous again**. No remainders of the viscous matter obtained after lysis of the cells (step 4) must be left.



- 6.) **Apply the neutralization mix from step 5 with all the precipitated matter in it into the Lysate Filter Unit (LFU) inserted into the previously equilibrated (*step 1*) JETSTAR / LFU column. Let the lysate run through by gravity flow until the flow stops or dripping becomes very slow (< 1 drop per 10 seconds).**

Do not force out any remaining liquid!

OPTIONAL: The final DNA yield can be somewhat improved when residual bacterial lysate that remains within the debris particles is washed out by applying 10 ml of buffer E5 into the filtration device as soon as gravity flow has stopped or became very slow.

If buffer E5 is used to wash out residual lysate, let the buffer flow through the column by gravity flow until the flow stops or dripping becomes very slow (< 1 drop per 10 seconds). Proceed to step 7.



- 7.) As soon as the JETSTAR column has stopped dripping, take the filtration device out of the column housing and discard it.

Wash the JETSTAR column by applying **20 ml of wash buffer E5**. Let the wash buffer flow through the column by gravity flow until all liquid has passed through the resin. **Do not force out any remaining buffer!**



- 8.) **OPTIONAL ,PRE-ELUTION STEP':** This ,pre-elution' step with a small portion of elution buffer is optional, but will improve the final DNA yield by up to 26%. **This optional step should only be carried out by experienced users.** If this optional ,pre-elution' step is NOT to be carried out, please go directly to step 9.

Apply precisely **1.5 ml of elution buffer E6** into the column and let this portion of elution buffer flow through the column into the waste tray by gravity flow until the column stops dripping. **Do not force out any remaining buffer!**

IMPORTANT NOTE: *Be very careful and precise with the volume of elution buffer required for this step, so that no plasmid DNA will be eluted into the waste tray.*



- 9.) **ELUTION:** Remove the waste tray and place a clean, sterile collection tube under the outlet nozzle of the JETSTAR 2.0 column. Elute the DNA from the column into the clean tube with **5 ml of elution buffer E6.**

Precipitate the DNA by mixing the eluate **thoroughly** with 0.7 vols. of isopropanol.



- 10.) Spin down the DNA for **30 min** at **≥12.000 g** at **4°C**. If centrifuged in glass tubes (e.g. Corex[®]), the plasmid DNA tends to be very "sticky" and may spread over the whole wall of the centrifuge tube if a fixed angle rotor is used. Therefore we recommend either the use of a swing out rotor (i.e. HB-4 or HB-6 for Sorvall centrifuges), or, if such a rotor is not available, the **siliconization** of the centrifuge tubes with dimethyldichlorosilane.

After precipitation the DNA is washed with 70-80% ethanol and dried briefly for 5 - 10 min either at elevated temperatures (50 – 60°C) or under vacuum (**be careful when using a speed vac evaporator !!!**). Dissolve the DNA in a suitable amount of 10 mM Tris-HCl or TE buffer (pH 8.0 – 8.5) or simply water.

Buffer Compositions:

Buffer E1: 50 mM Tris-HCl (pH 8.0), 10 mM EDTA, 100 µg/ml RNase A

Buffer E2: 200 mM NaOH, 1%(w/v) SDS

Buffer E3: 3.1 M potassium acetate (pH 5.5 with acetic acid)

Buffer E4: 100 mM Sodium acetate (pH 5.0 with acetic acid), 600 mM NaCl, 0.15% Triton X-100

Buffer E5: 100 mM Sodium acetate (pH 5.0 with acetic acid), 800 mM NaCl

Buffer E6: 100 mM Sodium acetate (pH 5.0 with acetic acid), 1.500 mM NaCl