

# Diagnosis protocol for the JETSTAR plasmid kits

If you encounter *low* or even *no* yield with the JETSTAR procedure, this test will help to determine whether the problem is due to the supplied reagents, columns or the procedure itself.

- 1.) Inoculate a plasmid-containing bacterial culture (high-copy number plasmid recommended; the culture should contain **at least 4-5 µg of plasmid per ml culture**) according to good microbiological practice in LB medium with the appropriate antibiotic and grow the bacteria for 14 - 16 h (usually overnight).

H

- 2.) Decide, what column type (**Mini/Midi/Maxi**) is necessary. For the different prep sizes use the following amounts of bacterial culture:

**-Mini: 3 ml,**

**-Midi: 25 ml,**

**-Maxi: 100 ml.**

H

- 3.) Equilibrate the column with **buffer E4** as described in the JETSTAR protocol.

H

- 4.) Take the appropriate amount of culture and spin down the bacterial cells as described in the JETSTAR protocol.

H

- 5.) Resuspend the cells in **400 µl (Mini) / 4 ml (Midi) / 10 ml (Maxi)** of **buffer E1 with 100 µg/ml RNase**. No cell clumps must be visible.

H

- 6.) Lyse the bacterial cells with **400 µl (Mini) / 4 ml (Midi) / 10 ml (Maxi)** of **buffer E2**. Mix gently but **thoroughly** until a **homogeneous mixture** is obtained. Due to the release of genomic DNA the mixture is very viscous at this stage. Incubate at **room temperature** for **5 min**.

H

- 7.) Neutralize the lysis mix from step 6 with **400 µl (Mini) / 4 ml (Midi) / 10 ml (Maxi)** of **buffer E3**. Mix gently but **thoroughly** until a **homogeneous mixture** is obtained. The liquid of the neutralized lysate must be **completely thin-bodied** again (not longer viscous).

H

- 8.) Centrifuge the lysate for **10 min** with **≥13,000 x g** at **25° C (room temperature)**. Collect the supernatant into a fresh tube.

## H

- 9.) Take an aliquot of the cleared lysate that corresponds to approximately 1 ml of the initial bacterial culture (based on the volumes given in step 2) and transfer it into a separate 1.5 ml tube:

*Mini: 400 µl,*

*Midi: 480 µl, (SAMPLE 1)*

*Maxi: 300 µl.*

Precipitate the DNA from this sample with 0.7 vol of isopropanol.

## H

- 10.) Apply the remaining cleared lysate of step 8 onto the previously equilibrated (*step 3*) JETSTAR column. Let the lysate run by gravity flow. Collect the flowthrough into a new tube.

## H

- 11.) *Mix the flowthrough of the column very well.* Take an aliquot of the flowthrough that corresponds to approximately 1 ml of the initial bacterial culture (based on the volumes given in step 2) and transfer it into a separate 1.5 ml tube:

*Mini: 400 µl,*

*Midi: 480 µl, (SAMPLE 2)*

*Maxi: 300 µl.*

Precipitate the DNA from this sample with 0.7 vol of isopropanol.

## H

- 12.) Wash the JETSTAR column as described in the protocol with the following modification:

Mini: 2 x 2.5 ml,

Midi: 2 x 10 ml,

Maxi: 2 x 30 ml.

*Mix each wash fraction very well!* Take aliquots of **each** wash fraction (→ **SAMPLES 3 and 4**) and precipitate them with 0.7 vol of isopropanol. The aliquots should be **900 µl (Mini), 900 µl (Midi), 900 µl (Maxi)**.

## H

- 13.) Elute the DNA from the JETSTAR column into a fresh tube. *Mix the eluate very well!* Transfer **900 µl** of the mixed eluate (→ **SAMPLE 5**) into a 1.5 ml tube and precipitate with **0.7 vol isopropanol** as described in the JETSTAR protocol.

## Analysis of the samples 1 - 5 / Expected Results

All sample DNAs taken during the procedure have to be precipitated as described with 0.7 vol of isopropanol. After spinning down the nucleic acids in a chilled microfuge for at least **30 min at  $\geq 13,000 \times g$  do not wash** the pellets with ethanol, but let all liquid drain from the pellets by inverting the open tubes for **at least 5 min** on a sheet of absorbent paper towel. Dry the nucleic acids further in vacuum for approximately 10 min. Be careful not to overdry the pellets. Dissolve the nucleic acids of the **samples 1-5** in **30-50  $\mu\text{l}$**  water or TE buffer for **10 min at 37°C**. The DNA content of the **eluate** should be checked **spectrophotometrically (see below)**. To all other samples and the remainder of the eluate add some dye mix for the gel analysis and run them on an 0.8 - 1% agarose gel.

Significant amounts of DNA can normally be expected only in samples/lanes **1** (original cleared lysate) and **5** (eluate).

**Lane 1** contains the amount of DNA present in **1 ml of the bacterial culture**. It allows an estimation of the overall plasmid content and so the expectable yield of the culture.

**Lane 2** contains the amount of DNA present in the **flowthrough** of the JETSTAR column. Taking into account the culture volumes given in step 2 of this test procedure and an approximate plasmid content of 4 - 5  $\mu\text{g/ml}$  bacterial culture (standard high-copy plasmid), only the bulk of degraded RNA, but **no or only a faint DNA band** should be visible on the gel.

If you get a DNA band nearly as big as in lane 1, the cleared lysate may contain too much salt, thus indicating a non-binding of the plasmid DNA. Check the ratio (1:1:1) and the volumes of buffers E1, E2 and E3 used for the procedure. If possible, reduce the amount of buffer E3 to 0.9 vol.

**Lanes 3 and 4** contain aliquots of the **wash fractions**. In lane 3 only degraded RNA and eventually trace amounts of plasmid DNA, in lane 4 no nucleic acids or only small amounts of degraded RNA should be visible.

If you encounter significant amounts of DNA in the wash fractions, the salt content of buffer E5 can be too high. Check the bottle used and/or prepare a new buffer E5 with the correct NaCl content of **800 mM in a 100 mM sodium acetate buffer with a pH of 5.0 (adjusted with acetic acid) (preparation protocol available on request)**.

**Lane 5** contains the DNA of the **eluate**. The precipitated DNA from the 900  $\mu\text{l}$ -eluate-aliquot should be sufficient to obtain readable data from the photometer (**see "Examples" below**). The user should use 50% of the DNA solution for spectrophotometric analysis and the remainder for gel analysis. The plasmid DNA in the eluate should be free of genomic DNA, RNA and be mostly supercoiled.

If you get **no DNA** in the eluate and **also no DNA** in the lanes 2, 3 and 4, check the bottle used and/or prepare a new buffer E6 with the correct NaCl content of **1250 mM in a Tris buffer with a pH of 8.5 (adjusted with HCl) (preparation protocol available on request)**.

## Examples

*Midi procedure:* A scientist normally expects up to **100 µg** of plasmid DNA from a JETSTAR Midi prep. If the bacterial culture contains these 100 µg, he will have them in the elution volume of **5 ml** after step 8 of the regular JETSTAR protocol. From a **900 µl-aliquot** of this eluate one can expect **18 µg of DNA**. Precipitated with isopropanol and spun down in a 1.5 ml polypropylene tube this will yield a very good visible DNA pellet. Dissolved in **500 µl of sterile water** the user will get an OD<sub>260</sub> reading of **0.720** in a 1 cm cuvette (1 O.D. at 260 nm are 50 µg/ml DNA). Taking into account that spectrophotometric data in the absorption range from **0.1 to 0.8** are valid, one can determine exactly DNA amounts down to **14 µg in the total eluate** (14 µg in a volume of 5 ml are meaning approximately 2.5 µg in the 900 µl aliquot. Precipitated and dissolved in 500 µl of water these 2.5 µg will give an OD<sub>260</sub> reading of **0.100** in a 1 cm cuvette).

If the total yield of the JETSTAR Midi prep is significantly higher than 100 µg, you will have to dissolve the precipitated DNA sample from the 900 µl-aliquot in a larger volume of water or make suitable dilutions to get readable data from the photometer.

*Maxi procedure:* A scientist normally expects up to **500 µg** of plasmid DNA from a JETSTAR Maxi prep. If the bacterial culture contains these 100 µg, he will have them in the elution volume of **15 ml** after step 8 of the regular JETSTAR protocol. From a **900 µl-aliquot** of this eluate one can expect **30 µg of DNA**. Precipitated with isopropanol and spun down in a 1.5 ml polypropylene tube this will yield a very good visible DNA pellet. Dissolved in **1 ml of sterile water** the user will get an OD<sub>260</sub> reading of **0.600** in a 1 cm cuvette (1 O.D. at 260 nm are 50 µg/ml DNA). Taking into account that spectrophotometric data in the absorption range from **0.1 to 0.8** are valid, one can determine exactly DNA amounts down to **84 µg in the total eluate** (84 µg in a volume of 15 ml are meaning approximately 5 µg in the 900 µl aliquot. Precipitated and dissolved in 1 ml of water these 5 µg will give an OD<sub>260</sub> reading of **0.100** in a 1 cm cuvette).

If the total yield of the JETSTAR Maxi prep is significantly higher than 500 µg, you will have to dissolve the precipitated DNA sample from the 900 µl-aliquot in a larger volume of water or make suitable dilutions to get readable data from the photometer.