

USER GUIDE

for

**Automated purification of genomic DNA
from Gram positive and Gram negative bacteria with**

**KingFisher 96 instrument
and
Invitrogen ChargeSwitch gDNA Mini Bacteria Kit**

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Description

Purification of genomic DNA from Gram negative and Gram positive bacteria with Invitrogen's ChargeSwitch® gDNA Mini Bacteria Kit can easily be automated using KingFisher® instruments (Thermo Fisher Scientific). The KingFisher platforms utilize patented technology where magnetic rods move particles through the processing steps. KingFisher 96 instrument operates on microplates and can process up to 96 samples per run. With the instrument the volumes handled can be up to 1 ml.

Typically, DNA isolation from 0.5 ml liquid culture using KingFisher instruments results up to 12 µg DNA. Generally, DNA yields vary according to sample type and storage conditions.

The protocol described here is designed for general use and can be modified according to customer individual needs using KingFisher® Software provided with the instrument.

Important notes

- See ChargeSwitch gDNA Mini Bacteria Kit user manual for reagent storage, product use limitations, safety information etc.
- Resuspend magnetic beads (DNA Binding Beads) thoroughly before use.

Importing protocols from the web

KingFisher Software protocols for Invitrogen ChargeSwitch gDNA Mini Bacteria Kit can be downloaded from the website (www.thermofisher.com/kingfisher). First you have to **save the file "IVGN_BacNeg_KF96"** for Gram negative Bacteria or **"IVGN_BacPos_KF96"** for Gram positive Bacteria **to your computer**.

1. Open KingFisher Software.
2. Select **Protocol** → **Import/Export data**.
3. Click **Read file**.
4. Select the database (*.KF2) by browsing in the **Open** dialog and click **Open**.
5. Select the protocol(s) you wish to import from the *Protocols in file* list. Use the SHIFT key together with the mouse button to select protocols between two clicked protocols and the CTRL key to select only the clicked protocols.

6. Tick **Update existing** if you wish to overwrite the protocols with identical protocol name(s) in the target database.
7. Click **Import**.

If there are protocols with identical names and you have not ticked the **Update existing** tick box, you will be prompted to change the name of the protocol that is being imported:

- Type in a new name and click **OK**.
 - o **Note:** Check that the name of the protocol does not exceed 17 characters.
- You will receive a message stating whether the database updating procedure was successful or not.

Sending or running a protocol

To send a protocol to the instrument memory, or to run the protocol directly without saving it to the instrument memory:

1. Check that the instrument has been configured correctly and that the instrument is connected to the correct COM port.
2. Open the **Transfer protocol to instrument** dialog from **Instrument** → **Send Protocol to Instrument...**
3. Select the target instrument from the *Select instrument* table.
4. Select a protocol from the *Protocols for selected instrument* table. The protocols in that table are the available protocols in the database.
5. Select either of the following:
 - a. Click **Send protocol** to transfer protocols to the instrument memory.
 - You can launch the protocol using the instrument keypad and display.
 - b. Click **Execute protocol** to launch the protocol directly without transferring it to the instrument memory
 - The protocol will launch after validation.

KingFisher 96 protocol

Sample preparation

- KingFisher DNA protocol **ChargeSwitch gDNA Mini Bacteria Kit** is designed to purify genomic DNA from Gram negative and Gram positive bacteria.
- Use KingFisher Microtiter™ deepwell microplate (Catalog No. 95040450), KingFisher deepwell tip comb (Catalog No. 97002534) and KingFisher 96 KF plate (Catalog No. 97002540) with ChargeSwitch gDNA Mini Bacteria protocol.
- Add sample and other reagents except Elution Reagent (E5) supplied by ChargeSwitch Plasmid ER Mini kit to Microtiter deepwell microplate according to table 1 and instructions below. Add the Elution Reagent (E5) to KingFisher KF96 plate and start the process.

KingFisher 96 process

Table 1 Pipetting instructions for KingFisher 96 and ChargeSwitch gDNA Mini Bacteria protocol.

Plate *	Plate	Content	Sample/ Reagent volume
A	1	Washing Buffer W12	700 µl
A	2	Washing Buffer W12	700 µl
A	3	Resuspension reagent R4 with RNase	100 µl
		Bacteria cell pellet	50 µl
		Proteinase K (added during first Pause step!)	10 µl
		Lysis Buffer L12 (added during first Pause step!)	500 µl
		ChargeSwitch Magnetic Beads (added during second Pause step!)	40 µl
		Binding Buffer B8 (added during second Pause step!)	300 µl
B	4	Elution Reagent E5	150 µl
A	5	Washing Buffer W12	700 µl

* A= Microtiter Deep well plate, B=KingFisher 96 KF plate

- Add 700 µl of Washing Buffer W12 to plates **1, 2 and 5.**

- Add 100 µl of Resuspension reagent and 50 µl of Bacteria cell pellet to plate **3.**
- Add 150 µl of ChargeSwitch Elution Reagent E5 to plate **4.**
- Combine the tip comb and The KingFisher plate. See KingFisher 96 User manual.
- Select the ChargeSwitch gDNA Mini Bacteria protocol using arrow keys from the instrument and press START button OR execute protocol using KingFisher software. Select either IVGN_BacPos_KF96 protocol for Gram positive bacteria or IVGN_BacNeg_KF96 for Gram negative bacteria.
- Load the plates according to protocol request and press START after every plate to confirm the action.
- Note!** Confirm that the plates are placed in correct orientation: A1 well to be pointed to upper right corner of the plate holder in turntable. A1 row of the plate is then always located in the inner circle of the turntable.
- The purification protocol will start when the last plate is loaded and START button is pressed.
- During **first Pause** step add 10 µl of Proteinase K and 500 µl of Lysis Buffer L12 to plate **3.**
- During **second Pause** step add 40 µl of ChargeSwitch Magnetic Beads and 300 µl of Binding Buffer B8 to plate **3.**
- After the purification process is completed the plates are removed according to instructions shown in instrument screen. Press START after each plate removal to confirm the action.
- When the last plate is removed text End_of_run will appear. Press STOP to complete the run.

Description of ChargeSwitch gDNA Mini Bacteria protocol for Gram positive and Gram negative bacteria with KingFisher 96

- Cells are incubated first with Resuspension reagent in plate 3 for 1 minutes with heating. Cells are resuspended during this incubation.
- Lysis buffer and Proteinase K is added during first Pause step to plate 3.
- Resuspended Gram positive bacteria cells are lysed during 10 minutes incubation at 55°C in plate 3. Respectively resuspended Gram negative bacteria cells are lysed during 60 minutes incubation at 80°C in plate 3.
- Magnetic beads and Binding buffer B8 are added to the plate 3 during second Pause step.

5. Lysed cell are incubated with magnetic beads and binding buffer in plate 3 for 3 minutes. Magnetic bead/DNA complexes are formed.
6. Magnetic beads are washed with ChargeSwitch Wash Buffer W12 in plates 1, 2 and 5.
7. DNA is released to ChargeSwitch Elution Reagent E5 in plate 4 for 8 minutes with heating.
8. Beads are discarded into plate 3.

Troubleshooting

1. Magnetic particles are remained in the sample well
 - If starting material is too viscose, the magnetic rods will not be able to collect the particles. Dilute the sample and check that the sample is properly homogenized/lysed.
2. Magnetic particles are attached to the tip combs
 - This will not affect the yield because the sample has been released from the particles.
3. The volumes of the reagent in one well exceeds the limit.
 - It is recommended to keep the given volumes within certain limits to avoid failure in performance of the chemical reactions and the processor.
4. Any steps of the protocol (e.g. sample incubation and elution times) and the reagent volumes can be modified with KingFisher® software.
5. The processor is not working properly
 - Refer to Kingfisher 96 User Manual
6. If you have questions related to ChargeSwitch chemistry see ChargeSwitch gDNA Mini Bacteria Kit User Manual for detailed troubleshooting instructions.

7. Ordering Information

Product no.	Product Description
Thermo Fisher Scientific	
540 05 00	KingFisher 96, 110V-240V, Magnetic particle processor
24073430	KingFisher 96 head for Deep Well plate
97002534	KingFisher 96 tip comb for DW magnets (10 x 10 pcs/box)
97002540	KingFisher 96 KF plate (200 µl), 48 plates/box
95040450	Microtiter Deep Well 96 plate, V-bottom, Polypropylene
Invitrogen	
CS11301	ChargeSwitch gDNA Mini Bacteria Kit

Contact information

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