# STANDARD OPERATING PROCEDURE (SOP)

## Qsep100 - Operation

| Document No: | CAF-SOP-002-V4 | Revision Date: | 02.06.2026 |
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## Policy, purpose and responsibilities

- The goal of this SOP is to ensure a proper operation of the specified instrument.
- Before using the Qsep100 for the first time, please ask Sarah Cattin for a mandatory training.
- The users must accept and sign the usage policies from the CAF before any instrument usage.
- Please immediately report any technical problems or questions to:

cellanalyticsfacility@unifr.ch

or

Sarah.cattin@unifr.ch

026/300.85.79

## Instrument procedure

### I. General information for the Qsep100

- The Qsep100 is a capillary electrophoresis instrument driven by pressure and high voltage.
- The instrument is a fully automated system using pen-shaped disposable gel-cartridges, specially designed for DNA or for RNA measurement.
- A run for single sample takes around 10min.

# II. Instrument Start-Up

- Switch on the Qsep100 (front blue button), the computer and the pump (if not already done)
- Start the Q-Analyzer software by double-clicking on it.
- Click the Connect button on the right to link the instrument
- Purge the system is the software ask for it
- Open the corresponding Cartridge with the needle on top (for RNA or DNA)
- Insert the Cartridge in the instrument following the guide until it clicks and close the cartridge door.
- Click the Latch button to collect the cartridge information
    - If the cartridge is new, run the **HV Check** and the **Calibrate** button
    - If the cartridge has already been used, run a **declog verification** (a drop should appear) and re-calibrate if needed

# III. RNA Cartridge High Sensitivity (NR1)

This cartridge is for a detection up to 1ng/ul, for 100 runs

- **Instrument preparation**
    - Prepare the different buffers and markers:
        - *Separation buffer RNA (1x) = dilute 1/10 in DEPC water*
        - *Dilution buffer RNA (1x) = dilute 1/10 in DEPC water*
        - *Lower Marker RNA (1x) = dilute 1/5 in dilution buffer (5ul in 20ul)*
    - Prepare the tray with the different buffers:
        - *P, W, C = DEPC water*
        - *S = 1x Separation buffer (3ml)*
    - Click the Change Buffer button and insert the Buffer tray once the plateau is in front
    - Insert the Alignment marker (100ul tube with minimum 20ul solution):
        - *1x Low RNA marker in MC-1 position*


- **Sample preparation**
    - Switch on the thermocycle (folder Sarah Cattin, program 0)
    - Samples preparation:
        - Dilute your sample 1/10 in 1x dilution buffer for a final 20ul injection volume (2ul sample volume).
        - Detection limit is 1ng/ul after dilution (best 1-100ng/ul, otherwise dilute 10x more).
    - Insert the diluted sample in the thermocycler and start the program (3min at 70°C, follow by 4°C).
    - Put on ice for minimum 5min

# IV. DNA Cartridge Standard (S2)

This cartridge is for a detection between 20-5000bp, up to 0.1ng/ul, for 200 runs

- Instrument preparation
    - Prepare the different buffers and markers:
        - *Separation buffer RNA (1x) = dilute 1/10 in DEPC water*
        - *Dilution buffer RNA (1x) = dilute 1/10 in DEPC water*
    - Prepare the tray with the different buffers:
        - *P, W, C = DEPC water*
        - *S = 1x Separation buffer (3ml)*
    - Click the Change Buffer button and insert the Buffer tray once the plateau is in front
    - Insert the Alignment markers (100ul tube with minimum 20ul solution, no dilution for DNA):
    - For sample size 20-1000bp (**PCR program**)
        - *1x Alignment 20-1000bp marker in MA-1 position*
        - *1x Size marker 15-622bp in MA-2 position (C109200)*
    - For sample size 20-5000bp (**library program**)
        - *1x Alignment 20-5000bp marker in MB-1 position*
        - *1x Size marker 50-3000bp in MB-2 position (C109300)*
- Sample preparation
    - Dilute your sample 1/10 in 1x dilution buffer for a final 20ul injection volume (2ul sample volume).
    - Detection is best for fragment sample of 0.1-10ng/ul after dilution and for smear samples of 2-50ng/ul.

# V. Sample acquisition

- Recalibrate the Cartridge Calibration Factor on top of the software window (check markers positions)
- Click the Change Sample button and insert your sample tubes (strips or plate, be careful that the lids are open)
- In the Sequence area, click the Open Button
- Select the program (=.seq) and click the Open Button:
    - *Library = for smear and marker 5kb*
    - *PCR = no smear and smaller then 1kb*
    - *mRNA = for RNA*
- "Do you want to append data to the current sequence" = Yes only if you have different experiment in a row, otherwise No
- Click into the Sample position field
- On the right grid, type the sample name or import an excel using the Load Button

- On the left grid, select the sample to be analyzed and click OK (selected place are red)
- In the Result Name upper field, write the systematic name (incorporate in all sample name)
- In the Result Name lower field, select where in the sample sheet to pick up the individual sample information (Sample ID is the default)
- Don't save! And the image is representative from the previous run…
- Click on Run to start the sample acquisition

## VI. Results analysis

- Click the Results tab on the left
- Click the Open File button
- Select the sample files, including the Size Marker file and open them
- RNA sample analysis:
    - **Alignment marker:** Make sure the Lower Marker (LM) and Upper Marker (UM) are well detected and assigned to all files. If needed, user can manually assign the peaks at "Peak" tab
    - Check RNA quality number (RQN): between 1 to 10
    - Check 18S/18S identification: if software canno't identify both regions correctly:
        - Auto assign
        - If needed, drag the red and green line
- DNA sample analysis:
    - **Size Marker Check** (cDNA): Make sure all peaks are well detected and assigned to all files.
    - **Library:** define the Z1 zone for fragment distribution check
- Create a report
    - Goes under Comparison and create a New Folder
    - Chose the samples and drag them on the right
    - Right click on the selected samples (on the right) and Export Report
    - Chose the desired reports format and click OK
    - Export the report in the dedicated folder and take it on a USB drive.

## VII. Instrument Shutdown

- Eject the Cartridge and place it back in vertical position in it's storage box, inside the gel.
- Empty the tray of any liquid
- Put the different buffer back in the fridge and loader back in the freezer
- Disconnect the instrument and quit the program

## SOP revision (annually at minimum)

| Revision | Date | Description of Changes | Reviewed By |
|---|---|---|---|
| V1 – V3 | 28.07.2021 | Initial release and update | Sarah Cattin |
| V4 | 02.06.2026 | Structural changes | Loïc Chentout / Sarah Cattin |
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