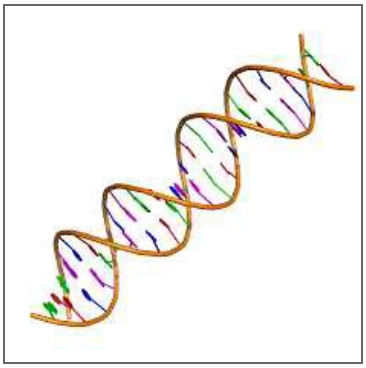

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Protocol status: Working
We use this protocol and it's working

DNA Extraction from Inoculated Broth for WGS using the Maxwell RSC Cultured Cells DNA Kit

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ABSTRACT

The Maxwell® RSC Cultured cells DNA Kit is used with the Maxwell RSC or Maxwell RSC 48 Instrument from Promega. This extraction method purifies genomic DNA (gDNA) efficiently using automation. The kit utilizes a silica-based paramagnetic particle that is used during the purification process.

This protocol will provide information for extraction from broth. Additional steps are performed when extracting DNA from gram-positive organisms.

This SOP is applicable to all GenomeTrakr laboratories with the intent of obtaining high quality, purified genomic DNA from gram-negative or gram-positive bacterial cells for use in subsequent sequencing protocols.

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PROTOCOL integer ID: 93810

Keywords: GenomeTrakr, DNA Extraction, Automation

GUIDELINES

Table 1. Supported Instruments.

Instrument	Cat.#	Technical Manual
Maxwell® RSC	AS4500	TM411
Maxwell® RSC 48	AS8500	TM510
Maxwell® CSC RUO Mode	AS6000	TM573
Maxwell® CSC 48 RUO Mode	AS8000	TM628
Maxprep™ Liquid Handler	AS9100, AS9101, AS9200, AS9201	TM509

PRODUCT	SIZE	CAT.#
Maxwell® RSC Cultured Cells DNA Kit	**48 preps**	**AS1620**

For Research Use Only. Not for use in diagnostic procedures. Sufficient for 48 automated isolations from samples containing up to 5×10^6 tissue culture cells or up to 2×10^9 bacterial cells. Cartridges are for single use only. Includes:

- 48 Maxwell® RSC Cartridge (RSCI)
- 1 Maxwell® RSC Plunger Pack (48 Plungers)
- 50 Elution Tubes (0.5ml)
- 20ml Elution Buffer

Storage Conditions: Store the Maxwell® RSC Cultured Cells DNA Kit at +15°C to +30°C.

MATERIALS

Reagents:

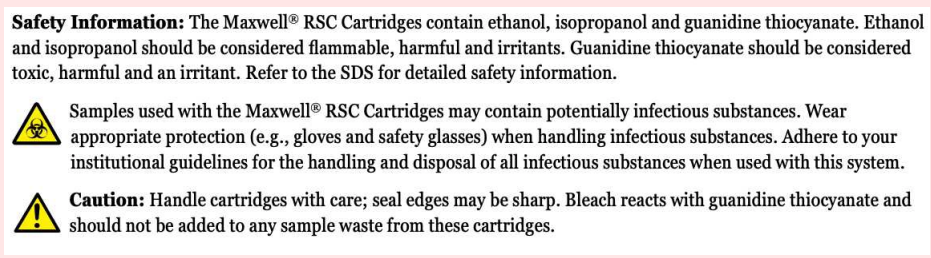
Maxwell® RSC Cultured Cells DNA Kit -- Cat# AS1620 [Promega]
DNase free, RNase free, molecular grade water -- Cat# 10977015 [Invitrogen] *or equivalent*
PureLink™ RNase A (20 mg/ml) -- Cat# 12091021 [ThermoFisher Scientific] *or equivalent*
TE, pH 7.0, RNase-free -- Cat# AM9861 [ThermoFisher Scientific] *or equivalent*
Lysozyme -- Cat# L4919 [Sigma Aldrich] *or equivalent*

Equipment:

Maxwell® RSC or Maxwell® RSC 48 Instrument
Pipettors
Centrifuge
Incubator
Heatblock
Vortexer

Consumables:
2.0 ml Eppendorf Safe-Lock Tubes -- Cat# 05-402-7 [ThermoFisher Scientific]
1.5 ml Eppendorf DNA LoBind Microcentrifuge Tubes -- Cat# 13-698-791 [ThermoFisher Scientific]
Pipette Tips

SAFETY WARNINGS



Refer to the full SDS for detailed safety information.

AS1620-en-US.pdf 369KB

Overnight Cultures

1 Inoculate 3 ml of Trypticase Soy Broth (TSB) or appropriate liquid media with a single colony of bacteria. Incubate for 16-24 hours at 35 ± 2ºC.

Note: This is a suggestion for the majority of bacteria sequenced by the GenomeTrakr network. Follow any specific protocols for other organisms as necessary (ie. *Campylobacter*)

Pre-processing of Gram Positive Cultures

2 Gram-Negative cultures do not require any steps prior to extraction on the Maxwell RSC instrument and can proceed to the **Cultured Cells DNA Cartridge Preparation** step. However, due to the thick peptidoglycan layer of **Gram-Positive** bacteria extra lysing is necessary. Follow the steps below for this pre-processing.

1. Resuspend the Lysozyme in TE buffer to a concentration of 100 mg/ml according to Table 1.
2. Transfer 400 µl of the overnight culture to a pre-labelled 1.5 or 2.0 ml microcentrifuge tube.
3. Centrifuge sample tubes at 8000 RPM for 5 minutes.
4. Remove the supernatant without disturbing the pellet.
5. Resuspend the pellet with 400 µl of the TE-Lysozyme buffer.
6. Vortex for 5-10 seconds
7. Incubate at 37 ºC for 30 minutes.
8. Proceed to **Cultured Cells DNA Cartridge Preparation**

# of Samples + 1 Extra	Total TE Volume (ml)	Lysozyme (g)
6	2.4	0.24
11	4.4	0.44
19	7.6	0.76
21	8.4	0.84
25	10	1
50	20	2

Table 1. Calculations for a 100 mg/ml solution of TE and Lysozyme

Cultured Cells DNA Cartridge Preparation

3 1. Turn on the instrument and associated PC. Start the RSC software and wait for the instrument to initialize.

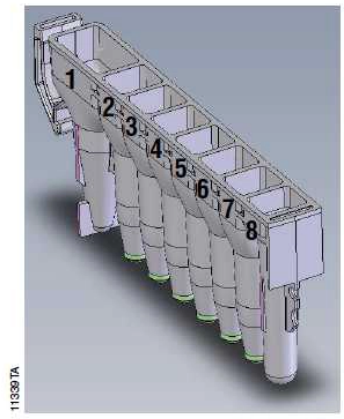
2. Open the door of the instrument by pressing the **Door** icon at the top right corner of the software.

3. Remove the deck tray(s) from the instrument. Press the **Door** icon to close the instrument door.

4. Place the number of cartridges needed for the number of samples into the deck tray. The cartridges are inserted with **Well 1 (lysis buffer)** at the top of the tray. Figure 1 illustrates the well locations on the cartridge. Press down on the cartridge to snap it into position.

5. Carefully peel back the seal on the cartridges. Ensure that the entire seal is removed.

6. Add a plunger to **Well 8** in each cartridge.



User Adds to Wells

1. Cultured Cell sample (up to 5×10^6 tissue culture cells; up to 2×10^9 bacterial cells)
8. RSC Plunger

Figure 1. Cartridge setup

7. Place elution tubes (provided with kit) into the elution tube position with the lid facing the bottom of the deck tray (Figure 2). Add 150 µl of **Elution buffer** to the each tube. Ensure there are no bubbles in the tubes, and the buffer is at the bottom of each tube.

Figure 2. Elution Buffer Tube and Lid positioning

8. Transfer 400 µl of the sample culture to the lysis buffer in **Well 1** of the corresponding cartridge and pipette gently up and down 10 times to mix. Proceed to the **Instrument Setup and Extraction Run** step.

9. **Optional: RNase A Treatment**
   a. Allow cells to lyse for 10 minutes in **Well 1**.
   b. Add 2 µl of RNase A to each sample in **Well 1.** Pipette up and down to mix.
   c. Incubate for an additional 10 minutes prior to starting the instrument.

Instrument Setup and Extraction Run

4

1. In the RSC Software, press **Start** to access the extraction method selection screen.

2. Select **Cultured Cells DNA** method, then press **Proceed**.

3. Select the lanes being used by touching them. Sample names can be inputted at this time.
   Note: For the RSC48, press the "BACK" and "FRONT" buttons on the bottom of the screen to move to each respective rack.

4. Press **Proceed** to continue. The instrument door will open.

5. Install the deck tray(s) into the platform by angling the back of the tray and inserting first. Press down the front of the tray until it snaps into place. Ensure the deck tray is level and fully seated.

Note: For the RSC48, ensure the tray labelled FRONT and BACK are placed in the correct places in the instrument.

6. Verify that all of the cartridges are properly inserted into the tray, the elution tubes are present and uncapped, and then plungers are in **Well 8.**

Note: Ensure there are no old plungers left on the instrument from a prior run.

7. Press **Start** to begin the extraction run.

8. Follow on-screen instructions at the end of the run.

9. Remove the deck tray(s) from the instrument. The DNA can be transferred to 1.5 ml LoBind Tubes for storage.

10. Remove the cartridges and plungers from the deck trays and discard in a biohazard container.

11. Clean the tray with 70% Ethanol. **Do NOT use bleach to clean the trays as this reacts with Guanidine Thiocyanate.**

12. Proceed to the **Cleaning and Decontamination** steps.

13. Proceed to quantify the DNA using the **DNA Quantification using the Qubit Fluorometer** SOP.

Cleaning and Decontamination

5 The Maxwell RSC instrument should be wiped down with 70% Ethanol as needed.

Follow up each extraction with the **Sterilization** process on the instrument.