Jul 08, 2019

BiomekFXp Robot Minipreps (RoboPreps)

James Angstman[1]

[1]Harvard University

Working dx.doi.org/10.17504/protocols.io.3zqgp5w

The Center for Genome Editing and Recording

James Angstman
Harvard University

ABSTRACT

RoboPreps are great for high-throughput cloning and subcloning, especially if you're going to be doing Maxipreps afterward anyway. RoboPreps must be done in 96 well format as of now, though future generations may one day break through this technological barrier.

RoboPreps.bmf qialyse.bmf BiomekFXp_Robot_Minipreps_(RoboPreps)_.pdf

GUIDELINES

qialyse.bmf

RoboPreps.bmf

.bmf files for program, also available in the Abstract.

MATERIALS

NAME	CATALOG #	VENDOR
Sera-Mag SpeedBead Carboxylate-Modified Magnetic Particles (Hydrophobic), 15 mL	65152105050250	Ge Healthcare
Biomek FX or FXP equipped with a 96-channel pod and P200 head		Beckman Coulter
Biomek AP96 P250 Pre-Sterile Tips with barrier	717253	Beckman Coulter
Abgene™ 96 Well 1.2mL Polypropylene Deepwell Storage Plate	AB1127	Thermo Scientific
Agilent filter microplate 96-well polypropylene with 0.7 µm glass fiber membrane 800 µL/well long drip 25/pk	200937-100	Agilent Technologies

MATERIALS TEXT

Filter block: - We use something similar to Agilent's 200937-100- but cheaper products should yield a similar result. All you need is something to filter out precipitates. As long as the total height of the filter stacked on top of the low-profile block is at least 55 mm, you're good to go.

A robot: - We use the BiomekFXp, with a shaking peltier device installed, but you can probably adapt other robots to perform similarly or train an undergrad to perform these steps. Use barrier tips if possible. Make sure you adapt the programs attached to fit your robot.

Low-profile 96-well blocks: -Thermo AB1127

SPRI Beads: - 20% PEG, 2.5 M NaCl buffer. Make it from scratch using Sigma 65152105050250, save your lab buckets of cash.

You will also need:
- 1ml 70% ethanol in a 96-well Axygen assay block
- 65 µl 0.1x Elution Buffer in a 96-well semi-skirted plate
- 165 µl of a 1:1 mixture of Bootleg SPRI Beads :Isopropanol in a 96-well semi-skirted plate
- An empty 96-well block for waste
- An empty semi-skirted plate to collect the eluted plasmid

- 3 200 µl barrier tip boxes

SAFETY WARNINGS

See SDS (Safety Data Sheet) for hazards and safety guidelines.

Cell Prep

1 Pick colonies into **1 ml LB cultures** into a 96-well assay block and grow at **37 °C** overnight with shaking.

2

Pellet the cells by spinning at **2000 x g** for 5-15 minutes **00:05:00** .

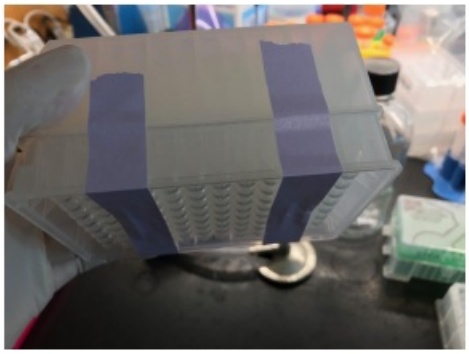
2.1 Remove the media by quickly inverting the block over a waste container.

If intended for later use, you can freeze the block in **-20 °C** for several weeks or keep it at **4 °C** for hours.

Pre Qialyse2

3 For a batch lysis, load the pelleted cultures onto the BiomekFXp robot using the **qialyse2** program.

3.1 For the **qialyse2** program, you need to assemble a two-component filter consisting of a low-profille **1.2 ml 96-well block** and a **96-well filter block**. They should be affixed to each other using tape as shown before loading on to the robot deck.



Like this.

And this.

For simplicity, use inverted tip-box covers as reservoirs for the resuspension, lysis, and neutralization buffers.

Qiagen Buffers P1, P2, and P3 can be used as the resuspension, lysis, and neutralization buffers (to be put in the reservoirs on the robot deck), but similar home-made buffers should be fine.

Qialyse2 Steps

4 Resuspend cell pellet in **80 µl P1 buffer** and shake.

5 Add **80 µl P2** and shake.

6 Pause for **00:02:00** .

7 Add **80 µl P3 buffer** and shake for **00:01:40** (100 seconds).

8 Transfer contents to assembled filter apparatus.

After Qialyse2

9 

Spin down the lysate through the filter block at **1000 x g** for at least **00:05:00** (longer is fine).

Make sure to balance with a blank filter. Another blank with improper dimensions may cause an imbalance error, even if the weight matches the filter.

10 Collect the block and remove the filter.

Take care not to jostle the low-profile 96-well block, since the flow-through can easily splash between wells.

11 Transfer the flow-through to an Axygen 96-well block and load it on to the robot using the RoboPrep program.

RoboPrep Steps

12 

Mix filtered cell lysate with **165 µl SPRI-Bead binding buffer**

13 

Incubate **Room temperature** for **00:05:00** .

14 Place on magnet and allow separation.

15 Remove Supernatant.

16 

Wash with 70% EtOH supplemented with 1% TritonX-114.

17 

Wash with 70% EtOH. (1/3)

18 Wash with 70% EtOH. (2/3)

19 

Wash with 70% EtOH. (3/3)

20 Remove all ethanol and dry beads for **00:05:00** .

21 Resuspend beads in **50 µl elution buffer**.

22 

Remove from beads.