QIAGEN Supplementary Protocol:

Purification of DNA from human whole blood and buffy coat using the FlexiGene® DNA AGF3000 Kit on the AutoGenFlex 3000

This protocol describes the steps for setting up the AutoGenFlex 3000 workstation and the steps for DNA purification from fresh or frozen human whole blood or buffy coat samples using the AutoGenFlex 3000 workstation.

IMPORTANT: Please read the *FlexiGene DNA AGF3000 Handbook*, paying careful attention to the "Safety Information" and "Important Notes" sections, before beginning this procedure.

Equipment and reagents to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

- AutoGenFlex 3000 workstation (AutoGen, www.autogen.com)
- 100% isopropanol (e.g., reagent R4 of the AutoGenFlex Blood DNA Finishing Kit, AutoGen, cat. no. AGFXPW)*
- 70% ethanol† (e.g., reagent R5 of the AutoGenFlex Blood DNA Finishing Kit, AutoGen, cat. no. AGFXPW)*
- High-quality distilled water
- Phosphate-buffered saline (PBS) may be required for adjusting sample volumes and is always required for buffy coat samples
- 5-Hole Tube Units (cat. no. 19589)
- 5-hole tube cap (AutoGen, cat. no. AGPC2000)
- 1.5 ml reaction tubes
- Pipets and sterile, DNase-free pipet tips with aerosol barrier
- Vortex mixer (e.g., Vortex-Genie® 2, VWR, cat. no. 444Q5900)*
- AutoGenFlex Vortex Adaptor (AutoGen, cat. no. AGFVA20, supplied with AutoGenFlex workstations)
- Heating block for 1.5 ml reaction tubes (e.g., Eppendorf® Thermomixer Compact, cat. no. 5350000.013)*

* This is not a complete list of suppliers and does not include many important vendors of biological supplies.

† Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

- The amount of QIAGEN Protease is sufficient for 16 runs of 40 samples each or 32 runs of 20 samples each. When processing batches of 20 samples, the runs must be performed in pairs with less than 48 hours between the 2 runs; otherwise extra QIAGEN Protease is required (cat. no. 19155 or 19157).
- For frozen samples: Water bath at 37°C

Protocol: Setting up the AutoGenFlex 3000 workstation

This protocol describes the steps for setting up the AutoGenFlex 3000 workstation. For setting up the AutoGenFlex STAR workstation, see the *FlexiGene DNA AGF3000 Handbook*.

Important point before starting

- Ensure that you are familiar with operating the AutoGenFlex 3000 workstation. Refer to the workstation user manual for operating instructions.

Things to do before starting

- Resuspend the lyophilized QIAGEN Protease in 7 ml Buffer FG3 (hydration buffer) to make a QIAGEN Protease stock solution. Dissolved QIAGEN Protease should be stored at 2–8°C or in aliquots at –20°C.
- Prepare a QIAGEN Protease working solution by diluting QIAGEN Protease stock solution 1/10 with high-quality distilled water (e.g., mix 3.5 ml QIAGEN Protease stock solution and 31.5 ml high-quality distilled water). The QIAGEN Protease working solution can be stored at room temperature on the instrument for up to 48 hours and reused in subsequent runs. If the solution has been stored more than 48 hours at room temperature, discard it and prepare a new QIAGEN Protease working solution.
- Dispense the QIAGEN Protease working solution into a 125 ml Nalgene® bottle (supplied with the instrument).

Procedure

1. **Connect the FlexiGene reagent bottles to the dispenser tubing of the AutoGenFlex workstation in the order given in Table 1 (page 3).**

   The volumes in the table are the minimal fill volumes of the reagent bottles required for a run of 10, 20, 30, or 40 samples. The instrument will consume only a portion of these volumes for priming the tubing and for purification, and it will pump the void volume back into the reagent bottles.

   Note: QIAGEN Protease working solution is stable at room temperature on the instrument for up to 48 h. If the solution has been kept longer at room temperature, prepare a fresh QIAGEN Protease working solution and use it.

Table 1. Minimal fill volumes of reagent bottles

Dispenser channel	Reagent	Minimal fill volume (ml)			
		10 samples	20 samples	30 samples	40 samples
1	Buffer FG1	60	100	140	180
2	Buffer FG2	30	40	50	60
3	Buffer FG3	110* 70†	200* 120†	290* 170†	380* 220†
4	Isopropanol	30	40	50	60
5	70% ethanol	30	40	50	60
6	QIAGEN Protease working solution	35‡	35‡	35§	35§
7	Not used	–	–	–	–

* When processing fresh whole blood or buffy coat samples.

† When processing frozen whole blood or buffy coat samples.

‡ After a single run of 10 or 20 samples, the bottle of QIAGEN Protease working solution can be left on the instrument and used for subsequent runs (up to 40 samples in total) if the subsequent runs will occur within 48 h. After the final run (and if subsequent runs will **not** occur within 48 h), remove the bottle of QIAGEN Protease working solution from the instrument, and store it at 2–8°C. For the next run, bring the volume up to 35 ml with fresh QIAGEN Protease working solution, and reconnect the bottle to the instrument.

§ After a run of 30 or 40 samples, remove the bottle of QIAGEN Protease working solution from the instrument, and store it at 2–8°C. For the next run, bring the volume up to 35 ml with fresh QIAGEN Protease working solution, and reconnect the bottle to the instrument.

2. **Make sure that the 4 centrifuge buckets are correctly inserted into the centrifuge. Make sure that the tube unit waste is empty and liquid waste containers have enough capacity for the waste from the run.**
3. **Switch on the instrument using the power switch.**
4. **In the instrument display, select "FG Blood Fresh" using the keypad, and press "ENTER".**
   On some instruments, this protocol is called "Protocol 1 (FG DNA Blood)".
5. **Select "MANUAL", and press "ENTER".**
   The instrument will perform a system check.
6. **After the LCD displays that the system check was successful, select "DISPENSER", and press "ENTER".**

7. **If all reagent bottles are already connected to the dispenser tubes from a previous run, proceed directly to step 8. If the QIAGEN Protease working solution is connected for the first time (or reconnected after storage at 2–8°C) and all other reagent bottles are already connected, follow step 7a. If the QIAGEN Protease working solution and other reagent bottles are connected for the first time (or reconnected after storage at 2–8°C), follow step 7b.**

7a. **All reagent bottles except QIAGEN Protease already connected: Select dispenser "6" and press "ENTER". Select speed "3" and press "ENTER". Type in *7.00 ml* and press "ENTER". Press "START". Repeat step 7a once more and then continue with step 8.**

7b. **QIAGEN Protease and other reagent bottles not already connected: Select dispenser "8" and press "ENTER". Select speed "3" and press "ENTER". Type in *7.50 ml* and press "ENTER". Press "START". Repeat step 7b once more and then continue with step 9.**

Selecting dispenser "8" will prime all channels simultaneously.

Skip step 8 and continue with step 9.

8. **Select dispenser "8" and press "ENTER". Select speed "3" and press "ENTER". Type in *1.00 ml* and press "ENTER". Press "START" and continue with step 9.**

Selecting dispenser "8" will prime all channels simultaneously.

Note: This step is not required after step 7b.

9. **Press "ESC" 3 times to return to the main menu.**

10. **Continue with "Protocol: Purification of DNA from 1–5 ml whole blood or buffy coat samples", below, or "Protocol: Purification of DNA from 1–5 ml frozen whole blood or buffy coat samples", page 7.**

Protocol: Purification of DNA from 1–5 ml whole blood or buffy coat samples

This protocol describes the steps for DNA purification using the AutoGenFlex 3000 workstation. For DNA purification using the AutoGenFlex STAR workstation, see the *FlexiGene DNA AGF3000 Handbook*.

Important point before starting

- This protocol is suitable for processing 1–5 ml fresh whole blood samples or fresh buffy coat samples derived from 1–5 ml fresh whole blood. An even number of 5-hole tube units must be used for balancing the AutoGenFlex centrifuge (i.e., for 10, 20, 30, or 40 samples).

Things to do before starting

- Set up the AutoGenFlex STAR workstation as described in "Protocol: Setting up the AutoGenFlex 3000 workstation", page 2.
- Equilibrate up to 40 whole blood or buffy coat samples to room temperature (15–25°C).

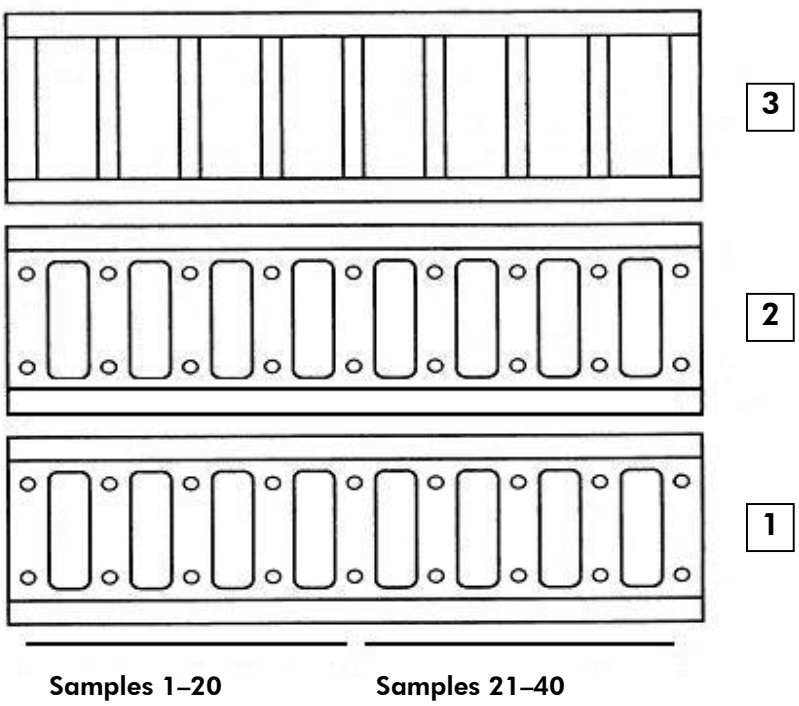

Figure 1. Schematic diagram of the AutoGenFlex worktable.
1. Rack position 1, with up to eight 5-hole tube units with blood samples.
2. Rack position 2, with up to eight empty 5-hole tube units for DNA recovery.
3. Heating block.

Procedure

1. **Open the upper left door of the AutoGenFlex workstation, and remove the tube unit rack 1 from the worktable.***

2. **Load the rack with up to eight 5-hole tube units.**
   One hole of a tube unit is used per sample. Up to 40 samples (8 tube units) can be prepared in a single run.

* See Figure 1, above.

3. **Pipet whole blood (1–5 ml) or buffy coat (derived from 1–5 ml whole blood), equilibrated to room temperature, into a hole of a 5-hole tube unit in a tube-unit rack. All samples in a single procedure must have the same volume. Adjust all buffy coat samples to 5 ml using PBS. If the volume of a whole blood sample needs to be adjusted, add the appropriate volume of PBS. Also add the appropriate volume of PBS to empty holes.**

   Note: An even number of 5-hole tube units must be used for balancing the AutoGenFlex centrifuge (i.e., for 10, 20, 30, or 40 samples).

4. **Load the tube-unit rack, containing up to 8 tube units filled with blood samples, into rack position 1 on the worktable of the AutoGenFlex workstation.***

5. **Load a tube-unit rack, containing the corresponding number of empty 5-hole tube units, into rack position 2 on the worktable of the AutoGenFlex workstation.***

6. **Close the upper left door and make sure that all doors on the workstation are closed.**

7. **Switch on the AutoGenFlex workstation using the power switch.**

8. **In the instrument display, select "FG Blood Fresh" using the keypad, and press "ENTER".**

   On some instruments, this protocol is called "Protocol 1 (FG DNA Blood)".

9. **Select "AUTOMATIC RUN", and press "ENTER".**

10. **Type in the total number of samples (*10*, *20*, *30*, or *40*), and press "ENTER".**

11. **Select file number "1", and press "ENTER".**

12. **Select system check number "2 (Circulate)", and press "ENTER".**

13. **Check that the reagent bottles contain the volumes of reagents listed in Table 1 (page 3), and press "ENTER" again.**

14. **Check that all buckets are inserted in the centrifuge rotor, and press "START".**

    The instrument will perform a system check.

15. **After the LCD displays that the system check was successful, press "START" again to start the automatic run.**

16. **At the end of the protocol run, switch off the AutoGenFlex workstation, and remove the tube-unit rack with the DNA samples from rack position 2 of the worktable.***

17. **Cap the 5-hole tube units with 5-hole tube caps (AutoGen, cat. no. AGPC2000), and place the 5-hole tube units into the AutoGenFlex Vortex Adaptor (AutoGen, cat. no. AGFVA20, supplied with the AutoGenFlex workstation).**

18. **Vortex the 5-hole tube units with the DNA samples for at least 10 min at full speed on a vortex mixer.**

19. **Transfer the eluates into 1.5 ml reaction tubes.**

    Make sure to transfer any undissolved DNA, which may have a jelly-like consistency.

* See Figure 1, page 5.

20. **If DNA is not completely dissolved after vortexing, heat the eluates for 1 h at 65°C with agitation, and vortex again for 10 min at high speed.**

    Note: For heating the eluates, a thermomixer is recommended. Use 1100 rpm for agitation speed.

21. **Clean the AutoGenFlex workstation.**

    Follow the maintenance instructions in the *AutoGenFlex 3000 User Manual*.

Protocol: Purification of DNA from 1–5 ml frozen whole blood or buffy coat samples

This protocol describes the steps for DNA purification using the AutoGenFlex 3000 workstation. For DNA purification using the AutoGenFlex STAR workstation, see the *FlexiGene DNA AGF3000 Handbook*.

Important point before starting

- This protocol is suitable for processing 1–5 ml frozen whole blood samples or frozen buffy coat samples derived from 1–5 ml fresh whole blood. An even number of 5-hole tube units must be used for balancing the AutoGenFlex centrifuge (i.e., for 10, 20, 30, or 40 samples).

Things to do before starting

- Set up the AutoGenFlex 3000 workstation as described in "Protocol: Setting up the AutoGenFlex 3000 workstation", page 2.
- Thaw and equilibrate up to 40 whole blood or buffy coat samples to room temperature (15–25°C). The samples should be thawed quickly in a 37°C water bath with mild agitation.

Procedure

1. **Open the upper left door of the AutoGenFlex workstation, and remove the tube unit rack 1 from the worktable.***

2. **Load the rack with up to eight 5-hole tube units.**

   One hole of a tube unit is used per sample. Up to 40 samples (8 tube units) can be prepared in a single run.

3. **Pipet whole blood (1–5 ml) or buffy coat (derived from 1–5 ml whole blood), equilibrated to room temperature, into a hole of a 5-hole tube unit in a tube-unit rack. All samples in a single procedure must have the same volume. Adjust all buffy coat samples to 5 ml using PBS. If the volume of a whole blood sample needs to be adjusted, add the appropriate volume of PBS. Also add the appropriate volume of PBS to empty holes.**

   Note: An even number of 5-hole tube units must be used for balancing the AutoGenFlex centrifuge (i.e., for 10, 20, 30, or 40 samples).

* See Figure 1, page 5.

4. **Load the tube-unit rack, containing up to 8 tube units filled with blood samples, into rack position 1 on the worktable of the AutoGenFlex workstation.***
5. **Load a tube-unit rack, containing a corresponding number of empty 5-hole tube units, into rack position 2 on the worktable of the AutoGenFlex workstation.***
6. **Close the upper left door and make sure that all doors on the workstation are closed.**
7. **Switch on the AutoGenFlex workstation using the power switch.**
8. **In the instrument display, select "FG Blood Frozen".**
9. **Select "AUTOMATIC RUN", and press "ENTER".**
10. **Type in the total number of samples (*10*, *20*, *30*, or *40*), and press "ENTER".**
11. **Select file number "1", and press "ENTER".**
12. **Select system check number "2 (Circulate)", and press "ENTER".**
13. **Check that the reagent bottles contain the volumes of reagents listed in Table 1 (page 3), and press "ENTER" again.**
14. **Check that all buckets are inserted in the centrifuge rotor, and press "START".**
    The instrument will perform a system check.
15. **After the LCD displays that the system check was successful, press "START" again to start the automatic run.**
16. **At the end of the protocol run, switch off the AutoGenFlex workstation, and remove the tube-unit rack with the DNA samples from rack position 2 of the worktable.***
17. **Cap the 5-hole tube units with 5-hole tube caps (AutoGen, cat. no. AGPC2000), and place the 5-hole tube units into the AutoGenFlex Vortex Adaptor (AutoGen, cat. no. AGFVA20, supplied with the AutoGenFlex workstation).**
18. **Vortex the 5-hole tube units with the DNA samples for at least 10 min at full speed on a vortex mixer.**
19. **Transfer the eluates into 1.5 ml reaction tubes.**
    Make sure to transfer any undissolved DNA, which may have a jelly-like consistency.
20. **If DNA is not completely dissolved after vortexing, heat the eluates for 1 h at 65°C with agitation, and vortex again for 10 min at high speed.**
    Note: For heating the eluates, a thermomixer is recommended. Use 1100 rpm for agitation speed.
21. **Clean the AutoGenFlex workstation.**
    Follow the maintenance instructions in the *AutoGenFlex 3000 User Manual*.

* See Figure 1, page 5.

Troubleshooting

For general troubleshooting, consult the Troubleshooting Guide in the *FlexiGene DNA AGF3000 Handbook*.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

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