invitrogen™

GeneCatcher™ gDNA 96 × 10 ml Automated Blood Kit

For automated purification of DNA from 3–10 ml human blood

Catalog no. CS21110-96

Version D
13 December 2007
25-0867

User Manual

Table of Contents

Kit Contents and Storage

Shipping and Storage

All components of the GeneCatcher™ gDNA 96 × 10 ml Automated Blood Kit are shipped at room temperature. Upon receipt, store components as follows:

- Store Protease at 4°C
- Store the remaining kit components at room temperature

All components are guaranteed stable for 6 months when stored properly.

Contents

The components supplied in the GeneCatcher™ gDNA 96 × 10 ml Automated Blood Kit are listed below.

The reagents supplied are sufficient to perform 96 purifications of 3-10 ml blood samples.

Component	Quantity
GeneCatcher™ Magnetic Beads (25 mg/ml in 16 mM acetate buffer, pH 4.0)	16 ml
Protease (25 mg/ml in 50 mM Tris-HCl, pH 8.5, 5 mM $CaCl_2$, 50% glycerol)	10 ml
Protease Buffer (20 mM Tris-HCl, pH 8.5, 6 M guanidine HCl)	2 x 310 ml
5X GeneCatcher™ Lysis Buffer (L12)	2 x 485 ml
GeneCatcher™ Wash Buffer (W12)	110 ml
GeneCatcher™ Elution Buffer (E5; 10 mM Tris-HCl, pH 8.5)	125 ml

Note

Since the amount of reagents used depends on the starting volumes of blood samples, some reagents might be in excess in the kit after performing the specified number of purifications.

Accessory Products

Additional Products

Special hardware and disposable items **required** for the Automated Purification can be purchased as "Carrier Sets" (see page 5).

The table below lists additional products available from Invitrogen that may be used with the GeneCatcher™ gDNA 96 x 10 ml Automated Blood Kit.

A large selection of ChargeSwitch® products for purification of nucleic acid and products for downstream applications such as PCR and qPCR are available from Invitrogen. For more information, visit www.invitrogen.com or contact **Technical Support** (page 25).

Product	Quantity	Catalog no.
GeneCatcher™ gDNA Blood Kit (0.3-1 ml)	96 preps	CS21101
GeneCatcher™ gDNA Blood Kit (3-10 ml)	200 ml	CS21110
Quant-iT™ DNA Assay Kit, High Sensitivity	1000 assays	Q33120
Quant-iT™ DNA Assay Kit, Broad-Range	1000 assays	Q33130
Quant-iT™ PicoGreen® dsDNA Assay	1 kit, 1 ml	P7589

Introduction

Overview

Introduction

The GeneCatcher™ gDNA 96 x 10 ml Automated Blood Kit allows scalable and automated extraction of genomic DNA (gDNA) from large volume blood samples including archived or poorly stored blood samples.

Genomic DNA is extracted from all types of blood samples using the cost-effective, user-friendly GeneCatcher™ Technology that eliminates the use of centrifugation and hazardous organic solvents. For more information on the GeneCatcher™ Technology, see next page.

The purified DNA is suitable for use in any downstream application of choice including qPCR, multiplex PCR and restriction enzyme digestion.

The automated purification of blood samples has been developed on the Tecan Freedom EVO™ liquid handling system capable of handling large volumes. See page 4 for further details.

Advantages

The GeneCatcher™ gDNA 96 x 10 ml Automated Blood Kit provides a flexible solution for large-scale genotyping and biobanking projects due to the following advantages:

- Automated scalable extraction of gDNA from up to 10 ml blood samples using a liquid handling robot capable of handling large volumes
- Capability to purify DNA from up to 40 blood samples per day
- Suitable for use on all types of blood samples including fresh, frozen, or poorly stored blood samples with a less than 1% sample failure rate
- Simple and robust purification procedure
- Minimal contamination with RNA
- High purity and yield of the extracted genomic DNA
- High long-term stability of the purified genomic DNA
- Reliable performance in downstream applications including southern blotting, RFLP analysis, PCR, qPCR, multiplex PCR, STR profiling, and restriction enzyme digestion

Continued on next page

The GeneCatcher™ Technology

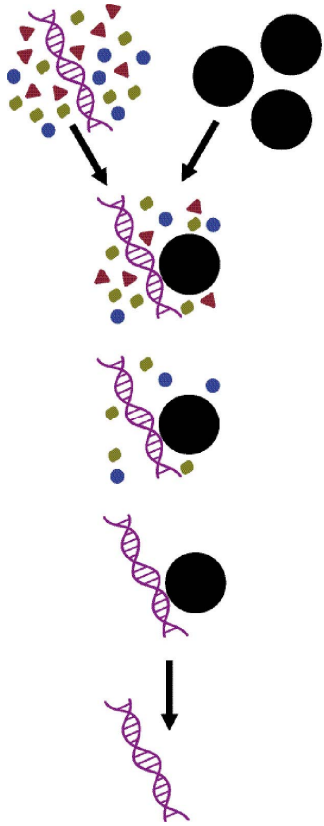
The GeneCatcher™ Technology is a novel magnetic bead-based technology that is designed to work on a wide range of blood samples including archived or poorly stored blood samples to facilitate genomic DNA purification.

See figure below for details.

Step 1–DNA Capture–Cells are lysed and crude DNA is captured on magnetic beads leaving most of the cell debris, hemoglobin, and other proteins behind in solution.

Step 2–DNA Purification–Any residual protein is digested using the Protease and then washed away to leave pure intact DNA.

Step 3–Elution–The pure DNA is then eluted into a small volume ready for use in any downstream applications.



System Specifications

Starting Material:	3-10 ml blood
Bead Binding Capacity:	>200 µg/mg beads
Bead Size:	~5 µm
Bead Concentration:	25 mg/ml
Elution Volume:	~1 ml
gDNA Yield*:	Up to 500 µg

*The gDNA yield depends on the sample volume and white blood cell count. Heparin blood may result in lower yields than blood treated with citrate or EDTA.

Automation Requirements

Automated Liquid Handling

The GeneCatcher™ chemistry is ideal for purification of DNA using liquid handling robots, without the need for centrifugation steps, vacuum manifolds and hazardous organic solvents.

You can use any liquid handling robot that is capable of aspirating and dispensing large volumes (5 ml) and allows flexibility for a deck configuration suitable for processing 3-10 ml blood samples.

The purification of gDNA from blood using the GeneCatcher™ gDNA 96 x 10 ml Automated Blood Kit has been developed and tested on the Tecan Freedom Evo® platform. The open platform of the system is capable of handling large volumes and offers flexibility for a specific deck configuration, required for the purification of gDNA from 3-10 ml blood samples. The system enables easy incorporation of new improved features onto the platform and to software scripts.

For help in setting-up the Tecan Freedom Evo® platform and acquiring the required hardware, disposable items, and software, contact Invitrogen Technical Support (page 25) and Tecan Customer Service (www.Tecan.com).

Hardware and Software Requirements

You will need the following hardware items and software to perform the automated processing of blood samples using the GeneCatcher™ gDNA 96 x 10 ml Automated Blood Kit:

- Tecan Freedom EVO® 1.5 m deck liquid handling robotic workstation
- GeneCatcher™ specific robotic hardware and disposable items of the "Carrier Sets" (see next page)
- Gemini™ or EVOware® software available from Tecan

  Note: The performance of the GeneCatcher™ gDNA 96 x 10 ml Automated Blood Kit was tested using the Gemini™ software.

We recommend that you work closely with the Tecan Costumer Support (www.tecan.com) to install and set-up the instrument for your requirements.

Read the recommendations on page 10 before you start operating Tecan Freedom EVO® platform to process your blood samples.

Continued on next page

Carrier Sets

Introduction

Carrier Sets contain specific hardware and disposable items that allow you to configure your Tecan Freedom EVO® platform for the automated purification using the GeneCatcher™ gDNA 96 x 10 ml Automated Blood Kit. You will need one Invitrogen Carrier Set and one Tecan Carrier Set for processing your samples (see **Note** below).

The Carrier Set items are not preinstalled when purchasing a Tecan Freedom EVO®.

If you are purchasing a new Tecan Freedom EVO® be sure to order the Carrier sets in addition to your instrument. If you already have a Tecan Freedom EVO® platform you need to purchase one of each the Carrier Sets to perform the automated purification using the GeneCatcher™ gDNA 96 x 10 ml Automated Blood Kit (see **Note** below).

The Invitrogen Technical Support (see page 25) will direct you to your sales representative, who will guide you through the process of getting all the items you need.

The Tecan Customer Support (www.tecan.com) will work closely with you to provide you with the Tecan Carrier Set and to set-up your Tecan Freedom EVO® platform according to your specific needs.

Note

The required quantities of some of the items in the Carrier Sets depend on the number of samples processed per day.

If you process up to 24 samples per day you will need to purchase only one of each Carrier Sets.

If you are processing 25 to 40 samples per day, we recommend purchasing one of each Carrier Set and the following additional items:

- GeneCatcher™ Automation Magnet (Invitrogen Carrier Set)
- Dry Block Heater Techne DB-2A (Tecan Carrier Set)
- Dry Block Heater Insert (Tecan Carrier Set)

Consult the Invitrogen Technical Support (see page 25) and Tecan Customer Support (www.tecan.com) for purchasing details and deck set-up guidance.

Continued on next page

Carrier Sets, Continued

Invitrogen Carrier Set

The table below lists the items of the **Invitrogen Carrier Set** available from Invitrogen that allows you to configure your Tecan Freedom EVO® platform.

Invitrogen Carrier Set Item	Quantity	Catalog no.
GeneCatcher™ Automation Magnet	1	CS21103
GeneCatcher™ 5 ml Disposable Tips	5 x 200*	IS91100

*You need 1,000 Tip adapters per 96 purifications

Tecan Carrier Set

The table below lists the items of the **Tecan Carrier Set** available from Tecan (www.tecan.com) for the deck set-up of your Tecan Freedom EVO® platform.

Tecan Carrier Set Item	Quantity
50 ml Tube Holder (8 positions)	1
Dry Block Heater Techne DB-2A	1
Dry Block Heater Insert	1
IKA Shaker KS130 including Base Plate	1
IKA Shaker Base Plate Modification	1
5 ml Tip Box Holder	6
Te Catch Modification (drip tray)	1
Large Volume Reagent Trough (500 ml)	1
Wash Station	2
Carrier for 1.5 ml Tubes (16 positions)	1
Carrier for Flat Beaker 3 Position, 384 MTP	1
Waste slide for 2 Disposable Tip Racks	2
Carrier for 3 Disposable Tip Racks	1
Carrier for 3 Additive Troughs	2
Carrier for Tubes 13 mm (16 positions)	1
Reagent Troughs (100 ml) (disposable)	5

Methods

General Information

Introduction

Review the information in this section before starting the **Automated Protocol 1** on page 16.

Blood Samples

The GeneCatcher™ gDNA 96 x 10 ml Automated Blood Kit is designed to purify high yields of gDNA from all types of human blood samples ranging in volume from 3-10 ml.

Examples for various types of blood samples that can be processed with this kit are listed below:

- Fresh, whole blood
- Blood collected in the presence of anti-coagulants such as EDTA, citrate, or heparin
- Frozen blood samples or blood samples exposed to repeated freeze-thaw cycles
- Old, archived, poorly stored blood samples

Handling Magnetic Beads

Follow the recommendations below for best results:

- Resuspend GeneCatcher™ Magnetic Beads before use by agitation. Vortex tube rigorously until beads are homogeneously dispersed.
- Perform mixing and washing of the GeneCatcher™ Magnetic Beads by gentle agitation using a plate shaker set to low speeds (300-400 rpm) or by pipetting up and down as directed in the **Automated Protocol 1**.
- Do not allow the beads to dry as drying diminishes the bead efficiency.
- Avoid disturbing the bead pellet or removing any beads when drawing off the supernatant by slowly aspirating the liquid from the opposite side of the tube.
- **Do not freeze the magnetic beads**, as freezing damages the beads and makes them unsuitable for nucleic acid purifications.

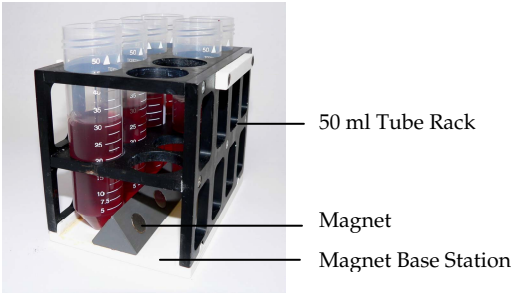
Continued on next page

GeneCatcher™ Automation Magnet

The GeneCatcher™ Automation Magnet is a magnetic base station containing 8 magnets (see figure below) for use in protocols with magnetic beads. You will need a GeneCatcher™ Automation Magnet (Invitrogen Carrier Set, page 6) and a 50 ml tube rack for automation (Tecan Carrier Set, page 6) for use of the GeneCatcher™ gDNA 96 x 10 ml Automated Blood Kit. The GeneCatcher™ Automation Magnet allows processing of eight 50 ml tubes and is designed for use with the Tecan Freedom EVO® platform. Other magnetic separators may not provide sufficient magnetic strength or may not be compatible with the volumes or numbers of samples used in the protocol.

During the automated sample processing, the removable tube rack containing the 50 ml sample tubes can be placed in two different positions relative to the GeneCatcher™ Automation Magnet. In the '**On the Magnet**' position, the rack is placed on the magnetic base station associating the 8 magnets with one side of each sample tube for simple sample processing using magnetic beads (see figure below). In the '**Off the Magnet'** position, the tube rack is placed away from the magnetic base station (see figure on page 8).

For more information, visit www.invitrogen.com contact **Technical Support** (page 25).



Continued on next page

Elution Buffer

The gDNA is eluted with Elution Buffer (E5; 10 mM Tris-HCl, pH 8.5). To obtain the best results, always use Elution Buffer (E5) to elute the gDNA. Do not use water for elution.

Elution Buffer Volume

The volume of elution buffer can be changed to obtain gDNA in the desired final concentration. Always use a volume of elution buffer that is equal or greater than the volume of beads used in the protocol. If the volume of elution buffer is lower than the volume of beads, gDNA elution is incomplete. For best results follow the guidelines provided in the **Automated Protocol 1**.

Safety Information

Follow the safety guidelines below when using the GeneCatcher™ gDNA 96 x 10 ml Automated Blood Kit.

- Treat all reagents supplied in the kit as potential irritants.
- Always wear a suitable lab coat, disposable gloves, and protective goggles when handling whole blood samples.
- Dispose of blood samples and washes during the purification procedure as biohazardous waste.
- Ensure that personnel using the liquid handling robot have been trained on its operation and follow all safety guidelines.

Setting Up the Deck Layout

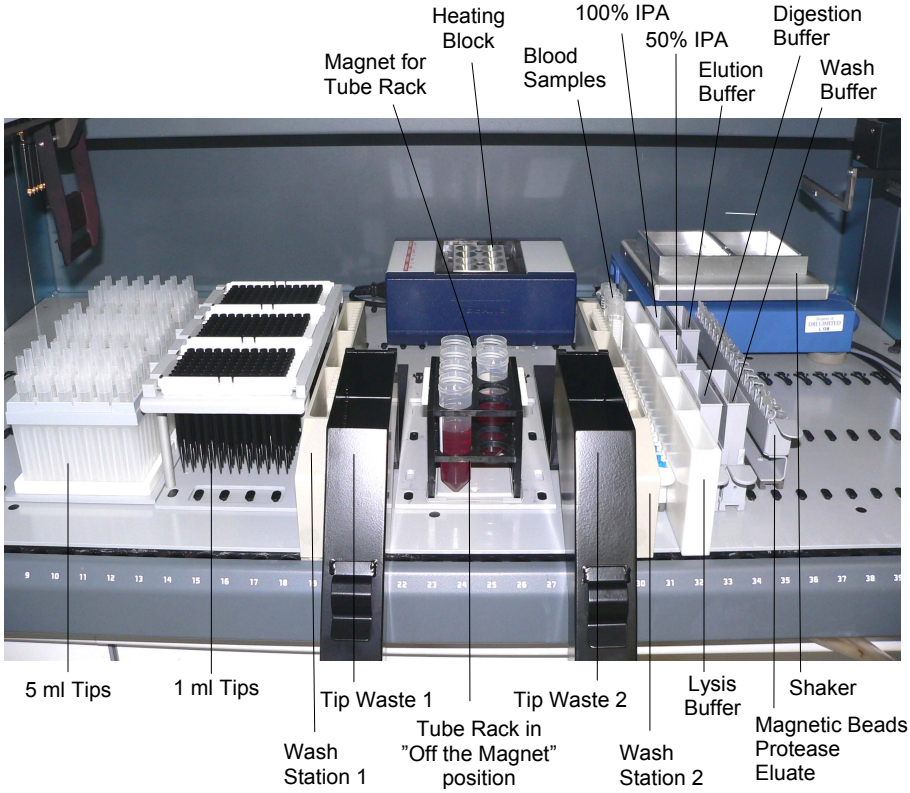
Introduction

This chapter provides you with guidelines for setting up the deck of your Tecan Freedom EVO® platform. An example of a deck layout is depicted below. If you are an experienced user you can change the deck layout for your specific sample processing requirements.

Deck Layout

The layout below is designed to minimize movements of the arms and risk of cross-contaminations by providing a Wash Station and Tube Waste on each side of the tube rack. This design prevents the arm from reaching over the open sample tubes. We recommend to set-up your Tecan Freedom EVO® deck accordingly.

The Tecan Technical Service (www.Tecan.com) will work closely with you in setting up the deck configuration according to your specific requirements.



Continued on next page

Setting Up the Deck Layout, Continued

Deck Set Up

Once you obtained the items of the Carrier Sets, configure the Tecan Freedom EVO® platform as described below. You may use any suitable configuration of your choice. The Tecan Customer Support (www.Tecan.com) will assist you setting up your specific deck configuration.

Note: The shaker and the heating block can be placed at the back of the deck out of the range of the tips arm but in the range of the robot gripper.

Location	Trough Content	Item
1-12		Boxes of 5 ml pipette tips
13-18		3 boxes of 1 ml pipette tips
19	Liquid waste	3 racks of Wash Station 1
20, 21	Tip waste	250 ml Tip Waste Troughs 1
23-26	Samples	8 x 50 ml Magnetic Rack and sample rack
28, 29		250 ml Tip Waste Troughs 2
30		3 racks of Wash Station 2
31	3-10 ml Blood sample	Carrier for Blood Containers
32	GeneCatcher™ Lysis Buffer (L12)	5 x 100 ml Reagent Troughs
33	100% IPA, 50% IPA, Protease Buffer	3 x 100 ml Reagent Troughs
34	GeneCatcher™ Elution Buffer (E5), GeneCatcher™ Wash Buffer (W12)	2 x 100 ml Reagent Troughs
36	GeneCatcher™ Magnetic Beads, Protease, Eluate	Carrier for 16 x 1.5 ml tubes

Continued on next page

Primary Liquid Handling Parameters

The table below lists the primary liquid handling parameters required to isolate gDNA using the **Automated Protocol 1**. Use the parameters and guidelines provided below as well as the **Automated Protocol 1** (page 16) to program your robot.

Parameter	Aim	Guidelines
[Mixing #1]	Used to mix bead/DNA pellet with buffer	• Shaker speed vigorously: 400 rpm • Shaker speed gently: 300 rpm
[Dispense liquid]	Normal liquid parameters for adding a reagent to each tube	• Aspirate/dispense at 750 µl/second • Transfer supernatant at 80 µl/second • Use multi-dispense if appropriate to save time
[Transfer supernatant to waste]	To remove and discard supernatant	• Aspirate slowly at 80 µl/second • Aspirate off the liquid using liquid detect and tracking or setting fixed height 1 mm above the tube bottom • Do not disturb the pellets • Discard to waste
[Final DNA Elution]	To dispense the eluate containing DNA	• Dispense at 80 µl/second • Aspirate from position fixed 1 mm above the tube bottom • Avoid bead carry-over • Dispense into new tube at 2 mm above the tube bottom

Isolating gDNA from 3-10 ml Human Blood

Introduction

This section provides instructions for isolating gDNA from 3-10 ml human blood samples using the Tecan Freedom EVO® automated liquid handling platform.

The procedure allows scalable purification of up to eight 3-10 ml blood samples simultaneously and the processing of **up to 24 samples** per day (**Automated Protocol 1**) or **up to 40 samples** per day (**Automated Protocol 2**).

The procedure is designed for isolating gDNA using the GeneCatcher™ Technology.

This section provides guidelines and a protocol that can be used to develop a script for your Tecan Freedom EVO® platform.

Materials Needed

Before starting, you will need the following items:

- Blood sample (3-10 ml, see page 7 for more details)
- 100% Isopropanol (IPA)
- 50% (v/v) Isopropanol
- Sterile 50 ml tube per sample
- Sterile 5 ml disposable tips (Rainin, catalog no. RT-5000)
- Sterile 1000 µl liquid sensing disposable tips (Tecan, catalog no. 612 512)
- Sterile 1.5 ml microcentrifuge tubes
- Vortex mixer

Components supplied with the kit

- GeneCatcher™ Magnetic Beads
- 5XGeneCatcher™ Lysis Buffer (L12)
- Protease
- Protease Buffer
- GeneCatcher™ Wash Buffer (W12)
- GeneCatcher™ Elution Buffer (E5)

Continued on next page

Isolating gDNA from 3-10 ml Human Blood,
Continued

Follow the guidelines below for handling DNA before starting the purification procedure:

- Clean the Tecan Freedom EVO® deck before starting
- Maintain a sterile environment when handling DNA to avoid any contamination from DNases
- Ensure that no DNase is introduced into the solutions supplied with the kit
- Use clean reagent troughs for the kit solutions
- Make sure that all equipment coming in contact with DNA is sterile, including pipette tips and tubes
- Ensure that the robotic tips enter the tubes without interfering with the bead pellet

Specific Reagent Volumes

Use the specified reagent volumes as indicated in the **Automated Protocol 1** based on the starting volume of the blood sample to obtain the best results. For some reagents, the same volume of reagent is used irrespective of the blood volume as indicated in the protocol.

Note

To purify genomic DNA from 2 ml blood sample, use the specified reagent volumes for 3 ml blood samples as described in the protocol. To obtain the best results we recommend to use a sample volume of 3-10 ml.

Continued on next page

Isolating gDNA from 3-10 ml Human Blood,
Continued

Before Starting

Follow the instructions below before starting your purification as described in the **Automated Protocol 1** or the **Automated Protocol 2** (see below for choosing the appropriate protocol):

- Set the Dry Block Heater (Tecan Carrier Set) at 70°C.
- Dilute 5XGeneCatcher™ Lysis Buffer (L12) using DNase/RNase-free water to 1XLysis Buffer (L12).
- Transfer 100% Isopropanol, 50% (v/v) Isopropanol, and buffers provided in the GeneCatcher™ gDNA 96 x 10 ml Automated Blood Kit into the respective troughs placed on the Tecan Freedom EVO® deck as indicated on pages 10-11.
- Open the tube containing the Protease and place the tube into the tube holder as indicated on pages 10-11.
- Vortex the tube containing the GeneCatcher™ Magnetic Beads to fully resuspend and evenly distribute the beads in the storage buffer. Open the tube and place tube into the tube holder as indicated on pages 10-11.

  Note: Beads stay in suspension for up to 30 minutes after resuspending.
- Invert the tubes containing the blood samples to mix, remove the tube caps, and place the tubes into the indicated tube holder on the automation deck (figure on page 10).

To maximize DNA yield, follow the recommendations below when processing samples:

- When removing supernatant, leave samples on the GeneCatcher™ Automated Magnet and aspirate slowly to ensure that the bead pellet is not disturbed.
- When mixing pelleted GeneCatcher™ Magnetic Beads, ensure that all beads are fully resuspended to maximize DNA recovery.
- Make sure that the GeneCatcher™ Wash Buffer (W12) is removed completely from the beads before adding the GeneCatcher™ Elution Buffer (E5).
- Ensure that beads are fully dispersed during the elution.

Continued on next page

Isolating gDNA from 3-10 ml Human Blood,
Continued

Choosing the appropriate Protocol

Depending upon your sample processing requirements choose one of the provided protocols.

To process **up to 24 samples** per day (1-3 sets of up to 8 samples) follow the **Automated Protocol 1**.

To process **25-40 samples** per day (4-5 sets of up to 8 samples) follow the **Automated Protocol 2** (page 21).

Automated Protocol 1

Follow the protocol below to purify gDNA from up to 3 sets of up to 8 samples per day (**1-24 samples**).

1. Aspirate the appropriate amount of resuspended **GeneCatcher™ Magnetic Beads** according to the volume of the blood sample as indicated in the table below.

Blood (ml)	3	4	5	6	7	8	9	10
Beads (µl)	45	60	75	90	105	120	135	150
1XLysis Buffer (L12) (ml)	9	12	15	18	21	24	27	30

2. Transfer and dispense the aspirated beads into a sterile 50 ml tube, placed in the GeneCatcher™ Magnetic Rack to the Off the Magnet position (see page 8).
3. Aspirate the appropriate volume of **1XGeneCatcher™ Lysis buffer (L12)** as directed in the table above.
4. Transfer and dispense the aspirated **1XGeneCatcher™ Lysis buffer (L12)** into the 50 ml tubes.
5. Aspirate 1 ml of the desired amount of blood from the open containers and dispense into the respective 50 ml tubes. Repeat adding the blood samples to the 50 ml tubes in sequential 1 ml aliquots. This allows proper mixing of the reagents.
6. Upon completion of adding the desired amount of blood to each 50 ml tube, place the tube rack on the shaker. Shake samples at 300 rpm for 1 minute.
7. Place the tube rack to the Off the Magnet position and incubate at room temperature for 4 minutes.

Procedure continued on the next page.

Continued on next page

Isolating gDNA from 3-10 ml Human Blood, Continued

Automated Protocol 1, continued

8. Place the tube rack to the On the Magnet position (see figure on page 8) for 3 minutes.
9. Without removing the tube rack from the magnet, carefully aspirate the supernatant using 5 ml tips without disturbing the bead pellet by slowly aspirating most of the supernatant (see **Note** below) from the opposite side of the tube. Discard supernatant into Wash Station and tips into tip waste containers.

   Note: It is acceptable to leave behind up to **≤ 5 ml** of supernatant to prevent the pellet from dislodging. Loss of any pellet would result in reduced yield of DNA.
10. Without removing the tube rack from the magnet, add 10 ml **1XGeneCatcher™ Lysis Buffer (L12)** irrespective of the blood volume to each tube.
11. Place the tube rack on the shaker. Shake samples gently at 300 rpm for 1 minute.
12. Place the tube rack to the On the Magnet position and incubate samples at room temperature for 30 seconds.
13. Without removing the tube rack from the magnet, carefully aspirate the supernatant using 5 ml tips without disturbing the bead pellet by slowly aspirating most of the supernatant (see **Note** below) from the opposite side of the tube. Discard supernatant into Wash Station and tips into tip waste containers.

    Note: It is acceptable to leave behind up to **≤ 5 ml** of supernatant to prevent the pellet from dislodging.
14. Repeat Steps 10-13 once.

    Note: It is acceptable to leave behind **≤ 1 ml** of supernatant to prevent the pellet from dislodging.
15. Place the tube rack to the Off the Magnet position.
16. Add 3 ml **Protease Buffer** and subsequently 40 µl of **Protease** irrespective of the blood volume to each tube.
17. Place the tube rack on the shaker and mix tube contents at 300 rpm for 1 minute to initiate bead dispersal.

Procedure continued on the next page.

Continued on next page

Isolating gDNA from 3-10 ml Human Blood, Continued

Automated Protocol 1, continued

18. Place the tube rack on the heating block, preheated at 65-70°C. Incubate samples for 5 minutes.
19. Without allowing the tube contents to cool, mix the tube contents by pipetting up and down 30 times using 1 ml tips to homogeneously disperse the bead/DNA pellet.
20. Place the tube rack on the preheated heating block, and incubate samples at 65-70°C for another 10 minutes.
21. Mix the tube contents by pipetting up and down 10 times using 1 ml tips to homogeneously disperse the bead pellet.
22. Add 3 ml **100% isopropanol** to each tube.
23. Place the tube rack on the shaker and mix tube contents at 400 rpm for 2 minutes by which time a visible aggregate would have formed.

    Note: Absence of a visible aggregate after 2 minutes of mixing indicates very low content of DNA in the sample.
24. Place the tube rack to the On the Magnet position and incubate for 1 minute.
25. Without removing the tube rack from the magnet, carefully aspirate the supernatant using 5 ml tips without disturbing the bead pellet by slowly aspirating the supernatant from the opposite side of the tube. Discard supernatant into Wash Station and tips into tip waste containers.

    Note: It is acceptable to leave behind **≤ 0.5 ml** of supernatant to prevent the pellet from dislodging.
26. Place the tube rack to the Off the Magnet position.
27. Add 3 ml **50% (v/v) isopropanol** to each tube irrespective of the blood volume.
28. Place the tube rack on the shaker. Shake samples gently at 300 rpm for 2 minutes.
29. Place the tube rack to the On the Magnet position and incubate for 1 minute.

Procedure continued on the next page.

Continued on next page

Automated Protocol 1, continued

30. Without removing the tube rack from the magnet, carefully aspirate the supernatant using 5 ml tips without disturbing the pellet of beads by slowly aspirating the supernatant from the opposite side of the tube. Discard supernatant into Wash Stations and tips into tip waste containers.
31. Without removing the tube rack from the magnet, carefully add 250 µl of **GeneCatcher™ Wash Buffer (W12)** to each sample without disturbing the bead pellet by pipetting down the side of the 50 ml tubes.
32. Incubate for 30 seconds at room temperature.
33. Without removing the tube rack from the magnet, carefully aspirate the supernatant using 1 ml tips without disturbing the bead pellet by slowly aspirating the supernatant from the opposite side of the tube. Discard supernatant into Wash Stations and tips into tip waste containers.
34. Without removing the tube rack from the magnet, carefully add 250 µl of **GeneCatcher™ Wash Buffer (W12)** to each tube without disturbing the bead pellet by pipetting down the side of the 50 ml tubes.
35. Incubate samples for 1 minute at room temperature.
36. Without removing the tube rack from the magnet, carefully aspirate the supernatant using 1 ml tips without disturbing the pellet of beads by slowly aspirating the supernatant from the opposite side of the tube. Discard supernatant into Wash Stations and tips into tip waste containers.
37. Place the tube rack to the Off the Magnet position.
38. Add the appropriate volume of **Elution Buffer (E5)** to each tube (see table below) using 1 ml tips.

Blood (ml)	3	4	5	6	7	8	9	10
Elution Buffer E5 (µl)	300	400	500	600	700	800	900	1000

Note: The volume of the Elution Buffer may vary depending on the volume of blood used and the desired DNA concentration.

Procedure continued on the next page.

Continued on next page

Automated Protocol 1, continued

39. Place the tube rack on the shaker. Shake samples gently at 300 rpm for 1 minute.

    Note: This step releases the bead pellet from the wall of the tube. The pellet is not completely dispersed at this step.

40. Place the tube rack on the preheated heating block, and incubate samples at 65-70°C for 30 minutes to 1 hour.
41. Mix the tube contents by pipetting up and down 20 times to homogeneously disperse the bead pellet.
42. Place the tube rack to the On the Magnet position and incubate samples for 15 minutes. The supernatant should be clear, colorless, and free of beads after 15 minutes.
43. Without removing the tube rack from the magnet, carefully aspirate the **supernatant containing the DNA** using 1 ml tips without disturbing the bead pellet by slowly aspirating the supernatant from the opposite side of the tube. Transfer the DNA to sterile microcentrifuge tubes.
44. Discard the used magnetic beads. **Do not** re-use the magnetic beads.

Continued on next page

Isolating gDNA from 3-10 ml Human Blood,
Continued

Guidelines for Processing Up to 40 Blood Samples

Follow the **Automated Protocol 2** below to purify gDNA from 4-5 of sets of up to 8 samples (25-40 samples) per day.

For processing 25-40 samples, the deck must be adjusted by adding a second GeneCatcher™ Automation Magnet, a Dry Block Heater, and Dry Block Insert. See pages 5-6 for additional information on these items.

Automated Protocol 2

Follow the protocol below to purify gDNA from up to 4-5 sets of up to 8 samples per day (**25-40 samples**).

1. Process 8 samples at a time following Steps 1-40 of the **Automated Protocol 1** (page 16).
2. After starting the heat incubation (Step 40), immediately proceed with processing the next 8 samples, following Steps 1-20 of the **Automated Protocol 1** (page 16).

   Note: Use the second heating block for incubating the second set of samples (Step 20).
3. During the heat incubation of the second set of samples (Step 20), proceed with the first set of samples by following Steps 41-42 of the **Automated Protocol 1** (page 20).
4. After starting the incubation of the first set of samples on the magnet (Step 42, page 20), proceed with processing the second set of samples by following Steps 21-40 of the **Automated Protocol 1** (page 17).
5. After starting the heat incubation (Step 40) of the second set of samples, proceed with completing the purification of the first set of samples by following Steps 43-44 of the **Automated Protocol 1** (page 20).
6. Immediately after removing the empty 50 ml tubes of the first set of samples, proceed with processing the third set of 8 samples following Steps 1-20 of the **Automated Protocol 1** (page 16).
7. Follow Steps 3-6 of this **Automated Protocol 2** while replacing the number of samples sets accordingly until processing of 5 sets of 8 samples is completed.

Storing DNA

- Place purified DNA at 4°C for immediate use or aliquot the DNA and store at -20°C for long-term storage.
- Avoid repeated freezing and thawing of DNA.

DNA Quantitation

DNA Yield

Perform DNA quantitation using UV absorbance at 260 nm or Quant-iT™ Kits.

UV Absorbance

1. Prepare a dilution of the DNA solution in 10 mM Tris-HCl, pH 7.5. Mix well. Measure the absorbance at 260 nm (A_{260}) of the dilution in a spectrophotometer (using a cuvette with an optical path length of 1 cm) blanked against 10 mM Tris-HCl pH 7.5.
2. Calculate the concentration of DNA using the formula:

   DNA (µg/ml) = $A_{260} \times 50$ x dilution factor

   For DNA, $A_{260} = 1$ for a 50 µg/ml solution measured in a cuvette with an optical path length of 1 cm.

Quant-iT™ Kits

The Quant-iT™ Kits (see page vi for ordering information) provide a rapid, sensitive, and specific fluorescent method for dsDNA quantitation. The kit contains a state-of-the-art quantitation reagent, DNA standards for standard curve, and a pre-made buffer to allow fluorescent DNA quantitation using standard fluorescent microplate readers or fluorometers.

Appendix

Troubleshooting

Introduction

Refer to the table below to troubleshoot problems that you may encounter when purifying gDNA with the kit.

Problem	Cause	Solution
Low DNA yield	Incorrect reagent amount	Use the appropriate amount of magnetic beads and reagents based on the starting material as directed in the protocol.
	Improper handling of GeneCatcher™ Magnetic Beads	• Vortex the tube containing the magnetic beads to fully resuspend the beads in solution prior to use.
	Pellet of beads disturbed or lost during binding or washing steps	• Keep the sample on the GeneCatcher™ Magnetic Rack when removing the supernatant during the binding or washing steps. • Remove the supernatant carefully without disturbing the bead pellet by aspirating the supernatant from the opposite side of the tube.
	Incorrect elution conditions	• Perform elution at 65-70°C. • After heat incubation of the sample in the Elution Buffer (E5) fully disperse the GeneCatcher™ Magnetic Beads. • Do not use water to elute DNA.
	Contamination with DNase	• Clean the deck before processing samples. • Use clean troughs for reagents. • Follow general guidelines for handling DNA (page 14). • Follow the recommendations on pages 14-15 for automated purification of gDNA.

Continued on next page

Problem	Cause	Solution
Cross-contamination of samples	Improper deck set-up	Set-up the deck by placing waste containers on each side of the tube rack (pages 10-11).
	Improper speed for tip mixing of samples	Set a low speed for aspiration and dispense as directed on page 12.
No DNA recovered	Water used for elution	• Do not use water for elution. • The elution buffer **must** have a pH of 8.5-9.0 or the DNA will remain bound to the GeneCatcher™ Magnetic Beads.
	Magnetic Beads stored or handled improperly	• Store beads at room temperature. **Do not freeze the beads,** as they will become irreparably damaged and unsuitable for nucleic acid purification. • Make sure that the beads are in solution at all times and are not dried. Dried beads are non-functional.
	DNA degraded due to DNase contamination	• Clean the deck before processing samples. • Use clean troughs for reagents. • Follow general guidelines for handling DNA (page 14). • Follow the recommendations on pages 14-15 for automated purification of gDNA.

Technical Support

Web Resources

Visit the Invitrogen website at www.invitrogen.com for:

- Technical resources including manuals, vector maps and sequences, application notes, MSDSs, FAQs, formulations, citations, handbooks, etc.
- Complete technical support contact information.
- Access to the Invitrogen Online Catalog.
- Additional product information and special offers.

Contact Us

For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on our website (www.invitrogen.com).

Corporate Headquarters	**Japanese Headquarters:**	**European Headquarters:**
Invitrogen Corporation 1600 Faraday Avenue Carlsbad, CA 92008 USA Tel: 1 760 603 7200 Tel (Toll Free): 1 800 955 6288 Fax: 1 760 602 6500 E-mail: tech_support@invitrogen.com	Invitrogen Japan LOOP-X Bldg. 6F 3-9-15, Kaigan Minato-ku, Tokyo 108-0022 Tel: 81 3 5730 6509 Fax: 81 3 5730 6519 E-mail: jpinfo@invitrogen.com	Invitrogen Ltd Inchinnan Business Park 3 Fountain Drive Paisley PA4 9RF, UK Tel: +44 (0) 141 814 6100 Fax: +44 (0) 141 814 6117 E-mail: eurotech@invitrogen.com

MSDS

MSDSs (Material Safety Data Sheets) are available on our website at www.invitrogen.com/msds.

Certificate of Analysis

Certificate of Analysis (CofA) for the product is available on our website at www.invitrogen.com/cofa.

Purchaser Notification

Limited Warranty

Invitrogen is committed to providing our customers with high-quality goods and services. Our goal is to ensure that every customer is 100% satisfied with our products and our service. If you should have any questions or concerns about an Invitrogen product or service, contact our Technical Support Representatives.

Invitrogen warrants that all of its products will perform according to the specifications stated on the certificate of analysis. The company will replace, free of charge, any product that does not meet those specifications. This warranty limits Invitrogen Corporation's liability only to the cost of the product. No warranty is granted for products beyond their listed expiration date. No warranty is applicable unless all product components are stored in accordance with instructions. Invitrogen reserves the right to select the method(s) used to analyze a product unless Invitrogen agrees to a specified method in writing prior to acceptance of the order. Invitrogen makes every effort to ensure the accuracy of its publications, but realizes that the occasional typographical or other error is inevitable. Therefore Invitrogen makes no warranty of any kind regarding the contents of any publications or documentation. If you discover an error in any of our publications, report it to our Technical Support Representatives.

Invitrogen assumes no responsibility or liability for any special, incidental, indirect or consequential loss or damage whatsoever. The above limited warranty is sole and exclusive. No other warranty is made, whether expressed or implied, including any warranty of merchantability or fitness for a particular purpose.

For research use only. Not intended for any animal or human therapeutic or diagnostic use.

Notes

Notes

invitrogen™

Corporate Headquarters
Invitrogen Corporation
1600 Faraday Avenue
Carlsbad, CA 92008
T: 1 760 603 7200
F: 1 760 602 6500
E: tech_support@invitrogen.com

For country-specific contact information visit our web site at **www.invitrogen.com**