Invitrogen™
life technologies

Instruction Manual

ChargeSwitch® gDNA 1 ml Blood Kit

For purification of genomic DNA from 1 ml samples of human blood

Catalog no. CS11001

Version B
14 February 2005
25-0833

Table of Contents

Kit Contents and Storage

Shipping and Storage

All components of the ChargeSwitch® gDNA 1 ml Blood Kit are shipped at room temperature. Upon receipt, store the Proteinase K at 4°C. Store all other components at room temperature.

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

Contents

The components supplied in the ChargeSwitch® gDNA 1 ml Blood Kit are listed below. The reagents supplied are sufficient to perform 20 purifications from 1 ml blood samples.

Note: Some reagents in the kit may be provided in excess of the amount needed.

Component	Amount
ChargeSwitch® 10X RBC Lysis Buffer (L8)	25 ml
ChargeSwitch® WBC Lysis Buffer (L12)	30 ml
ChargeSwitch® Magnetic Beads	2 x 1 ml
Proteinase K (20 mg/ml in 50 mM Tris-HCl, pH 8.5, 5 mM $CaCl_2$, 50% glycerol)	500 µl
ChargeSwitch® Purification Buffer (N5)	4.5 ml
ChargeSwitch® Wash Buffer (W12)	100 ml
ChargeSwitch® Elution Buffer (E5; 10 mM Tris-HCl, pH 8.5)	10 ml

Accessory Products

Additional Products

The table below lists additional products available from Invitrogen that may be used with the ChargeSwitch® gDNA 1 ml Blood Kit. In addition, the table lists a selection of ChargeSwitch® gDNA Kits that are available for purification of genomic DNA from other sources. For more information about these and other ChargeSwitch® gDNA Kits, refer to our Web site at www.invitrogen.com or call Technical Service (see page 16).

Product	Amount	Catalog no.
MagnaRack™	1 rack	CS15000
ChargeSwitch® gDNA 20 µl Blood Kit	96 purifications	CS11010
ChargeSwitch® gDNA 100 µl Blood Kit	50 purifications	CS11000
ChargeSwitch® gDNA 1 ml Serum Kit	50 purifications	CS11040
ChargeSwitch® gDNA 50 µl Sheep Blood Kit	50 purifications	CS11300
ChargeSwitch® gDNA Micro Tissue Kit	50 purifications	CS11203
ChargeSwitch® gDNA Mini Tissue Kit	25 purifications	CS11204
ChargeSwitch® gDNA Mini Bacteria Kit	50 purifications	CS11301
ChargeSwitch® gDNA Normalized Buccal Cell Kit	50 purifications	CS11020
ChargeSwitch® gDNA Buccal Cell Kit	50 purifications	CS11021
ChargeSwitch® Forensic DNA Purification Kit	100 purifications	CS11200
Quant-iT™ DNA Assay Kit, High Sensitivity	1000 assays	Q33120
Quant-iT™ DNA Assay Kit, Broad Range	1000 assays	Q33130

E-Gel® Agarose Gels and DNA Ladders

E-Gel® Agarose Gels are bufferless, pre-cast agarose gels designed for fast, convenient electrophoresis of DNA samples. E-Gel® agarose gels are available in different agarose percentages and well formats. In addition, a large variety of DNA ladders are available from Invitrogen for sizing DNA. For more information about these products, see www.invitrogen.com or call Technical Service.

Introduction

Overview

Introduction

The ChargeSwitch® gDNA 1 ml Blood Kit allows rapid and efficient purification of genomic DNA from 1 ml samples of human blood. After preparing the lysates, you may purify DNA in less than 15 minutes using the ChargeSwitch® Technology. For more information about the ChargeSwitch® Technology, see page 2.

Intended Use for the Kits

The ChargeSwitch® gDNA 1 ml Blood Kit is designed to allow isolation of up to 20 µg of genomic DNA from the following sources. The purified DNA is suitable for use in downstream applications including PCR, restriction enzyme digestion, and Southern blotting.

- One ml samples of human blood (fresh or frozen) treated with the anticoagulant EDTA or citrate
- Lysed blood cells
- Buffy coats equivalent to 1 ml of white blood cells

Important: Genomic DNA may be isolated from heparin-treated blood; however, the DNA **is not** suitable for use in downstream applications such as PCR due to the presence of the heparin.

Advantages

Use of the ChargeSwitch® gDNA 1 ml Blood Kit to isolate genomic DNA provides the following advantages:

- Uses a magnetic bead-based technology to isolate genomic DNA without the need for hazardous chemicals, centrifugation, or vacuum manifolds
- Rapid and efficient purification of genomic DNA from 1 ml of human blood in less than 15 minutes following sample preparation and lysis
- Simple lysis of blood cells with Proteinase K without the need for any mechanical lysis
- Minimal contamination with RNA
- The purified genomic DNA demonstrates improved downstream performance in applications including PCR, restriction enzyme digestion, and Southern blotting

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Overview, continued

System Specifications

Starting Material:	1 ml of fresh or frozen, EDTA- or citrate-treated human blood
Elution Volume:	300 µl
DNA Yield:	Up to 20 µg
DNA Size:	Varies (depends on the quality of the starting material)

The ChargeSwitch® Technology

The ChargeSwitch® Technology (CST®) is a novel magnetic bead-based technology that provides a switchable surface charge dependent on the pH of the surrounding buffer to facilitate nucleic acid purification. In low pH conditions, the CST® beads have a positive charge that binds the negatively charged nucleic acid backbone (see figure below). Proteins and other contaminants are not bound and are simply washed away in an aqueous wash buffer. To elute nucleic acids, the charge on the surface of the bead is neutralized by raising the pH to 8.5 using a low salt elution buffer (see figure below). Purified DNA elutes instantly into this elution buffer, and is ready for use in downstream applications.

Low pH High pH

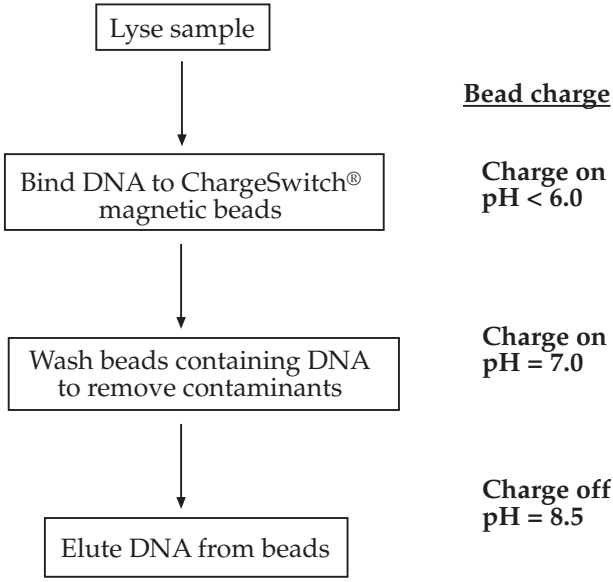
ChargeSwitch® Magnetic Bead Specifications

Bead Binding Capacity:	5-10 µg genomic DNA per mg
Bead Size:	< 1 µm
Bead Concentration:	25 mg/ml
Storage Buffer:	10 mM MES, pH 5.0, 10 mM NaCl, 0.1% Tween 20

Experimental Outline

Introduction

The figure below illustrates the basic steps necessary to purify genomic DNA from 1 ml blood samples using the ChargeSwitch® gDNA 1 ml Blood Kit.



Methods

General Information

User Supplied Materials

In addition to the reagents supplied with the kit, you will need to have the following materials on hand before beginning:

- 15 ml centrifuge tubes
- Centrifuge suitable for 15 ml centrifuge tubes
- A magnetic separation rack suitable for use with 1.5 ml microcentrifuge tubes (see below)
- Sterile, 1.5 ml microcentrifuge tubes
- Sterile 250 ml bottle (to prepare and store 1X RBC Lysis Buffer)
- Sterile water
- Vortex mixer
- 20 µl, 200 µl, and 1 ml sterile, pipette tips
- Water bath at 60°C

MagnaRack™

The MagnaRack™ available from Invitrogen (Catalog no. CS15000) is a two-piece magnetic separation rack for use in protocols with magnetic beads. The MagnaRack™ consists of a magnetic base station and a removable tube rack. The tube rack can hold up to 24 microcentrifuge tubes. The tube rack fits onto the magnetic base station in two different positions associating the row of 12 neodymium magnets with a single row of 12 tubes for simple 'on the magnet' and 'off the magnet' sample processing (see figure below). For more information, see www.invitrogen.com or call Technical Service (page 16).

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General Information, continued

Safety Information

Follow the safety guidelines below when using the ChargeSwitch® gDNA 1 ml Blood Kit.

- Treat all reagents supplied in the kit as potential irritants.
- Always wear a suitable lab coat, disposable gloves, and protective goggles.
- If a spill of the buffers occurs, clean with a suitable laboratory detergent and water. If the liquid spill contains potentially infectious agents, clean the affected area first with laboratory detergent and water, then with 1% (v/v) sodium hypochlorite or a suitable laboratory disinfectant.

Handling the ChargeSwitch® Magnetic Beads

Follow the guidelines below when handling the ChargeSwitch® magnetic beads.

- Do not freeze the beads as this irreparably damages them. Store the beads at room temperature.
- Always keep the beads in solution. Do not allow them to dry out as this renders them non-functional.
- When using the beads, resuspend thoroughly in the storage buffer by vortexing before removal.
- Discard beads after use. Do not reuse.

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General Information, continued

Elution Buffer

ChargeSwitch® Elution Buffer (E5; 10 mM Tris-HCl, pH 8.5) is supplied with the kit for eluting the DNA from the ChargeSwitch® Magnetic Beads. For best results, use Elution Buffer (E5) to elute the DNA. Alternatively, TE Buffer, pH 8.5-9.0 is acceptable. Note that the pH must be between 8.5-9.0 otherwise the DNA will not elute. **Do not use water for elution.**

The protocol recommends eluting the genomic DNA in 300 µl of ChargeSwitch® Elution Buffer (E5). You may vary the amount of ChargeSwitch® Elution Buffer (E5) used to obtain genomic DNA in the desired final concentration. **For best results, always use a volume of ChargeSwitch® Elution Buffer (E5) that is equal to or greater than the volume of ChargeSwitch® Magnetic Beads used in the protocol.** If the volume of ChargeSwitch® Elution Buffer (E5) is lower than the volume of beads used, DNA elution is incomplete. You may need to perform a second elution to recover all DNA.

Isolating Genomic DNA

Introduction

This section provides guidelines and instructions to isolate genomic DNA from 1 ml samples of human blood.

Starting Material

Use this procedure to isolate genomic DNA from:

- 1 ml of EDTA- or citrate-treated, fresh or frozen, human blood
- Buffy coats equivalent to 1 ml of white blood cells

Materials Needed

Have the following materials on hand before beginning:

- Blood sample(s) (see above)
- Sterile water
- Sterile 1.5 ml microcentrifuge tubes
- 15 ml centrifuge tubes and centrifuge
- MagnaRack™ (Catalog no. CS15000) or other magnetic separation rack
- Vortex mixer
- Sterile pipette tips (20 µl, 200 µl, and 1 ml)
- Water bath set at 60°C

Components Supplied with the Kit

- ChargeSwitch® 10X RBC Lysis Buffer (L8)
- ChargeSwitch® WBC Lysis Buffer (L12)
- Proteinase K
- ChargeSwitch® Magnetic Beads
- ChargeSwitch® Purification Buffer (N5)
- ChargeSwitch® Wash Buffer (W12)
- ChargeSwitch® Elution Buffer (E5) or TE Buffer (not supplied; 10 mM Tris-HCl, 1 mM EDTA, pH 8.5)

Preparing the 1X RBC Lysis Buffer

The first time you use the kit, prepare 1X RBC Lysis Buffer:

1. Mix the contents of the ChargeSwitch® 10X RBC Lysis Buffer (L8; 25 ml) with 225 ml of sterile water to prepare 1X RBC Lysis Buffer (total volume = 250 ml).
2. Use the 1X RBC Lysis Buffer (see **Preparing the Lysate**, next page) or store at room temperature.

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Isolating Genomic DNA, continued

Preparing the Lysate

Follow the procedure below to prepare a lysate from the 1 ml blood sample.

1. To a 15 ml centrifuge tube, add the 1 ml blood sample and 10 ml of 1X RBC Lysis Buffer (see previous page).
2. Mix by inverting 5 times, then incubate for 5 minutes at room temperature to lyse the red blood cells.
3. Centrifuge the sample for 5 minutes at 2,000 x g. Carefully pour away the supernatant, leaving a pellet of white blood cells (visible at the bottom of the tube).
4. Add 1 ml of ChargeSwitch® Wash Buffer (W12) by dispensing the liquid against the side of the tube. Take care not to disturb the white blood cell pellet.

   Note: If the pellet is dislodged from the bottom of the tube, centrifuge the sample for 1 minute at 2,000 x g.
5. Carefully pour away the supernatant containing heme, leaving a pellet of white blood cells.
6. Shake the bottle of ChargeSwitch® WBC Lysis Buffer (L12) to mix (solution will appear cloudy). Add 0.5 ml to the sample and mix by vortexing for 10 seconds (recommended) or pipetting up and down 10 times.
7. Transfer all of the liquid (and any lumps) to a sterile microcentrifuge tube containing 1 ml of ChargeSwitch® WBC Lysis Buffer (L12) and 20 µl of Proteinase K.
8. Pipet up and down twice to mix.
9. Incubate the sample for 10-30 minutes at 60°C with occasional mixing (by pipetting up and down, shaking, or vortexing) to lyse the white blood cells. Do not proceed until the sample is clear with no visible lumps.
10. Pipet up and down 10 times to thoroughly mix the sample.

    Note: Use a 1 ml pipette tip and allow as much sample as possible to enter the tip before aspirating the liquid.
11. Proceed to **Binding DNA**, next page.

continued on next page

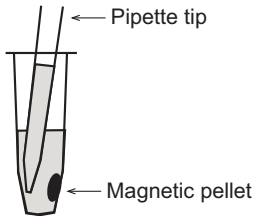
Isolating Genomic DNA, continued

Binding DNA

Follow the procedure below to bind the DNA to the ChargeSwitch® Magnetic Beads.

1. Vortex the tube containing the ChargeSwitch® Magnetic Beads to fully resuspend and evenly distribute the beads in the storage buffer. Make sure that all of the solution containing beads is at the bottom of the tube.
2. Add 100 µl of ChargeSwitch® Magnetic Beads to the digested sample (from Step 10, previous page), and pipet up and down gently twice to mix.
3. Add 100 µl of ChargeSwitch® Purification Buffer (N5) to the sample, and pipet up and down gently 5 times to mix.

   Note: Adding the ChargeSwitch® Purification Buffer (N5) lowers the pH of the sample, and optimizes the binding conditions.
4. Incubate at room temperature for 1 minute to allow the DNA to bind to the ChargeSwitch® Magnetic Beads.
5. Place the sample in the MagnaRack™ for 1 minute or until the beads have formed a tight pellet.
6. Without removing the tube from the MagnaRack™, carefully remove the supernatant and discard. Take care not to disturb the pellet of beads by angling the pipette such that the tip is pointed away from the pellet (see figure below).



7. Proceed immediately to **Washing DNA**, next page.

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Isolating Genomic DNA, continued

Washing DNA

1. Remove the tube containing the pelleted magnetic beads from the MagnaRack™ (Step 6, previous page). There should be no supernatant in the tube.
2. Add 1 ml of ChargeSwitch® Wash Buffer (W12) to the tube and pipet up and down gently 3 times to resuspend the magnetic beads.

   Important: Use a 1 ml pipette tip set to 900 µl to mix the sample. Make sure that the tip is submerged, and pipet up and down gently to avoid forming bubbles.
3. Place the sample in the MagnaRack™ for 1 minute or until the beads have formed a tight pellet.
4. Without removing the tube from the MagnaRack™, carefully remove the supernatant and discard. Take care not to disturb the pellet of beads by angling the pipette such that the tip is pointed away from the pellet (see figure on page 9).
5. Repeat Steps 1-4.
6. Proceed to **Eluting DNA**, next page.

continued on next page

Isolating Genomic DNA, continued

Eluting DNA

1. Remove the tube containing the pelleted magnetic beads from the MagnaRack™ (Step 5, previous page). There should be no supernatant in the tube.
2. Add 300 µl of ChargeSwitch® Elution Buffer (E5) (or TE Buffer, pH 8.5) to the tube and pipet up and down gently 10 times to resuspend the magnetic beads.

   Important: Do not use water for elution. The DNA will not elute due to the poor buffering capacity of water.
3. Incubate at room temperature for 5 minutes.

   Tip: For maximum yield, mix the suspension of beads (by pipetting up and down gently) half way through the incubation. Incubating the sample at 60°C may also improve yield.
4. Place the sample in the MagnaRack™ for 5 minutes or until the beads have formed a tight pellet.
5. Without removing the tube from the MagnaRack™, carefully remove the **supernatant containing the DNA** to a sterile microcentrifuge tube. Take care not to disturb the pellet of beads by angling the pipette such that the tip is pointed away from the pellet (see figure on page 9).

   Note: If the eluate containing the DNA is discolored, repeat Steps 4-5.
6. Discard the used magnetic beads. Do not reuse the beads.

Storing DNA

- Store the purified DNA at -20°C or use immediately for the desired downstream application.
- Avoid repeatedly freezing and thawing DNA. Store the purified DNA at 4°C for short-term use or aliquot the DNA and store at -20°C for long-term storage.

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Isolating Genomic DNA, continued

Quantitating DNA Yield

You may estimate the yield of purified genomic DNA by checking the UV absorbance at 260 nm or using one of the Quant-iT™ DNA Assay Kits.

UV Absorbance

1. Measure the A_{260} of the solution using a spectrophotometer blanked against 10 mM Tris-HCl, pH 8.5.
2. Calculate the amount of DNA using the formula:

   DNA (µg) = A_{260} × 50 µg/(A_{260} x 1 ml) x dil'n factor x total sample volume (ml)

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™ DNA Assay Kits

The Quant-iT™ DNA Assay Kits (see page vi for ordering information) provide a rapid, sensitive, and accurate method for dsDNA quantitation with minimal interference from RNA, protein, ssDNA (primers), or other common contaminants that affect UV absorbance. Each kit contains a state-of-the-art quantitation reagent, pre-diluted standards for a standard curve, and a pre-made buffer to allow fluorescence-based DNA quantitation. For more information, see www.invitrogen.com or call Technical Service (page 16).

Troubleshooting

Introduction

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

Problem	Cause	Solution
Low DNA yield	Incomplete lysis	• Decrease the amount of starting material used. • Be sure to add Proteinase K during lysis. • Increase the length of incubation at 60°C.
	Insufficient amount of ChargeSwitch® Magnetic Beads added	Vortex the tube containing the ChargeSwitch® Magnetic Beads to fully resuspend the beads in solution before adding them to your sample.
	Pellet of beads disturbed or lost during binding or washing steps	• Keep the sample in the MagnaRack™ when removing supernatant during the binding or washing steps. • Remove the supernatant without disturbing the pellet of beads by angling the pipette tip away from the pellet.
	Incomplete dissociation of DNA from the ChargeSwitch® Magnetic Beads	• Perform additional mixing of the suspension of beads (by pipetting up and down). • Elute DNA at 60°C.
	Incorrect elution conditions	• After adding ChargeSwitch® Elution Buffer (E5) to the sample, pipet up and down to resuspend the magnetic beads before incubation. • Elute at 60°C to improve DNA yield. • Do not use water to elute DNA. Use Elution Buffer (E5) or TE, pH 8.5.

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Troubleshooting, continued

Problem	Cause	Solution
No DNA recovered	Added WBC Lysis Buffer (L12) to sample instead of Purification Buffer (N5)	You must add ChargeSwitch® Purification Buffer (N5) to your sample (in the presence of magnetic beads) to lower the pH, allowing DNA to bind to the beads.
	Water used for elution	Do not use water for elution. The elution buffer **must** have a pH = 8.5-9.0 or the DNA will remain bound to the ChargeSwitch® Magnetic Beads. Use ChargeSwitch® Elution Buffer (E5) or TE, pH 8.5.
	ChargeSwitch® Magnetic Beads stored or handled improperly	• Store beads at room temperature. Do not freeze the beads as they will become irreparably damaged. • Make sure that the beads are in solution at all times and do not become dried. Beads that have dried out are non-functional.
DNA contains contaminants (*i.e.* $A_{260}/A_{280} < 1.7$)	White blood cells not completely lysed	During lysis step (with WBC Lysis Buffer (L12) and Proteinase K), increase length of incubation at 60°C. Mix sample occasionally by pipetting up and down, vortexing, or shaking.
	Beads not fully resuspended during washing steps	Thoroughly resuspend the pellet of magnetic beads by pipetting up and down.
Eluate containing DNA is discolored	Magnetic pellet disturbed during elution	Repeat the elution step (**Eluting DNA**, Steps 4-5, page 11).

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Troubleshooting, continued

Problem	Cause	Solution
DNA is sheared or degraded	Lysate mixed too vigorously or small pipette tips used during mixing	• Use a 1 ml pipette tip set to 900 µl to mix the sample. • Pipet up and down gently to mix.
	Bubbles formed during mixing steps	Make sure that the pipette tip is submerged in the solution during mixing.
	DNA repeatedly frozen and thawed	Aliquot DNA and store at 4°C or -20°C. Avoid repeated freezing and thawing.
	DNA contaminated with DNases	Maintain a sterile environment while working (*i.e.* wear gloves and use DNase-free reagents).

Appendix

Technical Service

World Wide Web

Visit the Invitrogen Web Resource using your World Wide Web browser. At the site, you can:

- Get the scoop on our hot new products and special product offers
- View and download vector maps and sequences
- Download manuals in Adobe® Acrobat® (PDF) format
- Explore our catalog with full color graphics
- Obtain citations for Invitrogen products
- Request catalog and product literature

Once connected to the Internet, launch your Web browser (Internet Explorer 5.0 or newer or Netscape 4.0 or newer), then enter the following location (or URL):

http://www.invitrogen.com

...and the program will connect directly. Click on underlined text or outlined graphics to explore. Don't forget to put a bookmark at our site for easy reference!

Contact Us

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

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continued on next page

Technical Service, continued

MSDS Requests

To request an MSDS, visit our Web site at **www.invitrogen.com.** On the home page, go to 'Technical Resources', select 'MSDS', and follow instructions on the page.

Limited Warranty

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Invitrogen warrants that all of its products will perform according to 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.

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Purchaser Notification and Product Qualification

Purchaser Notification

Limited Use Label License No. 265: ChargeSwitch® Technology

The use of this product may be covered by European Patent No. EP1036082B1 and foreign equivalents.

Product Qualification

Each kit is functionally tested to ensure conformance with the most current approved product specifications. Current specifications consist of tests for:

- Bead size, charge, and binding capacity
- Nucleic acid quality and quantity
- Buffer turbidity, volume, and absence of RNases and DNases
- Kit packaging and labeling accuracy

For individual lot test results and more information, visit www.invitrogen.com to download the Certificate of Analysis.

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Notes

Notes

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