

GenSolve User Manual: Recovery of Purified Genomic DNA, Purified Plasmids, Bacterial Culture and Glycerol/DMSO Stocks

For Use with:

GVGR-100, GenSolve for DNA recovery from GenPlate elements spotted with pure DNA or plasmids, 100 recoveries

GVPURSTART-1, GenVault starter kit for pure DNA

GVGRSAMPLE, GenSolve for purified DNA recovery, sample kit, 20 recoveries

GVPURSAMPLE, GenVault Sample starter kit

Note: For information on GenSolve kits for use with other types of biosamples, please refer to the GenSolve chart on page 12 of this manual.

Summary

GenSolve elutes DNA from GenPlate elements spotted with purified DNA or plasmids. It also recovers plasmids from elements spotted with glycerol/DMSO bacterial stocks, or overnight cultures grown in standard or rich media

FTA chemistry may interfere with some downstream applications; therefore, further purification after elution with GenSolve is recommended for applications such as PCR, sequencing or other genetic analysis.

TECHNICAL SUPPORT

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www.genvault.com

Expected Results for genomic and plasmid DNA Recovery

GenSolve is designed to meet certain performance specifications for DNA yield and quality when recovering from 6mm disks of FTA® paper.

- Genomic DNA Yield: ≥70% of spotted material recovered from a 1µg sample. This percentage may vary with higher or lower masses of DNA spotted, as well as fragment length sizes. Fragments greater than 100kb may not be efficiently recovered.
- Plasmid DNA Yield: 80-90% of spotted material is recovered from plasmids under 10kb in length.
- Size: Recovered fragment length is equivalent to applied fragment length.
- Reproducibility: <20%CV between triplicate samples and between assays.
- PCR Amplification: Successful amplification of at least 5kb with primers used in both research and clinical laboratories.

Safety Information

- These kits are intended For Laboratory Use.
- Certain chemical present in FTA paper may be hazardous: sodium dodecyl sulfate, uric acid and ethylenediaminetetraacetic acid.
- Care should be taken when working with hazardous chemicals, such as minimizing contact; wearing appropriate personal protective equipment (safety glasses, gloves or a lab coat). Contact with eyes and ingestion should be avoided.
- MSDS documents for GenSolve reagents are available at www.genvault.com.

GenSolve Kit Components

- Recovery Solution A, 4 vials
- Recovery Solution B
- Reconstitution Buffer 1
- Reconstitution Buffer 2
- Precipitation Buffer

Additional Materials Required

- GenVault Incubator/Shaker (Catalog #GVMXR-1) or VWR Pulsing Vortex mixer (Catalog #12620-862) recommended
- GenVault Spin Basket/Tube Assembly (Catalog #GVSPIN-250)
- Millipore Microcon YM-30 (Catalog # 42409)
- Millipore Microcon YM-100 (Catalog #42413)
- Microfuge Tubes, 1.7ml and 2.0ml
- P200 and P1000 pipets, pipet tips
- Microcentrifuge

Storage

- All reagents may be stored at room temperature

IMPORTANT NOTES BEFORE BEGINNING:

Sample Application

1. **GenPlates for PURIFIED DNA AND PLASMIDS** should be used for application of **Purified Genomic DNA and Plasmids**.
2. **GenPlates for BLOOD AND CLONES** should be used for application of **Glycerol/DMSO stocks and Bacterial Cultures**.

Reagent Preparation

1. Reconstitute **RECOVERY SOLUTION A** with 6 mL of **RECONSTITUTION BUFFER 1** (for plasmids) or **RECONSTITUTION BUFFER 2** (for genomic DNA, glycerol/DMSO stocks or bacterial cultures). Vortex to completely resuspend the lyophilized agent.
2. Following resuspension, Recovery Solution A should be discarded after 24 hours.

Element Preparation

1. Remove an element from the GenPlate utilizing a GenPunch and place into a 2.0 ml microtube.

SECTION 1:

GenSolve DNA RECOVERY FOR GENOMIC DNA

Note: For optimal recovery, ensure spotted elements have been cured for two weeks. Ensure that Recovery Solution A is reconstituted with Reconstitution buffer 2 and is used within 24 hours (see reagent preparation).

1. Aliquot 100 μ L of RECOVERY SOLUTION A, into each 2.0 ml microtube containing a single element.
2. Incubate at room temperature shaking at 1400 rpm for one hour.
3. Centrifuge 0.5 min @ 16,300 x g in microcentrifuge to get liquid off cap.
4. Prepare a Spin Basket/Tube Assembly for recovering the DNA: Add 1.0 μ L of RECOVERY SOLUTION B to a new microtube (per recovery) and insert a Spin Basket.
5. Transfer RECOVERY SOLUTION A/DNA liquid into Spin Basket making sure to transfer along the element by scooping it with pipet tip.
6. Centrifuge at 16,300 x g for 2.0 minutes.
7. Discard Spin Basket and element.
8. Pulse vortex each microtube.
9. Add 14 μ L of PRECIPITATION BUFFER. Mix gently by inversion.
10. Centrifuge at 16,300 x g for 5.0 minutes in microcentrifuge.
11. Transfer supernatant to *new* 1.7 ml microtube avoiding the pellet.
12. To ensure complete removal of the precipitate, centrifuge again at 16,300 x g for 2.0 minutes in microcentrifuge.
13. Transfer supernatant to *new* 1.7 ml microtube while avoiding the pellet, if any, and proceed to Genomic DNA buffer exchange and sample concentration.

Genomic DNA Buffer Exchange and Sample Concentration

*Note: **Microcon YM-100** (Catalog #42413) should be used for **GENOMIC DNA**. Do not allow membrane to dry with sample on it. Do not touch the membrane with pipet tip during sample addition or wash steps. When washing and concentrating gDNA samples **DO NOT** spin at more than 500xg (2400 rpm).*

Utilizing Microcon YM-100 for genomic DNA samples:

1. Insert MICROCON-YM-100 sample reservoir into a microfuge tube.
2. Add 50 µL of water and spin at 12,300 rpm (14,000 x g) for 3 minutes.
3. Apply SAMPLE onto MICROCON-YM-100.
4. Spin **2,400 rpm (500 x g)** for 15 minutes (LOW SPEED SPIN)
5. Transfer MICROCON-YM-100 to a new microtube.
6. Add 500 µL of water, spin at **2,400 rpm** for 15 minutes (LOW SPEED SPIN), and decant microtube
7. Repeat Step 6.
8. Continue to centrifuge until ~25 µL remains on the column (LOW SPEED SPIN). For maximum recovery do not spin to dryness. If processing multiple MICROCON-YM-100 units, independently monitor each unit, as they concentrate at different rates, to insure that the YM-100 membrane is not spun to dryness.
9. Gently pipet mix the ~ 25ul being careful not to touch the filter at the bottom of the filtration unit.
10. Invert MICROCON-YM-100 and transfer into a new microtube.
11. Spin at 3,500 rpm (1,100 x g) for 3 minutes to collect the DNA.

THE ELUATE IS READY FOR QUANTITATION AND DOWNSTREAM PROCESSING.

SECTION 2:

GenSolve DNA RECOVERY FOR PURIFIED PLASMIDS

Note: For optimal recovery ensure spotted elements have been cured for two weeks. Ensure that Recovery Solution A is reconstituted with Reconstitution Buffer 1 and is used within 24 hours (see reagent preparation).

1. Aliquot 100 µl of RECOVERY SOLUTION A into each 2.0 ml microtube containing a single element.
2. Incubate at room temperature shaking at 1400 rpm for one hour.
3. Centrifuge 0.5 min @ 16,300 x g in microcentrifuge to get liquid off cap.
4. Prepare a Spin Basket/Tube Assembly for recovering the DNA: Add 1.0 µl of RECOVERY SOLUTION B to a new microtube (per recovery) and insert a Spin Basket.
5. Transfer RECOVERY SOLUTION A/DNA liquid into the Spin basket making sure to transfer along the element by scooping it with pipet tip.
6. Centrifuge at 16,300 x g for 2.0 minutes.
7. Discard Spin Basket and element.
8. Pulse vortex each microtube.
9. 1-2 µl of eluate, or dilutions thereof pending input into GenPlate elements, can be added directly to chemically competent cells for transformation. Adding more than 2 µl of neat eluate can result in less robust transformation efficiency. For other applications and for bacmids, proceed to Plasmid DNA buffer exchange and sample concentration.

Plasmid DNA Buffer Exchange and Sample Concentration

*Note: **Microcon YM-30** (catalog #42409) should be used for **PLASMIDS**. Do not allow the membrane to dry with sample on it. Do not touch the membrane with pipet tip during sample addition or wash steps.*

Microcon YM-30 for plasmids requiring sequencing and PCR:

1. Insert MICROCON-YM-30 sample reservoir into a microfuge tube.
2. Add 50 μ l ultra-pure water and spin at 12,300 rpm (14,000 x g) for 3 minutes.
3. Apply SAMPLE onto MICROCON-YM-30.
4. Spin at 12,300 rpm for 5 minutes.
5. Transfer MICROCON-YM-30 to a new microtube.
6. Add 500 μ l ultra-pure water to MICROCON-YM-30 and gently mix by pipetting up and down. Be careful not to touch the filter.
7. Spin at 12,300 rpm for 5 minutes; decant the solution in the microtube.
8. Repeat steps 6 - 7.
9. Continue to centrifuge until \sim 25 μ L remains on the column. For maximum recovery **do not spin to dryness**. If processing multiple MICROCON-YM-30 units, independently monitor each unit, as they concentrate at different rates.
10. Gently pipet mix the \sim 25ul being careful not to touch the filter at the bottom of the filtration unit.
11. Invert MICROCON-YM-30, transfer to new tube
12. Spin at 12,300 rpm for 2 minutes to collect plasmid DNA.

THE ELUATE IS READY FOR DOWNSTREAM PROCESSING.

SECTION 3:

GenSolve DNA RECOVERY FOR GLYCEROL/DMSO STOCKS AND BACTERIAL CULTURES

Note: For optimal recovery, ensure spotted elements have been cured for two weeks and that Recovery Solution A is reconstituted with Reconstitution Buffer 2 and is used within 24 hours (see reagent preparation).

1. Aliquot 100 μ L of RECOVERY SOLUTION A, into each 2.0 ml microtube containing a single element.
2. Incubate at room temperature shaking at 1400 rpm for one hour.
3. Centrifuge 0.5 min @ 16,300 x g in microcentrifuge to get liquid off cap.
4. Prepare a Spin Basket/Tube Assembly for recovering the DNA. Add 1.0 μ l of RECOVERY SOLUTION B to a new microtube (per recovery) and insert a Spin Basket.
5. Transfer RECOVERY SOLUTION A/DNA liquid into Spin Basket making sure to transfer along the element by scooping it with pipet tip.
6. Centrifuge at 16,300 x g for 2.0 minutes.
7. Discard Spin Basket and element.
8. Pulse vortex each microtube.
9. Add 14 μ L of PRECIPITATION BUFFER. Mix gently by inversion.
10. Centrifuge at 16,300 x g for 5.0 minutes.
11. Transfer supernatant to *new* 1.7 ml microtube avoiding the pellet.
12. To ensure complete removal of the precipitate, centrifuge again at 16,300 x g for 2 minutes.
13. Transfer supernatant to new 1.7 ml microtube, avoiding transfer of any pellet.

1-2 μ l of eluate, or dilutions thereof, depending on input into GenPlate elements, can be added directly to chemically competent cells for transformation. Adding more than 2 μ l of neat eluate can result in less robust transformation efficiency. For other applications or difficulty with transformation, proceed to Plasmid DNA buffer exchange and sample concentration on page 9.

Frequently Asked Questions (FAQs)

HOW CAN I QUANTITATE MY RECOVERED DNA?

GenVault recommends a PicoGreen assay if expected DNA conc is < 5-10ng/ul as Spectrophotometric analysis using Optical Density (OD) at 260nm is not sensitive enough. PicoGreen assay uses an intercalating dye to specifically quantitate only double-stranded DNA.

HOW CAN I CONVERT RPM TO G (RCF)?

Incorrect spinning can lead to low yields and purity. Check your centrifuge setting using the following equation: $g(\text{rcf}) = 1.12 * r * (\text{rpm}/1000)^2$ where r is the radius of the rotor in mm.

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GenSolve Kit and Biosample Reference Chart

Biosample	Sample Type	Type of GenPlate for Application of Biosample	*GenPlate Catalog #	Type of GenSolve Kit for Recovery	**GenSolve Kit Catalog #
Purified Genomic DNA	Purified DNA	GenPlates for Purified gDNA and plasmid	GVGP-20 GVGNP-20	GenSolve for Purified Genomic DNA, Purified Plasmids, Bacterial Culture and Glycerol/DMSO Stocks	GVGR-100
Purified Plasmids	Purified DNA	GenPlates for Purified gDNA and plasmid	GVGP-20 GVGNP-20	GenSolve for Purified Genomic DNA, Purified Plasmids, Bacterial Culture and Glycerol/DMSO Stocks	GVGR-100
Bacterial Culture	Crude Sample (plasmid stock)	GenPlates for Blood and Clones	GVP-20 GVNP-20	GenSolve for Purified Genomic DNA, Purified Plasmids, Bacterial Culture and Glycerol/DMSO Stocks	GVGR-100
Glycerol Stock	Crude Sample (plasmid stock)	GenPlates for Blood and Clones	GVP-20 GVNP-20	GenSolve for Purified Genomic DNA, Purified Plasmids, Bacterial Culture and Glycerol/DMSO Stocks	GVGR-100
DMSO Stock	Crude Sample (plasmid stock)	GenPlates for Blood and Clones	GVP-20 GVNP-20	GenSolve for Purified Genomic DNA, Purified Plasmids, Bacterial Culture and Glycerol/DMSO Stocks	GVGR-100
Whole Blood	Crude Sample	GenPlates for Blood and Clones	GVP-20 GVNP-20	GenSolve for Whole Blood DNA Recovery from FTA paper, Guthrie cards and GenPlate Elements	GVR-100
Blood Products (such as Buffy Coat)	Crude Sample	GenPlates for Blood and Clones	GVP-20 GVNP-20	GenSolve for Whole Blood DNA Recovery from FTA paper, Guthrie cards and GenPlate Elements	GVR-100

*Catalog # for single region GenPlates with or without GenCode

**Catalog # for 100 sample kit