MegaLong™ Protocol for Isolation of >100kb Genomic DNA (Tissue Sample)

G-Biosciences

Abstract

MegaLong™ isolates high molecular weight (>100kb) genomic DNA from a variety of samples, including animal tissues, cultured cells, whole blood, bacterial and yeast.

The protocol described here is for tissue samples. Please refer to the appropriate protocol, depending on your application.

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Guidelines

INTRODUCTION

MegaLong™ isolates high molecular weight (>100kb) genomic DNA from a variety of samples, including animal tissues, cultured cells, whole blood, bacterial and yeast. MegaLong™ uses Genomic Tube-O-DIALYZER™, a unique micro dialysis device with a 0.45µm membrane, which minimizes sample manipulation, one of the main reasons for DNA breakage. MegaLong™ isolates nuclei under mild extraction conditions and releases genomic DNA by digestion of nuclear proteins with a highly active LongLife™ Proteinase K. The digestion is performed in the Tube-O-DIALYZER™ and after digestion the Tube-ODIALYZER™ is inverted to dialyze away digested protein and other impurities leaving behind highly pure and fully hydrated genomic DNA.

The fragile, high molecular weight genomic DNA can be stored in the Tube-O-DIALYZER™ to further minimize mechanical manipulation of the DNA. The DNA is suitable for Southern blot analysis, recovery of Lambda shuttle vectors from transgenic animals, PCR, analysis by pulsed-field electrophoresis or any application where genomic DNA is required.

APPLICATIONS

MegaLong™ kit can be used for the isolation of genomic DNA from animal tissues, cultured cells, whole blood, bacterial and yeast. For samples unsuitable for the isolation of high molecular weight DNA with MegaLong™, G-Biosciences recommends using the OmniPrep™ Genomic DNA isolation kit (Cat. # 786-136).

The kit is supplied as a Micro or Large packs to process either 25 or 50 1-25mg samples.

ITEM(S) SUPPLIED

Description	Cat. # 786-146	Cat. # 786-147
Nuclei Isolation Buffe	2 x 30ml	4 x 30ml
Suspension Buffer	1 x 10ml	2 x 10ml
Digestion Buffer	1 x 2ml	2 x 2ml
LongLife™ Proteinase K [5mg/ml]	2 x 0.5ml	4 x 0.5ml
Genomic Tube-O-Dialyzer™	25	50
Floats (Medi)	6	6
Caps (Medi)	25	50
Forceps	1	1

STORAGE CONDITION

The kit is shipped at ambient temperature. Upon arrival, store LongLife™ Proteinase K at -20°C and remaining components at 4°C. LongLife™ Proteinase K solution is stable for 1 year, if stored properly.

ADDITIONAL MATERIALS REQUIRED

- Microfuge tubes & pestles (Cat. # 786-138P)
- TE buffer

Materials

MegaLong™ 786-146 by G-Biosciences

Pestles & Tubes 786-138P by G-Biosciences

Protocol

Tissue Sample Preparation

Step 1.

For optimal yield, rapidly dissect tissue and proceed with DNA extraction immediately, keeping samples on ice or promptly freeze in liquid nitrogen and store at -70°C until required.

Tissue Sample Preparation

Step 2.

On ice, add 1-25mg ground frozen tissue or fresh diced tissue to a microcentrifuge tube containing 500µl Nuclei Isolation Buffer. Homogenize the sample with a microfuge pestle until a homogenous suspension is acquired.

NOTES

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NOTE: Do not twist the pestle or DNA shearing will occur. A Wheaton Dounce Homogenizer can also be used; First 5-15 strokes with a loose fitting pestle, then ~10 strokes with a tight fitting pestle. Do not twist

Tissue Sample Preparation

Step 3.

Incubate the sample at 4°C for >1 minute to sediment large tissue fragments without sedimenting the nuclei. During the incubation prepare the Tube-ODIALYZER™.

Tube-O-DIALYZER™ Preparation

Step 4.

Place the Tube-O-DIALYZER™ cap in a beaker of TE buffer and store at 4°C until required. Rinse the Tube-O-DIALYZER™ tube with TE buffer.

Tube-O-DIALYZER™ Preparation

Step 5.

With a pipette transfer the supernatant to the Tube-O-DIALYZER™, ensuring the settled cellular debris is left behind.

Tube-O-DIALYZER™ Preparation

Step 6.

Place a supplied cap on the tube and centrifuge at 16,000xg for 5 minutes to pellet the nuclei.

DURATION

00:05:00

Tube-O-DIALYZER™ Preparation

Step 7.

Carefully discard the supernatant and invert the tube on a paper towel to remove excess supernatant.

Tube-O-DIALYZER™ Preparation
Step 8.

Add 70µl Suspension Buffer to the nuclei and gently rock or tap the tube to dislodge the nuclei.

Tube-O-DIALYZER™ Preparation
Step 9.

Vortex the LongLife™ Proteinase K and add 10µl to the nuclei.

Tube-O-DIALYZER™ Preparation
Step 10.

Add 70µl Digestion Buffer and mix with gentle rocking.

Tube-O-DIALYZER™ Preparation
Step 11.

Incubate at 55°C for 2-4 hours with periodic rocking. Do not vortex.

DURATION
00:30:00

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NOTE: For periodic rocking, gently invert the tube 2-3 times every 30 minutes.

Tube-O-DIALYZER™ Preparation
Step 12.

After digestion is complete, centrifuge the tube for 20 seconds at 1,000g.

DURATION
00:00:20

Tube-O-DIALYZER™ Preparation
Step 13.

Replace the cap with the dialysis cap. Do not discard the storage cap as this will be required for storage of DNA.

Tube-O-DIALYZER™ Preparation
Step 14.

Place the Tube-O-DIALYZER™ upside down in a 50ml centrifuge tube and centrifuge at 1000xg for 30 seconds to bring the sample onto the dialysis membrane.

DURATION
00:00:30

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NOTE: Do not centrifuge longer or faster than stated to prevent damage to membrane and sample loss.

Tube-O-DIALYZER™ Preparation

Step 15.

Remove the Tube-O-DIALYZER™ from the 50ml tube with forceps and keeping it inverted slide into the provided float and dialyze in 500ml 1X TE buffer at room temperature for 18-24 hours with 2-3 buffer changes. Gently swirl tube to mix contents at each buffer change.

DURATION
18:00:00

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NOTE: Cloudy DNA is an indication of incomplete dialysis, therefore dialyze for an additional 24 hours. Change dialysis buffer and mix the content of the Tube-ODIALYZER™ by gently swirling every few hours.

Tube-O-DIALYZER™ Preparation

Step 16.

Following dialysis the genomic DNA may be concentrated in the Tube-O-DIALYZER™ using either Tube-O-DIALYZER™ Concentrator (Cat. # 786-144) or Concentrator Solution (Cat. # 786-143). Simply prepare the Concentrator as per the instructions and invert the Tube-O-DIALYZER™ containing your DNA in the solution.

Tube-O-DIALYZER™ Preparation

Step 17.

If concentration is not required or following concentration, centrifuge the tube at 1000xg for 1 minute. Replace the dialysis cap with the normal cap. The genomic DNA is now ready for use.

DURATION
00:01:00