MegaLong™ Protocol for Isolation of >100kb Genomic DNA (Cell Culture)

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 cell culture 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

Sample Preparation

Step 1.

On ice, add up to 2.5x10^6 cells to a Tube-O-DIALYZER™ and centrifuge at 5,000xg for 5 minutes to pellet cells. Discard the supernatant.

DURATION
00:05:00

Sample Preparation

Step 2.

Add 500µl Nuclei Isolation Buffer.

Sample Preparation

Step 3.

Invert the tube 2-3 times to suspend the cells, incubate for 10 minutes on ice.

DURATION
00:10:00

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

NOTES
Colin Heath 23 Jun 2016

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

NOTES
Colin Heath 23 Jun 2016

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

NOTES
Colin Heath 23 Jun 2016

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