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A Geno Technology, Inc. (USA) brand name

MegaLong™

For Isolation of >100kb Genomic DNA

(Cat. # 786-146, 786-147)

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-O-DIALYZER™ 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 Buffer	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

PROTOCOL

Tissue Sample Preparation

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. .
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.
   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*
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-O-DIALYZER™.

Tube-O-DIALYZER™ Preparation

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.
5. With a pipette transfer the supernatant to the Tube-O-DIALYZER™, ensuring the settled cellular debris is left behind.
6. Place a supplied cap on the tube and centrifuge at 16,000xg for 5 minutes to pellet the nuclei. Carefully discard the supernatant and invert the tube on a paper towel to remove excess supernatant.
7. Add 70µl Suspension Buffer to the nuclei and gently rock or tap the tube to dislodge the nuclei.
8. Vortex the *LongLife*™ Proteinase K and add 10µl to the nuclei.
9. Add 70µl Digestion Buffer and mix with gentle rocking.
10. Incubate at 55°C for 2-4 hours with periodic rocking. Do not vortex.
    NOTE: *For periodic rocking, gently invert the tube 2-3 times every 30 minutes.*
11. After digestion is complete, centrifuge the tube for 20 seconds at 1,000g.
12. Replace the cap with the dialysis cap. Do not discard the storage cap as this will be required for storage of DNA.

13. Place the Tube-O-DIALYZER™ upside down in a 50ml centrifuge tube and centrifuge at 1000xg for 30 second to bring the sample onto the dialysis membrane.
    NOTE: *Do not centrifuge longer or faster than stated to prevent damage to membrane and sample loss.*
14. 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.
    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-O-DIALYZER™ by gently swirling every few hours.*
15. 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 you DNA in the solution.
16. 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.

PROTOCOL VARIATIONS

Cell Culture

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.
2. Add 500µl Nuclei Isolation Buffer. Invert the tube 2-3 times to suspend the cells, incubate for 10 minutes on ice.
3. Continue protocol at the Tube-O-DIALYZER™ preparation stage (step 4).

Blood

1. On ice, add 5-400µl blood to a Tube-O-DIALYZER™ and centrifuge at 5,000xg for 5 minutes to pellet cells. Discard the supernatant.
2. Add 500µl Nuclei Isolation Buffer. Invert the tube 2-3 times to suspend the cells, incubate for 10 minutes on ice.
3. Continue protocol at the Tube-O-DIALYZER™ preparation stage (step 4).

Bacterial DNA

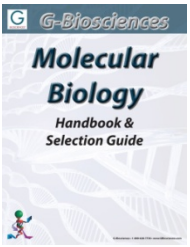
1. On ice, add 0.5ml bacteria culture to a Tube-O-DIALYZER™ and centrifuge at 5,000xg for 5 minutes to pellet cells. Discard the supernatant.
2. Add 50µl lysis solution containing 1% SDS, 0.1N NaOH and 10mM EDTA (not supplied), 25µl of Digestion Buffer and 10µl *LongLife*™ Proteinase K Solution. Proceed to protocol Step 10.

Yeast DNA

1. On ice, prepare spheroplasts from a 1.5ml overnight culture then begin the protocol at step 7.

RELATED PRODUCTS

Download our Molecular Biology Handbook.



http://info.gbiosciences.com/complete-molecular-biology-handbook/

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