Megabase DNA Extraction from Animal Blood

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Abstract

This protocol provides an efficient and reliable technique for obtaining HMW DNA(>1Mb) from animal blood. It accompanies:

Huang Zhihai, Xu Jiang, Xiao Shuiming, Liao Baosheng, Gao Yuan, Zhai Chaochao, Qiu Xiaohui, Xu Wen, Chen Shilin

(2016): Supporting data for 'Comparative optical genome analysis of two Pangolin species Manis pentadactyla and Manis javanica'. GigaScience Database.

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Protocol

sample collection

Step 1.

Collect 3mL animal's blood in 5 mL EDTA anticoagulative tube, and store at 4 °C

sample collection

Step 2.

Mix blood by gently rocking for 10min at room temperature to ensure a homogenous WBC distribution.

DURATION

00:10:00

sample collection

Step 3.

Transfer 3ml blood to 15ml screw cap tube containing 9ml RBC lysis solution (3 volumes), and mix by gently inverting 10x.

sample collection

Step 4.

Incubate 5 min at room temperature. Gently mix by inverting at least once during incubation.

DURATION

00:05:00

sample collection

Step 5.

Spin 5 min at 2000xg at 4^{0}C and carefully pipet out supernatant while not disturbing the pellet.

DURATION
00:05:00

sample collection

Step 6.
Resuspend cell pellet in 3ml PBS (1 blood volume):

sample collection

Step 7.
Add 9ml rbc lysis sol (3 volumes), invert to mix and incubate 5 min at room temperature.

DURATION
00:05:00

sample collection

Step 8.
Spin cell suspension for 5 min at 2000xg at 4^{0}C and carefully pipet out supernatant while not disturbing the pellet. Remove the last drop of liquid by tilting the tube and removing liquid with pipet tip.

DURATION
00:05:00

sample collection

Step 9.
Resuspend cell pellet in 580ul cell suspension buffer (Bio Rad plug lysis kit) until homogenous suspension.

sample collection

Step 10.
Transfer 375ul to a new microfuge tube and label (400). Transfer 187.5ul to another tube labeled (200); add 187.5ul cell suspension buffer, and pipet mix gently 5x. Keep both tubes on ice until ready to embed in agarose (section II).

sample collection

Step 11.
Melt 2% agarose (Bio Rad kit) by immersing in microwave-boiled water for 10-15min until agarose is completely melted.

DURATION
00:10:00

sample collection

Step 12.
Equilibrate both tubes from step 11 section I in 43°C water bath/heat block for at least 5 min before use.

DURATION
00:05:00

sample collection

Step 13.
After 5 min, prepare 5 plugs from one tube by performing the next steps rapidly to avoid solidification of the cell-agarose mixture before pipeting into plug mold:

sample collection

Step 14.
Place plug cast on ice for at least 45min or until agarose has solidified. Alternatively, can place plug cast on inverted metal microfuge ice block on ice for 45min, to avoid potential contact of agarose with ice

DURATION

00:45:00

sample collection

Step 15.

Wash away hemoglobin and cellular material with Proteinase K and WashBuffer.