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Bionano genome mapping from animal tissue

In 1 collection

Graham J Etherington¹, Darren Heavens¹, David Baker¹,
Ashleigh Lister¹, Rose McNelly¹, Gonzalo Garcia¹,
Bernardo Clavijo¹, Iain Macaulay¹, Wilfried Haerty¹,
Federica Di Palma¹

¹The Earlham Institute, Norwich Research Park, Norwich, NR4 7UZ, United Kingdom

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We use this protocol and it's working

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Graham Etherington
The Earlham Institute

ABSTRACT

Bionano genome map protocol

Keywords: polecat, vertebrate, non-model organism, Illumina, chromium, Bionano, assembly, sequencing

- 1 The Bionano genome maps were prepared by taking approximately 10 mg of DNA (in this case extracted from a polecat) from the sample stored in 100% EtOH.
- 2 The IrysPrep Animal Tissue DNA Isolation from Fibrous Tissue protocol was followed using the IrysPrep Animal Tissue DNA kit (RE-013-10) from Bionano Genomics.
- 3 The animal tissue sample was cut up into <3mm pieces for homogenisation and then fixed in a 2% formaldehyde solution in kit-provided Homogenisation Buffer (HB) for 00:30:00 On ice before blending using a Qiagen Tissuerruptor.
- 4 Spin at 1500x g for 00:05:00 in centrifuge, remove supernatant and resuspend in around 50 µL of HB buffer using wide bore tip to produce a total volume of 66 µL.
- 5 40 µL of LMP agarose was then melted at 70 °C and cooled to 43 °C before addition to the cell resuspension and mixing using a wide bore tip.
- 6 One plug of around 90 µL was cast using the Chef Mammalian Genomic DNA Plug Kit (Bio-Rad 170-3591).
- 7 Once cooled to 4 °C the plug was added to a lysis solution containing 200 µL proteinase K (QIAGEN 158920) and 2.5 mL of Bionano lysis Buffer.
- 8 This was incubated at 50 °C for 02:00:00 in a thermomixer, making a fresh proteinase

K solution and incubating Overnight .

- 9 The 50 mL tubes were then removed from the thermomixer for 00:05:00 before 50 µL RNase A (Qiagen158924) was added and to the tubes, returned to the thermomixer for a further hour at 37 °C .
- 10 The plugs were then washed 7 times in the Wash Buffer supplied with the Chef kit and 7 times in 1xTE.
- 11 The plug was removed and melted for 00:02:00 at 70 °C followed by 00:05:00 at 43 °C before adding 10 µL of 0.2U/µl of GELase (Cambio Ltd G31200).
- 12 After 00:45:00 at 43 °C the melted plug was dialysed on a 0.1 uM membrane (Millipore VCWP04700) sitting in 15 mL of 1xTE buffer in a small petri dish.
- 13 After 00:45:00 the sample was removed with a wide bore tip and mixed gently 00:45:00 and left Overnight at 4 °C . A small amount was removed to QC on an Opgen Argus Q-Card and Qubit HS to calculate the DNA concentration.
- 14 300 ng of DNA was taken into the NLRs (Nick, Label, Repair and Stain) reaction using 1 µL Nt.BspQI (NEB R0644S). The optical maps were then generated using the Bionano Irys platform.