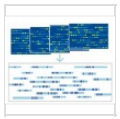

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Bionano genome mapping from animal tissue

In 1 collection

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1 *Works for me* dx.doi.org/10.17504/protocols.io.bd7ei9je

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ABSTRACT

Bionano genome map protocol

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 Tissueruptor.

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.

Citation: Graham J Etherington, Darren Heavens, David Baker, Ashleigh Lister, Rose McNelly, Gonzalo Garcia, Bernardo Clavijo, Iain Macaulay, Wilfried Haerty, Federica Di Palma (03/25/2020). Bionano genome mapping from animal tissue. https://dx.doi.org/10.17504/protocols.io.bd7ei9je

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.

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