Irys NLRS DNA labeling and Data collection

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

Abstract

This protocol is provided by BioNano Genomics and was used in:

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

Nicking (10 µL / ~2.5 hours)

Step 1.

Equilibrate DNA 30 min at room temp. Pipet mix 3x with wide bore tip.

DURATION

00:30:00

Nicking (10 µL / ~2.5 hours)

Step 2.

Prepare nicking master mix

One 10µL rxn: 1µL buffer + DNA + enzyme in 200µL thin-wall PCR tube.

Blood BspQI: 5-7u (BNG3)

Cell line BspQI: 3-5u (BNG3)

Other BspQI: 5-10u (BNG3)

BbvCI: 4-8u (BNG2)

Q+C: 3/2, 6/4, 9/6u (BNG2)

Nicking (10 µL / ~2.5 hours)

Step 3.

Mix 4x with Xplorer Plus Pipettor set to 8µL at lowest speed. Pulse spin 2sec.

Nicking (10 µL / ~2.5 hours)

Step 4.

Incubate in a thermal cycler for 2 hrs at 37°C with heated-lid.

DURATION

02:00:00

Labeling (15 µL / ~1.25 hours)

Step 5.

(For the rest of the protocol, protect from light)

Prepare the Labeling Master Mix.

reagent	amount
10x labeling buffer	1.5µL
10x labeling mix	1.5µL
Taq 5u/µL	1µL
H_20	1µL

Labeling (15 µL / ~1.25 hours)

Step 6.

Add 5µL Labeling Master Mix.

Step 7.

Mix 4x with Xplorer Plus Pipettor set to 13µL at lowest speed. Pulse spin 2sec.

Labeling (15 µL / ~1.25 hours)

Step 8.

Incubate in a thermal cycler for 60 min at 72°C with heated-lid."

DURATION

01:00:00

Repair (20 µL / ~45 minutes)

Step 9.

Prepare the Repair Master Mix.

reagent	amount
10x Thermo Pol	0.5µL
50x Repair Mix	0.4µL
50mM NAD+	0.4µL
Taq DNA ligase	1µL
H_20	2.7µL

NOTES

GigaScience Database 02 Nov 2016

Protect from light

Repair (20 µL / ~45 minutes)

Step 10.

Add 5µl of the Repair Master Mix.

Repair (20 µL / ~45 minutes)

Step 11.

Mix 4x with Xplorer Plus Pipettor set to 18µL at lowest speed. Pulse spin 2sec.

Repair (20 µL / ~45 minutes)

Step 12.

Incubate in a thermal cycler for 30 min at 37°C with heated-lid.

DURATION

00:30:00

Repair (20 µL / ~45 minutes)

Step 13.

Place NLR reaction on ice. Add 1µL Stop Solution. Mix by gently stirring 5 times with the pipet tip. Pulse spin 2sec."

Staining NLR (60 µL / ~16 hours)

Step 14.

Equilibrate Staining Master Mix components to room temp.

NOTES

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Protect from light

Staining NLR (60 µL / ~16 hours)

Step 15.

Prepare the Staining Master Mix.

reagent	amount
4x flow buffer	15µL
1M DTT	12µL
DNA stain	1.5µL
H_2O	11.5µL

Staining NLR (60 µL / ~16 hours)

Step 16.

Aliquot 40µL of the Staining Master Mix into a 0.6mL amber tube.

Staining NLR (60 µL / ~16 hours)

Step 17.

Transfer NLR to Staining Mix with standard tip. Pulse spin 2 sec.

Staining NLR (60 µL / ~16 hours)

Step 18.

Gently mix NLRS DNA 5x with wide bore tip set to 50µL. Pulse spin 2 sec.

Staining NLR (60 µL / ~16 hours)

Step 19.

Place staining reactions at 4°C overnight to ensure uniform DNA staining.

DURATION

16:00:00

NLRS DNA Conc.(6µL, ~0.5 hours)

Step 20.

Equilibrate NLRS to room temp for 30 min.

DURATION

00:30:00

NOTES

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Protect from light

NLRS DNA Conc.(6µL, ~0.5 hours)

Step 21.

Gently mix DNA 3x with wide bore tip set to 50µL. Pulse spin 2 sec.

NLRS DNA Conc.(6µL, ~0.5 hours)

Step 22.

Remove 2µL aliquots from the Top, Middle and Bottom to separate Qubit Assay tubes. Add 18uL of Qubit HS Buffer.

NLRS DNA Conc.(6µL, ~0.5 hours)

Step 23.

Sonicate 10 min in a bath sonicator.

DURATION

00:10:00

NLRS DNA Conc.(6µL, ~0.5 hours)

Step 24.

Add 180µL Qubit reagent mix. Vortex 5 seconds. Pulse spin 2 sec.

NLRS DNA Conc.(6µL, ~0.5 hours)

Step 25.

Incubate at least 2 min at room temp. Quantitate with Qubit 2.0 Fluorimeter.

NLRS DNA conc: 3-10ng/µL. CV < 25%.'

DURATION
00:02:00

Chip loading and data collection

Step 26.

Load sample into Irys chip and start data collection with a 30-cycle run on irys system