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10x Genomics Library Construction

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Graham J Etherington[1], Darren Heavens[1], David Baker[1], Ashleigh Lister[1], Rose McNelly[1], Gonzalo Garcia[1], Bernardo Clavijo[1], Iain Macaulay[1], Wilfried Haerty[1], Federica Di Palma[1]

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

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Graham Etherington
The Earlham Institute

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We use this protocol and it's working

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Abstract

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1　Input DNA (in this case Polecat) was diluted down from ⟦**[M]** 4.18 ng/ul⟧ to ⟦**[M]** 1 ng/ul⟧ using Qiagen EB and checked using a QuBit 2.0 Fluorometer (Thermo-Fisher). The sample was then diluted in half as it was denatured. Finally, ⟦🧪 2.5 µL⟧ of the diluted and denatured DNA was loaded onto the 10X chip which equated to ⟦🧪 1.25 ng⟧.

2　Gel beads, oil and master mix were loaded into the appropriate wells on the 10X chip. The DNA was loaded along with the master mix. The chip was then loaded into the 10X Genomics Chromium instrument.

3　The formed emulsion was removed and placed into the thermocycler for ⟦🕒 03:00:00⟧ at ⟦🌡 30 °C⟧. GEMs were cleaned up using the recovery reagent (10X Genomics), Silane beads (Thermo Fisher) and Tween-20 (Sigma-Aldrich) as per manufacturer's instructions. DNA is cleaned up using a 0.7X ratio of SPRISelect beads (Beckman Coulter).

4　The library underwent simultaneous End Repair and A tailing on the thermocycler at ⟦🌡 20 °C⟧ for ⟦🕒 00:30:00⟧ and then ⟦🌡 65 °C⟧ for ⟦🕒 00:30:00⟧. Adapters were then ligated at ⟦🌡 20 °C⟧ for ⟦🕒 00:15:00⟧. A 0.8X ratio SPRISelect clean-up was performed. Sample Indexes were added during the final 8 cycle PCR amplification.

Temperature	Time
98°C (step 1)	45 secs
98°C (step 2)	20 secs
54°C (step 3)	30 secs
72°C (step 4)	20 secs
Steps 2-4 for 7 or 8 cycles in total	
72°C (step 5)	60 secs
4°C (step 6)	Hold

5　A dual-SPRI size selection was performed at 0.5X then 0.7X ratios.

6   Library sizes were checked using the High Sense DNA bioanalyzer (Agilent). Molarity was checked using qPCR (KAPA Library Quant kit (Illumina), using ABI Prism qPCR Mix (Kapa Biosystems).

7   The library was clustered on the flow cell using the qPCR molarity. It was clustered at 8pM along with 1% PhiX spike-in (Illumina). Two lanes of a Rapid Run v2 flow cell were run on an Illumina HiSeq2500. 150bp PE, 8bp i7 index read, 0bp i5 index read. The libraries clustered at around 1100K/mm2.
Manufacturer's instructions:
https://assets.contentful.com/an68im79xiti/4z5JA3C67KOyCE2ucacCM6/d05ce5fa3dc4282f3da5ae7296f2645b/CG00022_GenomeReagentKitUserGuide_RevC.pdf