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A new targeted capture method using bacterial artificial chromosome (BAC) libraries as baits for sequencing relatively large genes

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ABSTRACT

To analyze a specific genome region using next-generation sequencing technologies, the enrichment of DNA libraries with targeted capture methods has been standardized. For enrichment of mitochondrial genome, a previous study developed an original targeted capture method that use baits constructed from long-range polymerase chain reaction(PCR) amplicons, common laboratory reagents, and equipment. In this protocol, a new targeted capture method is presented, that of bacterial artificial chromosome (BAC) double capture (BDC), modifying the previous method, but using BAC libraries as baits for sequencing a relatively large gene.

GUIDELINES

This targeted enrichment was conducted following the modified protocol of Maricic et al., (2010) and the NimbleGen technical note "Double Capture: High Efficiency Sequence Capture of Small Targets for use in SeqCap EZ Library, Applications on 454 Sequencing Systems." Please get the two protocols and check those in detail.

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MATERIALS

MATERIALS

⊗ MinElute PCR Purification Kit **Qiagen Catalog #28004**

⊗ NucleoBond BAC100 **Macherey and Nagel**

⊗ human Cot-1 DNA **Contributed by users**

⊗ PCR grade water **Contributed by users**

⊗ Dynabeads M-270 Streptavidin **Invitrogen - Thermo Fisher**

⊗ KAPA HiFi DNA Polymerase **Kapa Biosystems**

⊗ KAPA Library Quantification Kit for Illumina NGS platforms **Kapa Biosystems**

⊗ Solutions to biotinylate sheared BAC (Maricic et al., 2010) **Contributed by users**

⊗ Blocking oligonucleotide solutions (Maricic et al., 2010) **Contributed by users**

⊗ Primers: Sol_bridge_P5 and Sol_bridge_P7 (Maricic et al., 2010) **Contributed by users**

⊗ SeqCap EZ Hybridization and Wash Kit **Roche**

Bait production

- 1 NucleoBond BAC 100 (Macherey-Nagel; Düren, Germany) was used to purify the BAC.
- 2 The concentration was measured using a NanoPhotometer (Implen).
- 3 The total amount of 5 µg of BAC was sheared using a Covaris S2 sonicator (Covaris) using default settings of the peak from 350 to 750 bp.
- 4 The sheared BACs were purified using a MinElute PCR Purification Kit (Qiagen).
- 5 1.5-µg sheared BACs per capture reaction were prepared.

- 6 the products were then biotinylated according to **the protocol used in Maricic et al., (2010).**

BAC double capture (BDC)

- 7 **IMPORTANT:** In BDC section, please check the details of reagents and oligos with '**Maricic et al., Multiplexed DNA Sequence Capture of Mitochondrial Genomes Using PCR Products (2010)**' and the NimbleGen technical note "**Double Capture: High Efficiency Sequence Capture of Small Targets for use in SeqCap EZ Library, Applications on 454 Sequencing Systems.**"
- 8 Blocking oligonucleotide solutions (Maricic et al., 2010) and human Cot-1 DNA were added to the library solution.
- 9 The solution was dried out using a heat block at 95C.
- 10 Hybridization buffer and formamide added to the dried DNA, and the mixture was suspended by vortex mixing.
- 11 The suspended mixture was single-stranded using a heat block at 95C for 10 min.
- 12 Biotinylated BAC baits (500 ng) eluted by 4.5 µL PCR grade water was added to the single-stranded DNA mixture and mixed by pipetting.
- 13 The solution was heated in a thermal cycler to 95C for 10 min and incubated at 65C overnight

(12–16 h).

- 14 Following incubation, Dynabeads M-270 Streptavidin (Invitrogen; CA, USA) was added to the hybridization mixture.
- 15 Bound DNA fragments were washed and eluted using NGS MagnaStand (Nippon Genetics, Tokyo, Japan).
- 16 After the wash and the elution, PCRs were run of the enriched library (26 μ L) before removing magnetic beads. The 5 μ L of the eluted library was used as a template for PCR in a 50 μ L solution containing deoxynucleotide (dNTP) 0.3 mM, 0.5 μ M of each primer, Sol_bridge_P5 and Sol_bridge_P7 in Maricic et al. (2010), 1.0 U of KAPA HiFi DNA Polymerase (Kapa Biosystems). The 1st post-capture PCR was carried out following the cycling reaction: an initial denaturing step at 98C for 2 min, 8 cycles of denaturation at 98C for 10 sec, annealing at 60C for 15 sec, extension at 68C for 50 sec.
- 17 The PCR amplicon was purified using a MinElute PCR Purification Kit (Qiagen).
- 18 The 2nd capture was conducted using the enriched and purified library using the same steps as in the 1st capture. The 2nd post-capture PCR of the 2nd captured library was run in 13 cycles using the same cycling condition as in the 1st post-capture PCR.
- 19 The PCR amplicon was purified using a MinElute PCR Purification Kit (Qiagen).
- 20 Quantification of the amplified capture library was conducted with a KAPA Library Quantification Kit for Illumina NGS platforms (Kapa Biosystems) and a 2100 Bioanalyzer (Agilent Technologies).