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.

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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.

Materials

- MinElute PCR Purification Kit 28004 by Qiagen
- NucleoBond BAC100 by Macherey and Nagel
- human Cot-1 DNA by Contributed by users
- PCR grade water by Contributed by users
- Dynabeads M-270 Streptavidin by Invitrogen - Thermo Fisher

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KAPA HiFi DNA Polymerase by Kapa Biosystems

KAPA Library Quantification Kit for Illumina NGS platforms by Kapa Biosystems

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

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

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

SeqCap EZ Hybridization and Wash Kit by Roche

Protocol

Bait production

Step 1.

NucleoBond BAC 100 (Macherey-Nagel; Düren, Germany) was used to purify the BAC.

Bait production

Step 2.

The concentration was measured using a NanoPhotometer (Implen).

Bait production

Step 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.

Bait production

Step 4.

The sheared BACs were purified using a MinElute PCR Purification Kit (Qiagen).

Bait production

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Step 5.
1.5-µg sheared BACs per capture reaction were prepared.

Bait production

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

BAC double capture (BDC)

Step 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."**

BAC double capture (BDC)

Step 8.
Blocking oligonucleotide solutions (Maricic et al., 2010) and human Cot-1 DNA were added to the library solution.

BAC double capture (BDC)

Step 9.
The solution was dried out using a heat block at 95C.

BAC double capture (BDC)

Step 10.
Hybridization buffer and formamide added to the dried DNA, and the mixture was suspended by vortex mixing.

BAC double capture (BDC)

Step 11.
The suspended mixture was single-stranded using a heat block at 95C for 10 min.

BAC double capture (BDC)

Step 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.

BAC double capture (BDC)

Step 13.
The solution was heated in a thermal cycler to 95C for 10 min and incubated at 65C overnight (12–16 h).

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BAC double capture (BDC)

Step 14.

Following incubation, Dynabeads M-270 Streptavidin (Invitrogen; CA, USA) was added to the hybridization mixture.

BAC double capture (BDC)

Step 15.

Bound DNA fragments were washed and eluted using NGS MagnaStand (Nippon Genetics, Tokyo, Japan).

BAC double capture (BDC)

Step 16.

After the wash and the elution, PCRs were run of the enriched library (26 µL) before removing magnetic beads. The 5 µL of the eluted library was used as a template for PCR in a 50 µL solution containing deoxynucleotide (dNTP) 0.3 mM, 0.5 µM 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.

BAC double capture (BDC)

Step 17.

The PCR amplicon was purified using a MinElute PCR Purification Kit (Qiagen).

BAC double capture (BDC)

Step 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.

BAC double capture (BDC)

Step 19.

The PCR amplicon was purified using a MinElute PCR Purification Kit (Qiagen).

BAC double capture (BDC)

Step 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).

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