Gmail

Alec Steep <alec.steep@gmail.com>

MiSeq data available -- run 20181128_Amplicon_PE250

4 messages

Carr, Kevin <carrk@msu.edu> Mon, Dec 3, 2018 at 2:20 PM
To: "Steep, Alexander Cordiner" <steepale@msu.edu>

Dear Alec,

Sequencing is complete for samples submitted to the RTSF Genomics Core, project ID STE7140 (Amplicon_MiSeq). You submitted 34 dsDNA 1° PCR amplicons prepared in your lab. Each of these 34 samples represented multiple amplicon amplicon products from a single sample/line. Your PCR primers all incorporated the Fluidigm CS1/CS2 universal oligomers at their 5' ends. The Genomics Core added dual indexed, Illumina compatible adapters via 2° PCR with primers targeting the CS1/CS2 ends. After PCR all samples were batch normalized using an Invitrogen SequalPrep DNA Normalization Plate. Product recovered from the normalization plate was pooled and the pool was cleaned up and concentrated using AmpureXP magnetic beads. The pool was QC'd and quantified using a combination of Qubit dsDNA HS, Agilent 4200 TapeStation High Sensitivity DNA 1000 and Kapa Illumina Library Quantification qPCR assays. The pool was loaded onto an Illumina MiSeq Standard v2 flow cell and sequencing performed in a 2x150bp paired end format using a MiSeq v2 300 cycle reagent cartridge. Custom sequence and index primers complementary to the CS1/CS2 oligos were added to appropriate wells of the reagent cartridge. Base calling was done by Illumina Real Time Analysis (RTA) v1.18.54 and output of RTA was demultiplexed and converted to FastQ format with Illumina Bcl2fastq v2.19.1. A summary of the run output is attached below. Basic QC information about your sequence data is provided by the accompanying FastQC reports. Please see the FastQC Tutorial and FAQ (https://rtsf.natsci.msu.edu/genomics/tech-notes/fastqc-tutorial-and-faq/) for information regarding interpretation of these reports.

The FastQC reports reveal that a large fraction of the reads across all 34 of your samples come from amplicons with products ranging form 40-60bp in length. The proportion products in this size range varied depending on sample from just below 20% to as high as 65% of the total reads per sample. In all 34 of the samples 80% or more of the sequenced fragments were < 150bp.

You may download your data using the Cheng lab account on the Genomics Core FTP server. Login credentials are:

hostname: titan.bch.msu.edu
username: chengh
password: bu29eBeF

Please retain this information for future use. Data for this project is in subdirectory 20181128_Amplicon_PE250. You must use secure FTP (FTPS) when connecting to the RTSF server. See the Genomics FAQ for general instructions (https://rtsf.natsci.msu.edu/genomics/data-retrieval/). Sequence data typically remain available on the FTP server for 60 days. It is the responsibility of the researcher to download and store their data long term, including a safe backup copy. The RTSF only guarantees retention of sequence data for one year from the date of availability.

Regards,

Kevin M. Carr

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Bioinformatics Specialist
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We have created a new email list to allow us to easily communicate with Genomics Core users about news and seminars that are relevant to the Core. You may sign up to this mailing list by sending the text "SUBSCRIBE GENOMICS-CORE"

on a single line and as the only text in the body of an email to LISTSERV@LIST.MSU.EDU. It is important that "SUBSCRIBE GENOMICS-CORE" (without quotes) is the only text in the email body. Once your email is successfully received by the listserver, an automatic response will be sent back to you. This automatic response email will contain a URL that you _must_ click in order to complete the sign-up process. If you have difficulties signing up to the email list yourself, you may write to kchilds@msu.edu to request to be added to the email list.

20181128_SeqProduction_Cheng.xlsx
59K

Alec Steep <alec.steep@gmail.com> Mon, Dec 3, 2018 at 2:24 PM
To: Hans Cheng <hcheng@msu.edu>

Hi Hans,
Our sequencing came back from the core. Looks like they pushed it through for us.

I'll have to examine the specifics and see how the sequences map, likely after the dissertation.

Kind regards,
Alec
[Quoted text hidden]
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Alec Steep
Genetics Program
Michigan State University

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steepale@msu.edu

20181128_SeqProduction_Cheng.xlsx
59K

Cheng, Hans <hcheng@msu.edu> Mon, Dec 3, 2018 at 3:07 PM
To: Alec Steep <alec.steep@gmail.com>

Good news and good luck

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Hans H. Cheng

Research Geneticist

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FAX: (517) 337-6776

E-mail: hcheng@msu.edu

or Hans.Cheng@ars.usda.gov

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From: Alec Steep <alec.steep@gmail.com>
Date: Monday, December 3, 2018 at 2:27 PM
To: Hans Cheng <hcheng@msu.edu>
Subject: Fwd: MiSeq data available -- run 20181128_Amplicon_PE250

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Alec Steep <alec.steep@gmail.com> Mon, Dec 3, 2018 at 4:40 PM
To: "Kevin M. Carr" <carrk@msu.edu>

Hi Kevin,
Thank you for all your help in this study.

We'll let you know how the results turned out and if we find what we're looking for.

We really appreciate the core pushing this through for us.

Our sincere thanks.

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