There are totally three machines including Ion Chef, Ion S5 XL and Server. Ion Chef is for template preparation(notice: before template preparation, we need to finish library preparation), amplification and positive clone isolation (everything is done on the Chip); Ion S5 XL is used to sequencing (Take the Chip out from Ion Chef, and put it in Ion S5 XL for sequencing); Server is utilized to analyze NGS data (can use both website and terminal, account & password are ionadmin);

The Ion workflow:

DNA -----> Compatible Library Prep ------> Template Prep ----->Sequencing ------>Compatible data

The machine in our lab is ion S5 XL, Chip used in the training is Ion 540 Chip.

Ion S5XL machine needs 2.5 hr to finish 200 bp sequencing.

Ion 520 Chip can product 1-2 Gb data, 3-5 million reads, the read length is up to 400 bp, need 1hr to analyze this data(generating bam file);

Ion 530 Chip products 3-5 Gb data, 15-20 million reads, the read length is up to 400 bp, need 2.5 hr to finish analyzation(generating bam file);

Ion 540 Chip yield 10-15 Gb data, 60-80 million reads, the read length is up to 200 bp, need 5 hr to analyze(generating bam file);

Ion Chef machine is used for template preparation, clonal amplification & positive clone isolation. Take out Chip, and put it in sequencer for sequencing.

Data analysis flow:

Single flow data (DAT) --🡪 many flows data (DAT) --🡪raw signals per flow (WELLS) ---🡪base calling & get unmapped BAM file --🡪 BAM file ---🡪 VCF file

Library preparation (#Notice: which is different from template preparation):

1. Fragment library;

b. Amplicon Library ; (standard PCR amplicons; Ion AmpliSeq DNA technology Exome, Custom; Fusion primers; Ion AmpliSeq RNA)

c. RNA Library; (Ion AmpliSeq RNA; Samll RNA library; Transcriptome library)

1. Library Prep: Fragment workflow for gDNA

50-100 ng or 1ug DNA –(shear DNA)-->Fragmented DNA –(Blunt End Repair which is for mechanical shearing only)-->End Polished Fragments---(adapter ligation, nick translation & size selection)--->adapter ligated library –(PCR amplify, if needed)--->amplified, adapter ligated library ---->template preparation (using Ion Chef)

Enzymatic and Ultrasonic, two methods are for fragmentation; Should consider read length;

1. Template Preparation:

Prepare Library---> Clonal Amplification (use Ion Chef)---->Isolate Positive Ion Sphere particles(use Ion Chef)---->load Chip and sequence---->Data analysis

Template preparation usually need 10.5 hr to finish. We can make it overnight. It’s automated template Prep, enrichment and loading.

In this step, firstly turn on the Ion Chef machine, and put reagents in the machine according to the instruments, totally there are eight parts needed to be putted reagents, the detailed information can accorded to ppt named 04a\_Ion U\_OT2\_Instrument Workflow.

After finishing this step, take out everything, just preserve the Chip, take it out, and put it in the Ion S5 XL.

Each time, the Chip should be scanned by scanner which can input the Chip information into server, and we can find these information in PC.

1. Sequencing

Firstly turn on the Ion S5 XL machine, and do initialization, after finishing, check reagents according to the instructions, and put the Chef in. Click run.

Notice: how to take the Chef, don’t touch the central region of the Chef.

1. Data analyzing

After finishing sequencing, we can use server to analyze these data.

Account : ionadmin

Password: ionadmin

IP address: 132.18.70.50

Some concepts

1. Flow: each time, only one nucleotide goes through the Chip, it means one flow;
2. Cycle: each cycle have same flow order, for example, 32 flows contain a cycle, these 32 flows are same in every cycle.
3. Key seq: has four nucleotides which is utilized to distinguish experiment sample and control sample?

Steps in practice:

1. Dilute the library to correct concentration(there two tubes for sample, just add this sample in 1# tube), the final volume is 25 ul.
2. Input IP address: 132.183.70.50 (torrent server) to browser and make some settings

For example: use 540# Chip, use scanner to scan barcode on the tube.

1. Turn on Chef: “setup run”-->”step by step”---->”template”--->”next”----->”next”

Putting all necessary materials into Chef. :this sign means open the door.

1. Turn on Ion S5 XL (sequencer):

Initialization (totally four steps): make sure reagents are full in each bottles.

Tips: how to turn off sequencer? Home menu : “setting”--->”system tools”---->”shutdown”

1. Chef in finished (2 steps)

Sample tube is used for QC, take the chip out & remove all trashes, clean Chef (step 2)

1. Before running, “setting”--->”system tools”--->”reagent checking
2. Go back to home --->follow up the instrument --->load chip (step1)--->choose a run plan ---> sequencing

How to log on the Torrent Server?

Input the IP address: 132.18.70.50

And input the user name: ionuser

And input the password: ionuser