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© Workflow for bulk RNAseq of human fallopian tube and uterine endomyometrium

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Protocol status: Working

We use this protocol and it's working

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Abstract

Described here is the workflow used by the Female Reproductive Tissue Mapping Center at UCSD to generate bulk RNAseq data from human fallopian tube and uterine endomyometrium.

Troubleshooting



Tissue preparation

As soon as possible after sterilization (salpingectomy or tubal ligation), prepare fallopian tube tissue according to the following protocol:

<u>Human Pregnant Fallopian Tube Tissue Collection and Preservation Methods - UCSD</u> Female Reproductive TMC

At the time of C-section, prepare uterine endomyometrium tissue according to the following protocol:

<u>Human Pregnant Uterine Myometrium Tissue Collection and Preservation Methods - UCSD Female Reproductive TMC</u>

For this protocol, use tissue that has been collected in RNAlater.

Total RNA isolation

Isolate total RNA using a bead beater to disrupt the tissue, followed by organic extraction and ethanol precipitation. Use the following protocol, which was originally written for placenta:

Total RNA extraction from frozen placenta tissue

After passing quality control, proceed to library construction.

Note

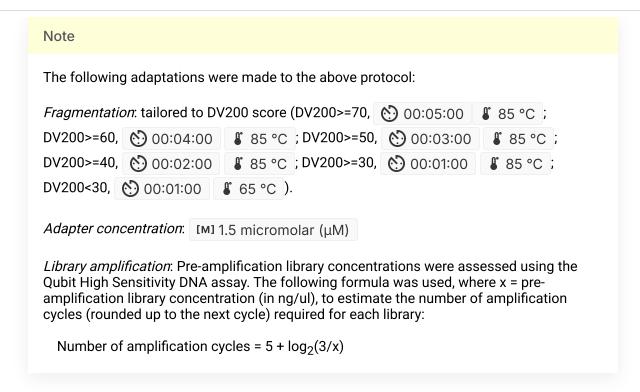
In our hands, RIN scores for fallopian tube and uterine endomyometrium were quite low. Bioanalyzer DV200 scores (the percentage of fragments >200 nucleotides) were instead used to assess RNA quality, and libraries were constructed according to a ribodepletion method which is more appropriate for low-quality RNA.

Library construction

3 Construct libraries using the KAPA RNA HyperPrep Kit with RiboErase (HMR), according to the following protocol, which was originally written for placenta:

Library construction for human placenta bulk RNAseq





After passing quality control, proceed to sequencing.

Sequencing

For HuBMAP bulk RNAseg samples, the multiplexed pool was sequenced on a NovaSeg 6000 S4 lane using a 100bp paired-end run configuration. Reads were aligned using STAR, and transcript abundances were quantified using RSEM.