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Workflow for human fallopian tube and uterine endomyometrium bulk ATACseq

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

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See accompanying protocols.

Tissue preparation

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

Human Pregnant Fallopian Tube Tissue Collection and Preservation Methods - UCSD Female

Reproductive TMC

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

Human Pregnant Uterine Myometrium Tissue Collection and Preservation Methods - UCSD Female Reproductive TMC

For this protocol, use tissue that has been snap-frozen.

Nuclei isolation

2 Isolate nuclei from 4 samples at a time according to the following protocol:

Nuclei isolation from snap-frozen human placental tissue for bulk ATACseq

Proceed to tagmentation immediately.

Protocol above was written for human placental tissue but can be used for fallopian tube and uterine endomyometrium. Isolated nuclei are paler than those from placenta and should be counted manually with a hemocytometer instead of using an automated cell counter which can struggle to detect them. Nuclei also have a different morphology to placenta, and are much sparser in uterine endomyometrium.

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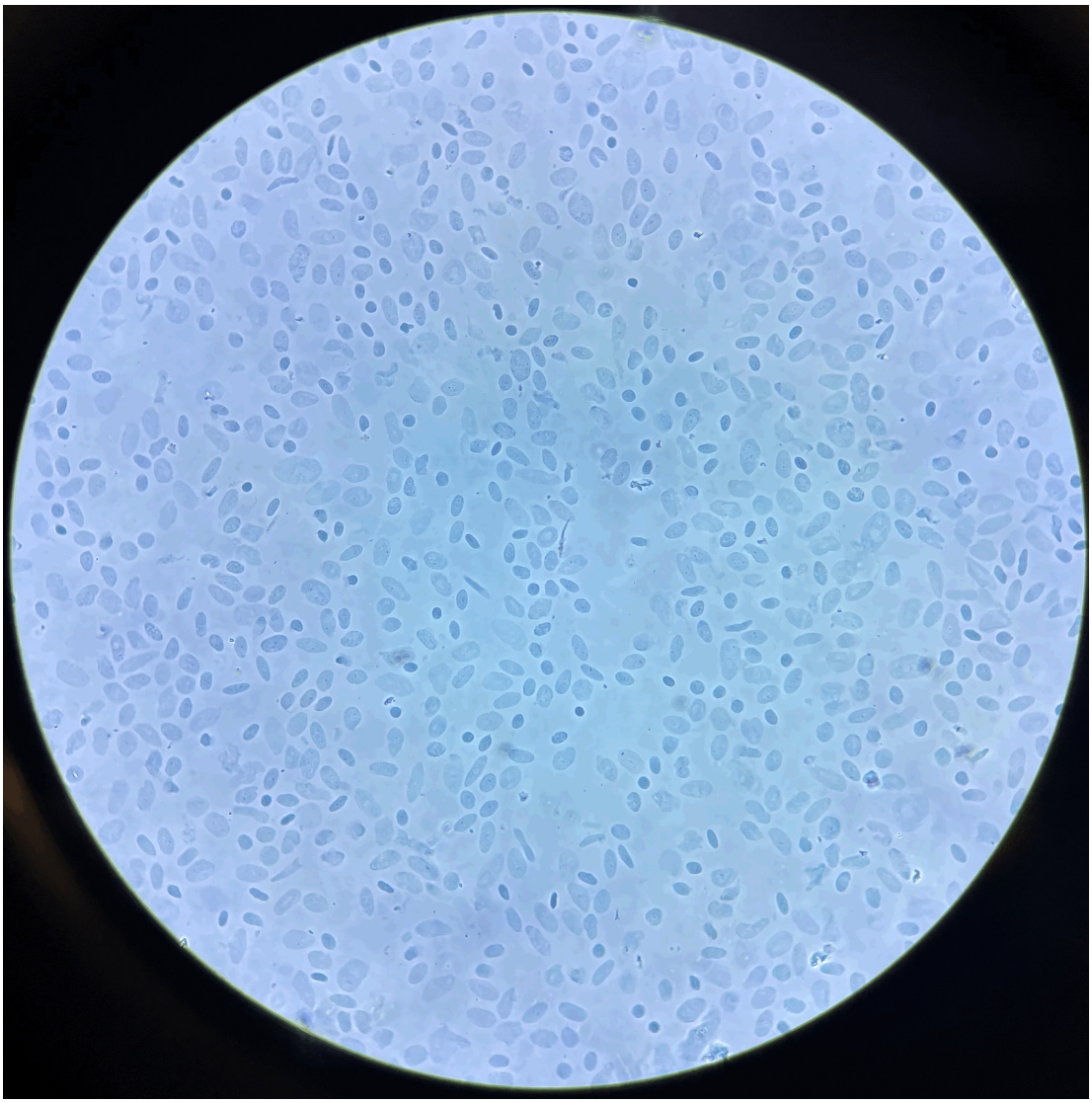

Representative image of nuclei isolated from fallopian tube.

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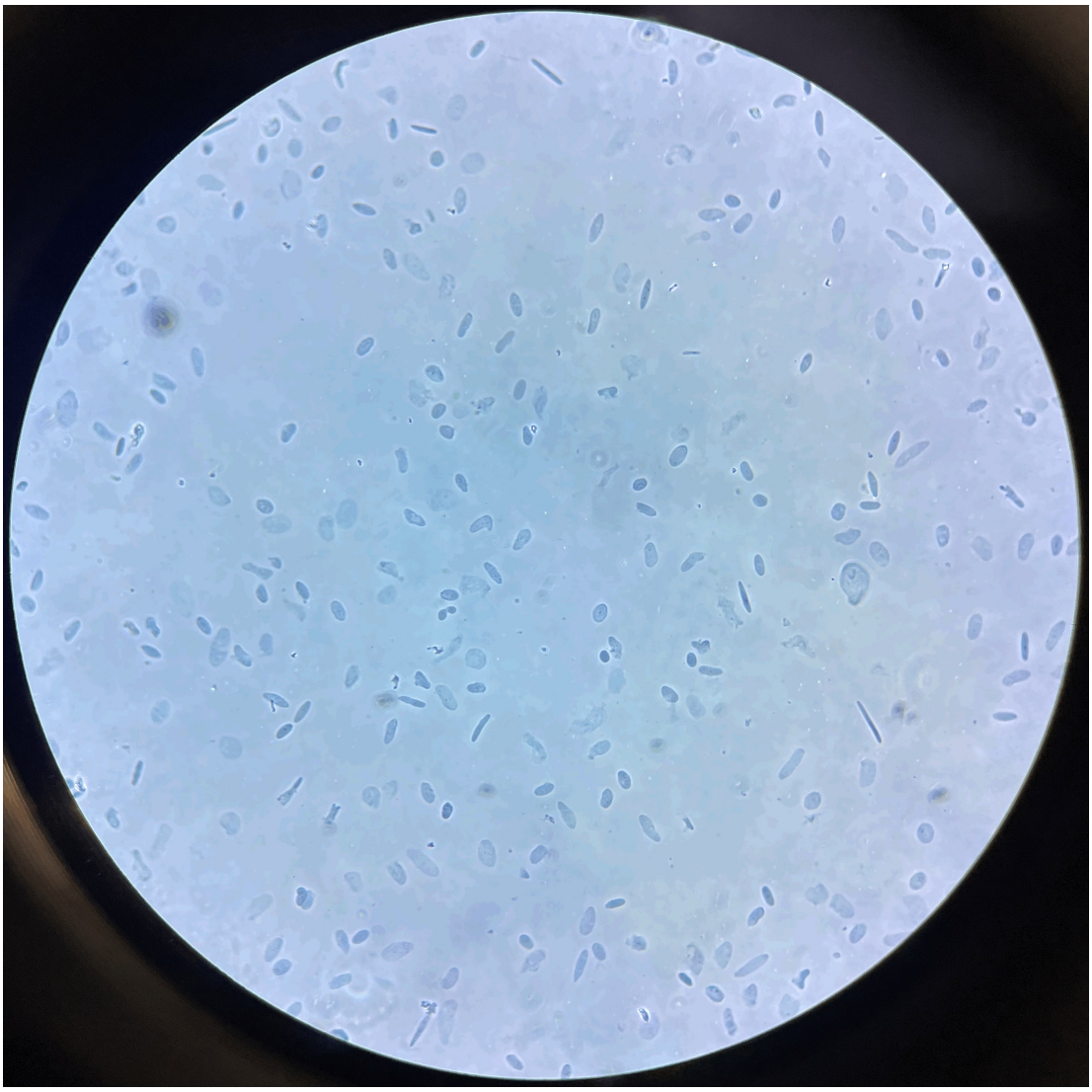

Representative image of nuclei isolated from uterine endomyometrium.

Tagmentation and library generation

3 Perform tagmentation and library generation according to the following protocol:

Tagmentation and library generation for human placental bulk ATACseq

After passing quality control, proceed to sequencing.

Protocol above was written for human placental tissue but can be used for fallopian tube and uterine endomyometrium. Bioanalyzer traces typically show a less obvious nucleosomal laddering pattern when compared to those from placenta.

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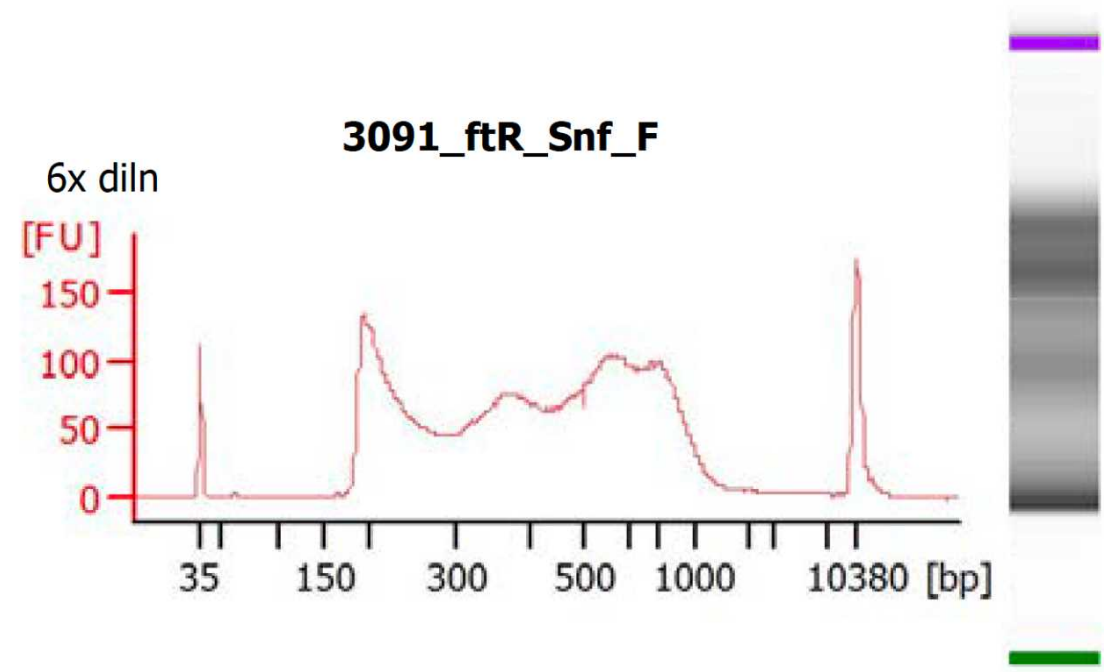
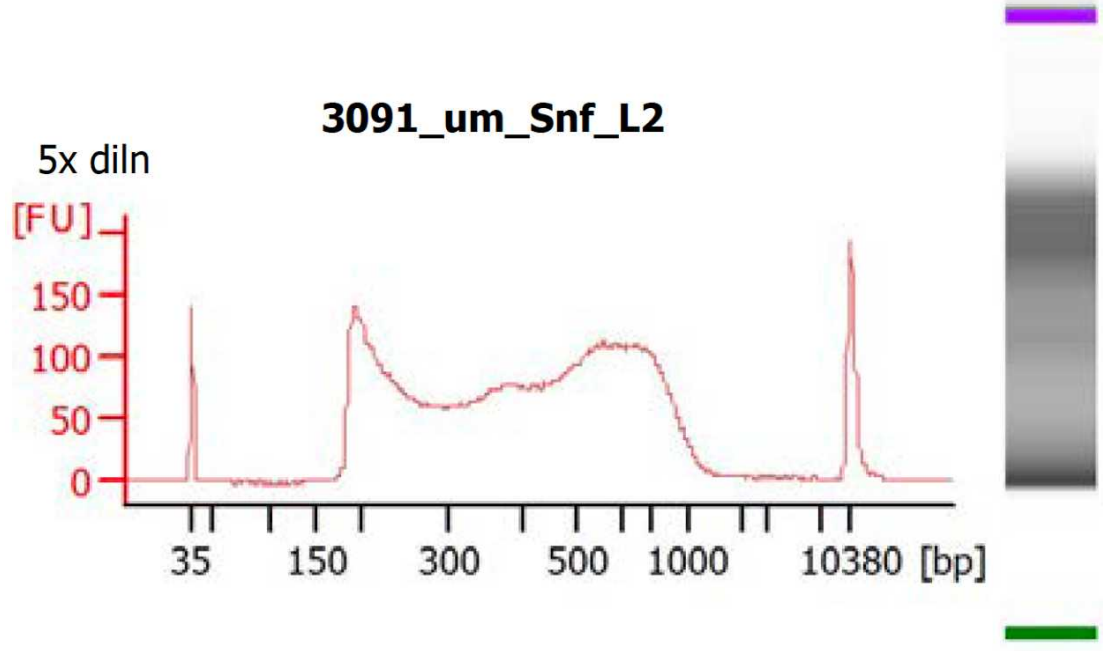


Typical Bioanalyzer trace for a fallopian tube bulk ATACseq library.



Typical Bioanalyzer trace for a uterine endomyometrium bulk ATACseq library.

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Sequencing

4 For HuBMAP bulk ATACseq samples, the multiplexed pool was sequenced on a NovaSeq 6000 S4 lane using a 100bp paired-end run configuration. Alignment and peak-calling were performed using the ATAC-seq pipeline within the bcbio Python package.

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