Collecting gut content (e.g. caecum) for microbiome sequencing

How to collect, preserve and ship animal gut content for 16S amplicon or shotgun metagenomics without contamination or DNA degradation.

1. Before you start

- **Ethics:** animal work must be approved by your institutional animal ethics committee, and euthanasia must use an approved method.
- **Sterile tools:** scissors, forceps, dissecting board, tips and tubes, cleaned with 70% ethanol or autoclaved. Dissect in a clean area or laminar-flow hood.
- **Tubes:** DNA-free 1.5 or 2 mL tubes, labelled with animal ID, date and sample type.

2. Dissection and collection

1. After euthanasia, place the animal on its back on a clean surface.
2. Make a midline incision from the lower abdomen to the ribcage and expose the gut without rupturing organs.
3. Find the caecum (the pouch where the small and large intestines meet) and remove it with sterile scissors.
4. Open it lengthwise and collect 100–300 mg of content with a sterile tip or spatula into the labelled tube.

3. Preservation

- DNA extracted within 2–4 hours: keep at 4 °C.
- Otherwise: freeze at −80 °C immediately.
- If a freezer is not available, use a preservative such as DNA/RNA Shield or RNAlater.

4. Avoiding common problems

Problem	Solution
Contamination from tools, gloves or surfaces	Separate or cleaned tools for each animal (bleach or ethanol); change gloves often
DNA degradation by gut enzymes	Work quickly and on ice; freeze at −80 °C as soon as possible
Low DNA yield	Use stool/gut extraction kits with bead-beating (for example Qiagen, Zymo)
Cross-contamination between samples	Single-use consumables; one animal at a time; wipe surfaces
PCR inhibitors (bile salts, polysaccharides)	Kits with inhibitor removal, or an extra clean-up

> Include controls: an extraction blank and, where possible, a sample from a reference animal or environment.

Questions before sending samples? WhatsApp / phone +880 1308-222824 · bdgenome@yahoo.com · bdgenomes.com/guides/gut-content/