Dear Participants,
As part of our upcoming practical module on Next-Generation Sequencing (NGS), it is essential to understand the fundamentals of **Target Enrichment and Primer Specificity**. Your task is to design a high-quality PCR primer set for a specific human gene. Attached to this email, you will find an **Activity Sheet** titled *"Primer Design for Human GAPDH"*. This sheet summarizes the step-by-step workflow using NCBI Gene and Primer-BLAST to ensure high specificity and minimal non-specific binding.
Your Assignment:

1. **Select a Gene:** You may choose any gene of interest or pick one from the list of 10 housekeeping/small genes provided below.
2. **Design Primers:** Follow the parameters outlined in the GAPDH activity sheet (target T_m ~60°C, GC% 40-60%, product size 80-200 bp).
3. **Specificity Check:** Use the Primer-BLAST tool against the **Human Genome (taxid:9606)** to ensure no significant off-target binding occurs.
4. **Submission:** Please upload them on GitHub and have your primer sequences, T_m, and product length ready for our next session on Monday.

Recommended Housekeeping & Small Gene Options:
If you do not have a specific gene in mind, we recommend selecting one of the following frequently used controls:

1. **ACTB** (Beta-actin) – Cytoskeletal structural gene.
2. **B2M** (Beta-2-microglobulin) – Component of MHC class I molecules.
3. **HPRT1** (Hypoxanthine phosphoribosyltransferase 1) – Purine salvage pathway.
4. **RPL13A** (Ribosomal protein L13a) – Protein synthesis component.
5. **TBP** (TATA-box binding protein) – General transcription factor.
6. **GUSB** (Beta-glucuronidase) – Lysosomal enzyme.
7. **PGK1** (Phosphoglycerate kinase 1) – Glycolytic enzyme.
8. **HMBS** (Hydroxymethylbilane synthase) – Heme synthesis pathway.
9. **PPIA** (Cyclophilin A) – Protein folding chaperone.
10. **SDHA** (Succinate dehydrogenase complex flavoprotein subunit A) – Citric acid cycle.

Designing primers for these genes will help you understand how to manage conserved sequences and avoid common pseudogene interference during NGS library prep.

We look forward to reviewing your designs!

Best regards,
EYM Life Sciences Team