

The diagram illustrates the experimental design for four groups: Tide Pool 1 (S1), Tide Pool 2 (S2), Nearshore (N), and Field Blank. Each group has three replicates. The process flow is as follows:

- Tide Pool 1 (S1):** Three replicates of samples are collected. Each replicate has three sub-replicates. The first sub-replicate is stored in 100% ethanol, and the other two are stored in -80°C freezer. All samples are then analyzed by DNA extraction, PCR, and sequencing.
- Tide Pool 2 (S2):** Three replicates of samples are collected. Each replicate has three sub-replicates. The first sub-replicate is stored in 100% ethanol, and the other two are stored in -80°C freezer. All samples are then analyzed by DNA extraction, PCR, and sequencing.
- Nearshore (N):** Three replicates of samples are collected. Each replicate has three sub-replicates. All sub-replicates are stored in -80°C freezer. All samples are then analyzed by DNA extraction, PCR, and sequencing.
- Field Blank:** Three replicates of samples are collected. Each replicate has three sub-replicates. All sub-replicates are stored in -80°C freezer. All samples are then analyzed by DNA extraction, PCR, and sequencing.