Turtle gonad culture method

Perform the dissections in dissecting media (Leibovit’s L-15 + 25 μg/ml ampicillin) when possible, or transfer tissue from PBS to this media as soon as possible. Gonads are separated from the adjacent mesonephros using a sharp needle prior to culture. Isolated gonads are then carefully laid in thin wells shaped in strips of agar gel (1.5% agar in Leibovitz’s L-15 medium (Gibco); approximately 0.3 ml in volume each) placed in 35-mm tissue culture dishes. The gels are made by pouring melted 1.5% gel on molds of raised ridges that conform to the approximate width of stage 17 turtle gonads. Our molds have three ridges each; after the gel hardens, the full cast is cut into three pieces. This allows us to increase our number of replicates and reduces the impact of random contamination events. Each strip is bathed in 0.3 ml of culture medium comprised of 10% charcoal-stripped FBS in Leibovitz with 50 μg/ml ampicillin and 1.25 μg/ml Fungizone (Gibco). Excess medium is removed from the wells so that the gonads remain fairly dry. The samples are then cultured at the desired incubation temperature in ambient CO2. Approximately 5 μl of medium is pipetted directly onto the gonads and incubated for ~5 min before removal on each day of the culture period. The culture medium is fully aspirated and replaced every other day. Wearing a surgical mask while changing the medium or dripping on the tissues also reduces the incidence of contamination.