Chick Floating Filter Culture

This is good for embryos of st. 2, and you can keep them going to about st. 8. For much earlier embryos, consider New culture. For later embryos, consider filter paper+vitelline membrane culture.

1) Make sure you have eggs which aren't too old. Incubate them to desired stage (fresh eggs are about 12-15 hours to get to early streak stages). Culturing before you see the beginnings of a streak does not seem to work. From st. 2^- or so, you can culture fine.
2) Crack the eggs into a tray full of 1X PBS. Cut out the embryo and transfer it into a dish of PBS. Do not worry about keeping the vitelline membrane whole (as you would for New culture). As you crack the eggs, save the thin albumin into a 50 ml Falcon tube – you might need it for culture medium.
3) Under the dissecting scope, carefully peel the embryo off the vitelline membrane.
4) Cut around the embryo with iridectomy scissors so that only a small border of the area opaca exists around the area pellucida (if there is an area opaca at all, that is). Put the embryo in a dish of 40% thin albumin / 60% PBS. It might float to the top, so gently pull it down again. This helps survival – I think the albumin wash dries off some of the PBS which isn't good to have on the embryo once it's on the filter.
5) Then, take your finger (in a glove), touch it into a dish of PBS, and wet the top surface of a petri dish top just a little. Place a filter disk (shiny side upwards) onto the wet surface of the dish lid - this will cause it to stick down onto the petri dish top. You can do this for a few filters at once, on a small petri dish top. Note: you will have to get a brand new dish top every few discs – it begins to attract water off the filter (a change in the electrostatic properties as you use it?).
6) Move the dish top under a dissecting scope. Use a P-1000 with a blue tip (with the tip cut off with a razor blade to make the opening of the appropriate size for your embryos) to transfer a trimmed embryo with a drop of albumin/PBS onto the middle of the filter disk.
7) Under the scope, use forceps to make sure it is yolk-side upwards (ectoderm onto the filter). Use a P-200 to suck off most of the liquid from the embryo; be careful - too strong, and you may suck the embryo into the tip, killing it. When it is fairly dry, it will stick onto the filter.
8) Use forceps to pick up the filter by the edge; if the embryo is attached well and won't fall off, touch its bottom edge onto a paper towel (the remaining liquid will disappear), and then float it gently onto a well of a 24-well plate (by holding the filter at one end with forceps, resting the opposite end on the edge of the well, and then lowering the filter so that it goes onto the surface of the medium flat and does not dip under the liquid). Each well of the dish should have 2-3 ml of medium in it.
9) Keep the plate at 37 °C with 5% CO_2 - the medium will turn dark red if they're out too long (this only happens if the medium is Alpha MEM).
10) Culture until needed – you can take them out from time to time to check the staging under the scope, and then put them back. If you do this, you can take the opportunity to let excess water drain off of the edge of the filter (touch it to a paper towel) – this will help survival.

Materials

The filters are bought from Costar/Corning, and they are PC nucleopore track-etch membrane 13mm 1.0 μm, item #110410. The medium in the well is just Alpha MEM (Gibco #1251056). For later stages, you could make it more complex: 83 ml Alpha MEM, 10 ml Fetal Calf Serum, 2 ml chick extract (= 1 bottle of Gibco #15115-017 powder + 10 ml water, with the crap spun out of it), 1 ml pen/strep, 1 ml L-glutamine. The embryos grow better with that extra stuff in it, but it sometimes randomizes LR, so I leave it out (it was batch-dependent, probably the FCS has factors in it).

Also, you can solve the problem of pH changes due to being out of the CO_2 incubator by (according to Mark Mercola) putting a bit of HEPES in there to keep the pH constant: make it 1M, pH to desired value (Takashi Mikawa says it should be 7-7.4), and make it 10 mM in the final medium. Claudio Stern says you should use albumin instead of Alpha MEM and then you don't need HEPES (or CO_2 for that matter!). I am now routinely using thin albumin with a bit of Alpha MEM (in which drugs, etc.) are mixed. Oligos are precipitated by albumin, so you'd have to use straight Alpha MEM if you want to culture in the presence of oligos etc.