**Casamino Acids Media (Hv-Ca)**  
5 or 10 bottles of 333 mlHv-Ca Agar

### 1. Make up 30% SW For details see page 1.

### 2. Make up 10X Ca

Distilled H2O ~130 ml  
**C**asamino Acids 8.5 g

Slowly add 4 ml 1 M KOH while stirring.  
Add distilled H2O to final volume of 170 ml.

Distilled H2O ~250 ml  
**C**asamino Acids 17 g

Slowly add 8 ml 1 M KOH while stirring.  
Add distilled H2O to final volume of 340 ml.

Do not autoclave, but use the same day.

### 3. Hv-Ca Agar

Into 500 ml Duran bottles measure:

Agar 5 g  
Distilled H2O 100 ml  
30% SW 200 ml

Microwave until agar has dissolved  
(20 minutes on 100%, 20 minutes on 70% and 2x 10 minutes on 50% – see page 2).

Then add to each 500 ml bottle:

10X Ca 33 ml

Add a large stir bar to each 500 ml bottle.  
Autoclave as soon as possible in benchtop autoclave.

After cooling to ~57°C, add slowly to each 500 ml bottle:

0.5 M CaCl2 2 ml  
Thiamine & Biotin 300 µl

To avoid bubbles in agar, use a magnetic stirrer and let liquid run down side of bottle.

If additives are required, add at this point as well.  
e.g. per 500 ml bottle (333 ml agar) add:

Thymidine & Hypoxanthine (4 mg/ml each in 50 mM NaOH) 3.4 ml (Red) (40 µg/ml)  
Tryptophan (10 mg/ml in dH2O) 1.7 ml (Brown) (50 µg/ml  
Uracil (50 mg/ml in DMSO) 340 µl (Pink) (50 µg/ml)  
5-FOA\* (50 mg/ml in DMSO) 340 µl (2x Pink) (50 µg/ml)  
 \*Note: Hv-Ca + 5-FOA agar normally requires addition of 67 µl uracil (50 mg/ml) to 10 µg/ml

Pour thick plates (i.e. ~9 plates per 333 ml) as soon as possible.

Mark the plates with a **red** stripe using the thick marker pen.  
If additives are included, indicate on the plate and add a thin stripe according to the key (above).

Once the plates have set, seal in plastic bags and store in the cold room in a cardboard box.