doi: 10.1387/ijdb.120098ra



### **SUPPLEMENTARY MATERIAL**

### corresponding to:

# Successful whole embryo culture with commercially available reagents

HANNAH C. GLANVILLE-JONES, NGAI WOO and RUTH M. ARKELL\*

#### TABLE S1

### DEVELOPMENTAL SCORING SCHEME FOR EMBRYOS CULTURED FROM 7.5DPC

Feature	Scoring system
Yolk-sac circulation	0.00 = no yolk-sac circulation 1.00 = yolk-sac circulation established
Somites	0.00 = no somites grown 1.00 = the expected number of somites grown, (embryos entering culture at mid- to late-streak stage are expected to develop 2 - 4 somites, and embryos entering culture at zero- to early-bud stage are expected to develop 4 - 8 somites in 24 hours (Downs and Davies 1993; Kaufman 2002))
Heart	0.00 = not present 1.00 = present
Yolk sac	0.00 = abnormal, (ruffled, granular) 1.00 = normal, (smooth)
Allantois	0.00 = not present 0.50 = present, abnormal 1.00 = present, normal
Forebrain: forebrain/midbrain junction or neural folds visible	0.00 = not present 0.50 = present, neural folds 1.00 = present, complete
Midbrain: pre-otic sulcus visible	0.00 = not present 0.50 = present, truncated 1.00 = present, complete
Hindbrain: otic sulcus visible	0.00 = not present 0.50 = present, truncated 1.00 = present, complete

#### TABLE S2

## DEFORMITY SCORING SCHEME FOR EMBRYOS CULTURED FROM 7.5 DPC

Feature	Scoring system
Major Deformities	
Pyknosis	0.00 = no pyknosis 1.00 = pyknosis present
Allantois protruding from yolk sac	0.00 = no protrusion 1.00 = protrusion
Head/tail fusion	0.00 = no fusion 1.00 = fusion

#### TABLE S3

## DEVELOPMENTAL SCORING SCHEME FOR EMBRYOS CULTURED FROM 8.0 OR 8.5 DPC

Feature	Scoring system
Somites	0.00 = no somites grown 1.00 = the expected number of somites grown, (embryos entering culture at 2-somite stage onwards are expected to develop 1 somite pair per 2 hours of WEC (Takahashi and Osumi 2010))
Heart	0.00 = not beating 1.00 = beating
Eyes	0.00 = both absent 0.50 = 1 eye present 1.00 = 2 eyes present
Branchial arches	0.00 = 0 arches present 1.00 = 1 arch present in 8.0 dpc embryos, 2 arches present in 8.5dpc embryos, or 3 arches in 9.0/9.5 dpc embryos
Anterior neural tub closure	be 0.00 = no fusion of anterior neural tube 1.00 = closure initiated for 8.0 dpc (4 – 5 somite-stage) embryos, partially completed for 8.5 dpc embryos, or complete for 9.0 dpc (15 – 18 somite-stage) embryos onward
Embryonic turning	$0.00 = turning \ not initiated$ $1.00 = turning \ initiated for 8.0 dpc (6 somite-stage) embryos, partially completed for 8.5 dpc embryos, or complete for 9.0 dpc (14 - 16 somite-stage) embryos onward$
Forelimb buds	0.00 = no buds present by 9.5dpc 1.00 = no forelimb buds present in 9.0dpc or younger embryos, or forelimb buds present in 9.5dpc or older embryos

### TABLE S4

### DEFORMITY SCORING SCHEME FOR EMBRYOS CULTURED FROM 8.0 OR 8.5 DPC

Feature	Scoring system
Major Deformities	
Head truncation	0.00 = no truncation 1.00 = truncated
Wrinkled neural folds	0.00 = no wrinkling 1.00 = wrinkling
Haemorrhage	0.00 = no haemorrhage 1.00 = haemorrhage
Pyknosis	0.00 = no pyknosis 1.00 = pyknosis
Minor Deformities	
Blood spotting	0.00 = no blood spotting 0.05 = minor blood spotting 0.10 = significant blood spotting
Ectodermal blebbing	0.00 = no blebbing 0.05 = minor blebbing 0.10 = significant blebbing