All gBlock data

2021-06-08

Contents

Instructions

This file recreates the analyses in the publication, 'A Statistical Model for Calibration and Computation of Detection and Quantification Limits for Low Copy Number Environmental DNA samples' by Lesperance, Allison, Bergman, Hocking, Helbing, *eDNA*, 2021.

- Create a folder call Outputs in your working directory.
- Put files in working directory: PoissonCalib-Functions-7April2021.R, GEDWG_LOD_DATA3.csv
- Data set csv file requirements. Columns: Target, Lab, Cq, Sq
- DO NOT DUPLICATE Target names over different Labs!!
- Uses observations with nonempty data and where phat <1 (num detect=num technical replicates)
- Only the SQ's up to the first one with phat==1 are used.
- Allows for variable numbers of SQ levels per Target.
- Assumes SQs == NA are zero, i.e. are negative controls.

- Adds in Negative Control (ntc) zeroes (24/(48 for Monroe) technical replicates).
- The code uses the R function optim. A convergence code 0 indicates successful completion.

- Ignore warnings if results are sensible.

Process/Summarize samples by Lab; Compute the Poisson estimates of SQ

Hindson et al "High-Throughput Droplet Digital PCR System for Absolute Quantitation of DNA Copy Number", Anal. Chem., 2011, 83 (22), pp 8604–8610 use a Poisson approximation for quantitation. Before that, Dube et al. 2008, "Mathematical analysis of copy number variation in a DNA sample using digital PCR on a nanofluidic device", POlS One, Vol 3, Issue 8, e2876, model the number of molecules in each chamber as a Poisson process, giving the relationship between p and λ.

```
## [1] 300   6
```

```
## dim, before negative controls added
```

```
##      Target          SQ              detect            n         
##  Hno    : 14   Min.   :      0   Min.   : 0.00   Min.   : 1.00  
##  MYPI-6 : 12   1st Qu.:      2   1st Qu.: 8.00   1st Qu.:24.00  
##  MYPI-6t: 12   Median :     50   Median :24.00   Median :24.00  
##  eASMO9 : 10   Mean   :   8414   Mean   :27.73   Mean   :36.58  
##  eASTR4 : 10   3rd Qu.:   1250   3rd Qu.:48.00   3rd Qu.:48.00  
##  eFISH1 : 10   Max.   :1000000   Max.   :96.00   Max.   :96.00  
##  (Other):232                                                    
##      Cqmean           Lab       
##  Min.   :16.32   UVIC   :110  
##  1st Qu.:29.06   Monroe : 79  
##  Median :33.85   CERC   : 36  
##  Mean   :33.02   UMESC  : 31  
##  3rd Qu.:37.70   Lance  : 14  
##  Max.   :47.20   Wilson : 14  
##  NA's   :24      (Other): 16  
```

```
## [1] 325  13
```

```
## dim, Added 24/48 negative controls for Targets with zero ntc
```

```
##
##
## Table: APHIS
##
## Target       SQ   detect    n    phat
## -------  ------  -------  ---  ------
## D-loop      0.0        0   24   0.000
## D-loop      0.1        7   48   0.146
## D-loop      0.5       18   47   0.383
## D-loop      1.0       33   48   0.688
## D-loop     10.0       48   48   1.000
## D-loop    100.0       48   48   1.000
##
##
## Table: CERC
##
##      Target              SQ   detect    n    phat
```

```
##      ----------  ----------  -------  ---  ------
## 7    AC1_CID_1        0.000        0   24   0.000
## 8    AC1_CID_1        4.000        3   22   0.136
## 9    AC1_CID_1        8.000       12   23   0.522
## 10   AC1_CID_1       19.200       19   24   0.792
## 11   AC1_CID_1      192.000       24   24   1.000
## 12   AC1_CID_1     1920.000       24   24   1.000
## 13   AC1_CID_1    19200.000       24   24   1.000
## 14   CID              0.000        0   24   0.000
## 15   CID             15.625       22   22   1.000
## 16   CID             31.250       24   24   1.000
## 17   CID             62.500       24   24   1.000
## 18   CID            125.000       24   24   1.000
## 19   CID            250.000       24   24   1.000
## 20   CID            500.000       24   24   1.000
## 21   MYPI-6           0.000        0   24   0.000
## 22   MYPI-6           0.244        0   24   0.000
## 23   MYPI-6           0.489        0   24   0.000
## 24   MYPI-6           0.978        0   24   0.000
## 25   MYPI-6           1.955        0   24   0.000
## 26   MYPI-6           3.906        0   24   0.000
## 27   MYPI-6           7.812        0   24   0.000
## 28   MYPI-6          15.625        3   24   0.125
## 29   MYPI-6          31.250        2   24   0.083
## 30   MYPI-6          62.500       10   24   0.417
## 31   MYPI-6         125.000        4   24   0.167
## 32   MYPI-6         250.000       23   24   0.958
## 33   MYPI-6         500.000       24   24   1.000
## 34   MYPI-6t          0.000        0   24   0.000
## 35   MYPI-6t          0.244        3   24   0.125
## 36   MYPI-6t          0.488        4   24   0.167
## 37   MYPI-6t          0.975        9   24   0.375
## 38   MYPI-6t          1.950        9   24   0.375
## 39   MYPI-6t          3.906       22   24   0.917
## 40   MYPI-6t          7.812       22   24   0.917
## 41   MYPI-6t         15.625       24   24   1.000
## 42   MYPI-6t         31.250       24   24   1.000
## 43   MYPI-6t         62.500       24   24   1.000
## 44   MYPI-6t        125.000       24   24   1.000
## 45   MYPI-6t        250.000       24   24   1.000
## 46   MYPI-6t        500.000       23   24   0.958
##
##
## Table: Goldberg
##
##      Target       SQ   detect    n    phat
## ---  -------  ------  -------  ---  ------
## 47   NZMS          0        0    1   0.000
## 48   NZMS          5       13   20   0.650
## 49   NZMS         10       22   22   1.000
## 50   NZMS         50       20   20   1.000
## 51   NZMS        100       22   22   1.000
## 52   NZMS       1000        2    2   1.000
```

```
## 53   NZMS      10000        2    2   1.000
## 54   SAFO          0        0   24   0.000
## 55   SAFO          5       14   19   0.737
## 56   SAFO         10       20   20   1.000
## 57   SAFO         50       20   20   1.000
##
##
## Table: Lance
##
##      Target         SQ   detect    n    phat
## ---  ----------  -----  -------  ---  ------
## 58   Elod-2-NU       0        0   48   0.000
## 59   Elod-2-NU       2       22   48   0.458
## 60   Elod-2-NU      10       47   47   1.000
## 61   Elod-2-NU      50       48   48   1.000
## 62   Elod-2-NU     250       48   48   1.000
## 63   Elod-2-NU    1250       48   48   1.000
## 64   Elod-2-NU    6250       48   48   1.000
## 65   MYPI            0        0   48   0.000
## 66   MYPI            2       31   48   0.646
## 67   MYPI           10       48   48   1.000
## 68   MYPI           50       48   48   1.000
## 69   MYPI          250       48   48   1.000
## 70   MYPI         1250       48   48   1.000
## 71   MYPI         6250       48   48   1.000
##
##
## Table: Monroe
##
##       Target          SQ   detect    n    phat
## ----  -------  ---------  -------  ---  ------
## 72    AC1            0.0        0   48   0.000
## 73    AC1            0.4       26   48   0.542
## 74    AC1            2.0       45   48   0.938
## 75    AC1           10.0       48   48   1.000
## 76    AC1           50.0       48   48   1.000
## 77    AC1          250.0       48   48   1.000
## 78    AC1         1250.0       48   48   1.000
## 79    AC1         6250.0       48   48   1.000
## 80    AC1        31250.0       48   48   1.000
## 81    AC3            0.0        0   48   0.000
## 82    AC3            0.4       25   48   0.521
## 83    AC3            2.0       45   48   0.938
## 84    AC3           10.0       48   48   1.000
## 85    AC3           50.0       48   48   1.000
## 86    AC3          250.0       48   48   1.000
## 87    AC3         1250.0       48   48   1.000
## 88    AC3         6250.0       48   48   1.000
## 89    AC3        31250.0       48   48   1.000
## 90    BH1            0.0        0   48   0.000
## 91    BH1            0.4       19   48   0.396
## 92    BH1            2.0       38   48   0.792
## 93    BH1           10.0       47   48   0.979
```

```
## 94    BH1           50.0       48   48   1.000
## 95    BH1          250.0       48   48   1.000
## 96    BH1         1250.0       48   48   1.000
## 97    BH1         6250.0       48   48   1.000
## 98    BH1        31250.0       48   48   1.000
## 99    BH2            0.0        0   48   0.000
## 100   BH2            0.4       20   48   0.417
## 101   BH2            2.0       34   48   0.708
## 102   BH2           10.0       47   48   0.979
## 103   BH2           50.0       48   48   1.000
## 104   BH2          250.0       48   48   1.000
## 105   BH2         1250.0       48   48   1.000
## 106   BH2         6250.0       48   48   1.000
## 107   BH2        31250.0       48   48   1.000
## 108   GCTM10          0.0        0   48   0.000
## 109   GCTM10          0.4       24   48   0.500
## 110   GCTM10          2.0       43   48   0.896
## 111   GCTM10         10.0       48   48   1.000
## 112   GCTM10         50.0       48   48   1.000
## 113   GCTM10        250.0       48   48   1.000
## 114   GCTM10       1250.0       48   48   1.000
## 115   GCTM10       6250.0       48   48   1.000
## 116   GCTM10      31300.0       48   48   1.000
## 117   GCTM22          0.0        0   48   0.000
## 118   GCTM22          0.4       22   48   0.458
## 119   GCTM22          2.0       44   48   0.917
## 120   GCTM22         10.0       48   48   1.000
## 121   GCTM22         50.0       48   48   1.000
## 122   GCTM22        250.0       48   48   1.000
## 123   GCTM22       1250.0       48   48   1.000
## 124   GCTM22       6250.0       48   48   1.000
## 125   GCTM22      31300.0       48   48   1.000
## 126   GCTM32          0.0        0   48   0.000
## 127   GCTM32          0.4       20   48   0.417
## 128   GCTM32          2.0       44   48   0.917
## 129   GCTM32         10.0       48   48   1.000
## 130   GCTM32         50.0       48   48   1.000
## 131   GCTM32        250.0       48   48   1.000
## 132   GCTM32       1250.0       48   48   1.000
## 133   GCTM32       6250.0       48   48   1.000
## 134   GCTM32      31300.0       48   48   1.000
## 135   Goby            0.0        0    6   0.000
## 136   Goby            1.0       26   46   0.565
## 137   Goby           10.0       46   46   1.000
## 138   Goby          100.0       46   46   1.000
## 139   Goby         1000.0       46   46   1.000
## 140   Goby        10000.0       46   46   1.000
## 141   Goby       100000.0       46   46   1.000
## 142   SC4            0.0        0   48   0.000
## 143   SC4            0.4       13   48   0.271
## 144   SC4            2.0       41   48   0.854
## 145   SC4           10.0       48   48   1.000
## 146   SC4           50.0       48   48   1.000
```

```
## 147   SC4          250.0       48   48   1.000
## 148   SC4         1250.0       48   48   1.000
## 149   SC4         6250.0       48   48   1.000
## 150   SC4        31250.0       48   48   1.000
## 151   SC5            0.0        0   48   0.000
## 152   SC5            0.4       14   48   0.292
## 153   SC5            2.0       42   48   0.875
## 154   SC5           10.0       48   48   1.000
## 155   SC5           50.0       48   48   1.000
## 156   SC5          250.0       48   48   1.000
## 157   SC5         1250.0       48   48   1.000
## 158   SC5         6250.0       48   48   1.000
## 159   SC5        31250.0       48   48   1.000
##
##
## Table: UMESC
##
##       Target       SQ   detect    n    phat
## ----  -------  ------  -------  ---  ------
## 160   BHC           0        0   96   0.000
## 161   BHC           1       25   96   0.260
## 162   BHC           5       59   96   0.615
## 163   BHC          10       96   96   1.000
## 164   BHC         100       96   96   1.000
## 165   BHC        1000       96   96   1.000
## 166   BHC       10000       96   96   1.000
## 167   Dre16s        0        1   96   0.010
## 168   Dre16s        1        9   96   0.094
## 169   Dre16s        5       42   96   0.438
## 170   Dre16s       10       68   96   0.708
## 171   Dre16s      100       96   96   1.000
## 172   Dre16s     1000       96   96   1.000
## 173   Dre16s    10000       96   96   1.000
## 174   SS            0        0   96   0.000
## 175   SS            1       44   96   0.458
## 176   SS           10       96   96   1.000
## 177   SS          100       96   96   1.000
## 178   SS         1000       96   96   1.000
## 179   SVC           0        0   96   0.000
## 180   SVC           1       25   96   0.260
## 181   SVC           5       59   96   0.615
## 182   SVC          10       96   96   1.000
## 183   SVC         100       96   96   1.000
## 184   SVC        1000       96   96   1.000
## 185   SVC       10000       96   96   1.000
## 186   YPC           0        0   96   0.000
## 187   YPC           1       31   96   0.323
## 188   YPC          10       94   96   0.979
## 189   YPC         100       96   96   1.000
## 190   YPC        1000       96   96   1.000
##
##
## Table: UVIC
```

```
##
##       Target          SQ   detect    n    phat
## ----  -------  ---------  -------  ---  ------
## 191   eASMO9    0.00e+00        0   24   0.000
## 192   eASMO9    3.20e-02        0   24   0.000
## 193   eASMO9    1.60e-01        6   24   0.250
## 194   eASMO9    8.00e-01       11   24   0.458
## 195   eASMO9    4.00e+00       22   24   0.917
## 196   eASMO9    2.00e+01       24   24   1.000
## 197   eASMO9    1.00e+02       24   24   1.000
## 198   eASMO9    5.00e+02        8    8   1.000
## 199   eASMO9    2.50e+03        8    8   1.000
## 200   eASMO9    1.25e+04        8    8   1.000
## 201   eASMO9    6.25e+04        8    8   1.000
## 202   eASTR4    0.00e+00        0   24   0.000
## 203   eASTR4    3.20e-02        0   24   0.000
## 204   eASTR4    1.60e-01        4   24   0.167
## 205   eASTR4    8.00e-01        9   24   0.375
## 206   eASTR4    4.00e+00       19   24   0.792
## 207   eASTR4    2.00e+01       24   24   1.000
## 208   eASTR4    1.00e+02       24   24   1.000
## 209   eASTR4    5.00e+02        8    8   1.000
## 210   eASTR4    2.50e+03        8    8   1.000
## 211   eASTR4    1.25e+04        8    8   1.000
## 212   eASTR4    6.25e+04        8    8   1.000
## 213   eFISH1    0.00e+00        0   24   0.000
## 214   eFISH1    3.20e-02        1   24   0.042
## 215   eFISH1    1.60e-01        0   24   0.000
## 216   eFISH1    8.00e-01       10   24   0.417
## 217   eFISH1    4.00e+00       18   24   0.750
## 218   eFISH1    2.00e+01       24   24   1.000
## 219   eFISH1    1.00e+02       24   24   1.000
## 220   eFISH1    5.00e+02        8    8   1.000
## 221   eFISH1    2.50e+03        8    8   1.000
## 222   eFISH1    1.25e+04        8    8   1.000
## 223   eFISH1    6.25e+04        8    8   1.000
## 224   eLIPI1    0.00e+00        0   24   0.000
## 225   eLIPI1    3.20e-02        0   24   0.000
## 226   eLIPI1    1.60e-01        5   24   0.208
## 227   eLIPI1    8.00e-01       11   24   0.458
## 228   eLIPI1    4.00e+00       23   24   0.958
## 229   eLIPI1    2.00e+01       24   24   1.000
## 230   eLIPI1    1.00e+02       24   24   1.000
## 231   eLIPI1    5.00e+02        8    8   1.000
## 232   eLIPI1    2.50e+03        8    8   1.000
## 233   eLIPI1    1.25e+04        8    8   1.000
## 234   eLIPI1    6.25e+04        8    8   1.000
## 235   eMIDO1    0.00e+00        0   24   0.000
## 236   eMIDO1    3.20e-02        0   24   0.000
## 237   eMIDO1    1.60e-01        2   24   0.083
## 238   eMIDO1    8.00e-01       16   24   0.667
## 239   eMIDO1    4.00e+00       23   24   0.958
## 240   eMIDO1    2.00e+01       24   24   1.000
```

```
## 241   eMIDO1    1.00e+02       24   24   1.000
## 242   eMIDO1    5.00e+02        8    8   1.000
## 243   eMIDO1    2.50e+03        8    8   1.000
## 244   eMIDO1    1.25e+04        8    8   1.000
## 245   eMIDO1    6.25e+04        8    8   1.000
## 246   eMISA2    0.00e+00        0   24   0.000
## 247   eMISA2    3.20e-02        0   24   0.000
## 248   eMISA2    1.60e-01        3   24   0.125
## 249   eMISA2    8.00e-01       10   24   0.417
## 250   eMISA2    4.00e+00       21   24   0.875
## 251   eMISA2    2.00e+01       24   24   1.000
## 252   eMISA2    1.00e+02       24   24   1.000
## 253   eMISA2    5.00e+02        8    8   1.000
## 254   eMISA2    2.50e+03        8    8   1.000
## 255   eMISA2    1.25e+04        8    8   1.000
## 256   eMISA2    6.25e+04        8    8   1.000
## 257   eONKI4    0.00e+00        0   24   0.000
## 258   eONKI4    3.20e-02        3   24   0.125
## 259   eONKI4    1.60e-01        6   24   0.250
## 260   eONKI4    8.00e-01       14   24   0.583
## 261   eONKI4    4.00e+00       21   24   0.875
## 262   eONKI4    2.00e+01       24   24   1.000
## 263   eONKI4    1.00e+02       24   24   1.000
## 264   eONKI4    5.00e+02        8    8   1.000
## 265   eONKI4    2.50e+03        8    8   1.000
## 266   eONKI4    1.25e+04        8    8   1.000
## 267   eONKI4    6.25e+04        8    8   1.000
## 268   eRAAU1    0.00e+00        0   24   0.000
## 269   eRAAU1    3.20e-02        1   24   0.042
## 270   eRAAU1    1.60e-01        2   24   0.083
## 271   eRAAU1    8.00e-01        7   24   0.292
## 272   eRAAU1    4.00e+00       20   24   0.833
## 273   eRAAU1    2.00e+01       24   24   1.000
## 274   eRAAU1    1.00e+02       24   24   1.000
## 275   eRAAU1    5.00e+02        8    8   1.000
## 276   eRAAU1    2.50e+03        8    8   1.000
## 277   eRAAU1    1.25e+04        8    8   1.000
## 278   eRAAU1    6.25e+04        8    8   1.000
## 279   eRACA2    0.00e+00        0   24   0.000
## 280   eRACA2    3.20e-02        0   24   0.000
## 281   eRACA2    1.60e-01        0   24   0.000
## 282   eRACA2    8.00e-01       11   24   0.458
## 283   eRACA2    4.00e+00       21   24   0.875
## 284   eRACA2    2.00e+01       24   24   1.000
## 285   eRACA2    1.00e+02       24   24   1.000
## 286   eRACA2    5.00e+02        8    8   1.000
## 287   eRACA2    2.50e+03        8    8   1.000
## 288   eRACA2    1.25e+04        8    8   1.000
## 289   eRACA2    6.25e+04        8    8   1.000
## 290   eRALU2    0.00e+00        0   24   0.000
## 291   eRALU2    3.20e-02        1   24   0.042
## 292   eRALU2    1.60e-01        2   24   0.083
## 293   eRALU2    8.00e-01        5   24   0.208
```

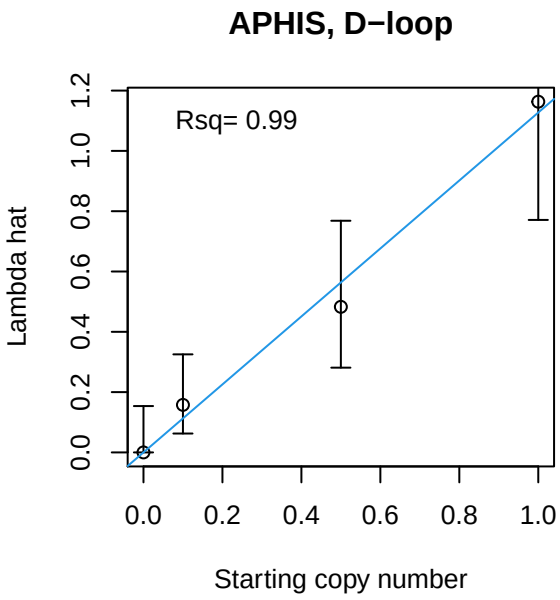
```
## 294   eRALU2    4.00e+00       20   24   0.833
## 295   eRALU2    2.00e+01       24   24   1.000
## 296   eRALU2    1.00e+02       24   24   1.000
## 297   eRALU2    5.00e+02        8    8   1.000
## 298   eRALU2    2.50e+03        8    8   1.000
## 299   eRALU2    1.25e+04        8    8   1.000
## 300   eRALU2    6.25e+04        8    8   1.000
## 301   eRAPR2    0.00e+00        0   24   0.000
## 302   eRAPR2    3.20e-02        1   24   0.042
## 303   eRAPR2    1.60e-01        2   24   0.083
## 304   eRAPR2    8.00e-01       12   24   0.500
## 305   eRAPR2    4.00e+00       22   24   0.917
## 306   eRAPR2    2.00e+01       24   24   1.000
## 307   eRAPR2    1.00e+02       24   24   1.000
## 308   eRAPR2    5.00e+02        8    8   1.000
## 309   eRAPR2    2.50e+03        8    8   1.000
## 310   eRAPR2    1.25e+04        8    8   1.000
## 311   eRAPR2    6.25e+04        8    8   1.000
##
##
## Table: Wilson
##
##       Target           SQ   detect    n    phat
## ----  -------  ----------  -------  ---  ------
## 312   Hno             0.0        0   48   0.000
## 313   Hno             0.2        7   24   0.292
## 314   Hno             1.0       37   48   0.771
## 315   Hno             5.0       24   24   1.000
## 316   Hno            10.0       24   24   1.000
## 317   Hno            25.0       24   24   1.000
## 318   Hno           100.0       24   24   1.000
## 319   Hno           125.0       24   24   1.000
## 320   Hno           625.0       24   24   1.000
## 321   Hno          1000.0       24   24   1.000
## 322   Hno          3125.0       24   24   1.000
## 323   Hno         10000.0       24   24   1.000
## 324   Hno        100000.0       24   24   1.000
## 325   Hno       1000000.0       24   24   1.000
```

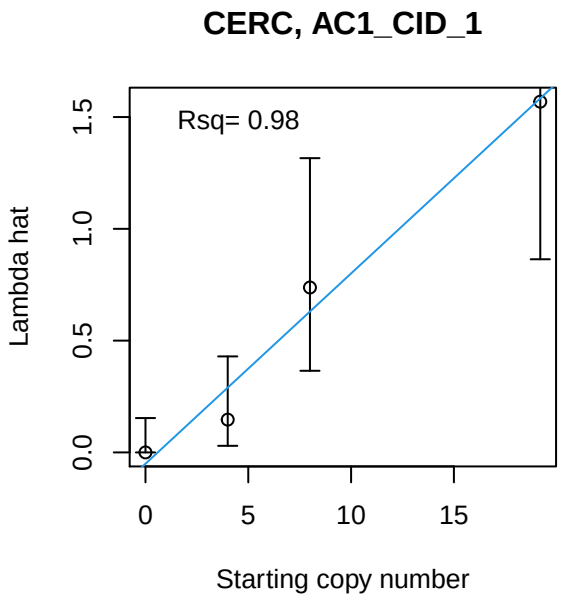
Plot the Poisson estimates (and CI) of SQ for levels that had non-detects

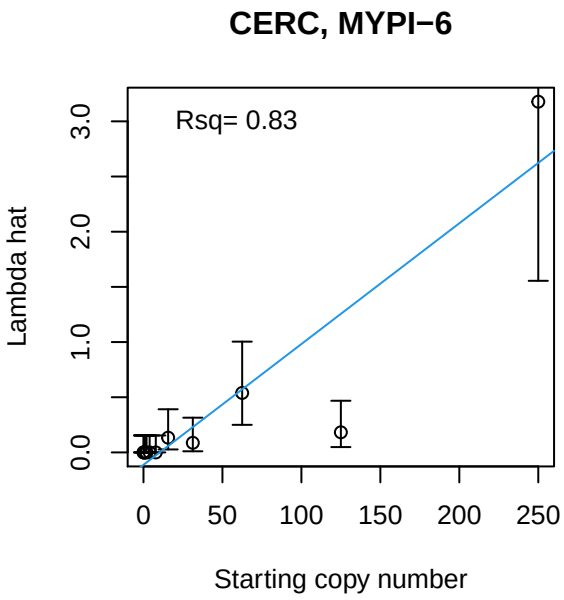
Only the first levels of SQ that had non-detects are analyzed. Blue line is least squares linear regression line.

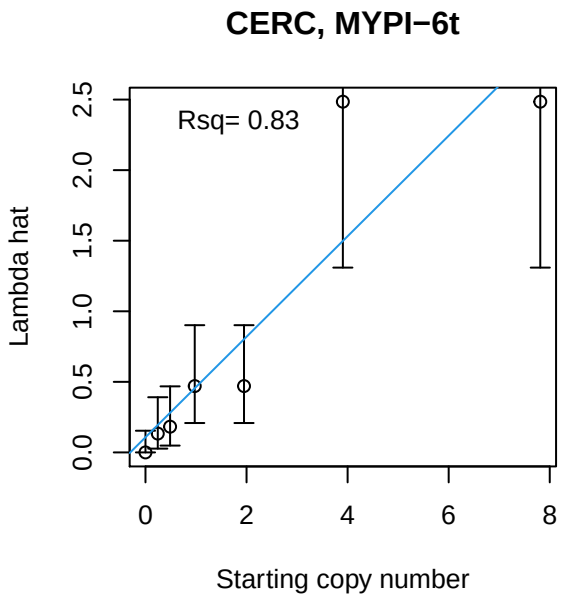
ML ??will error if all phats==1

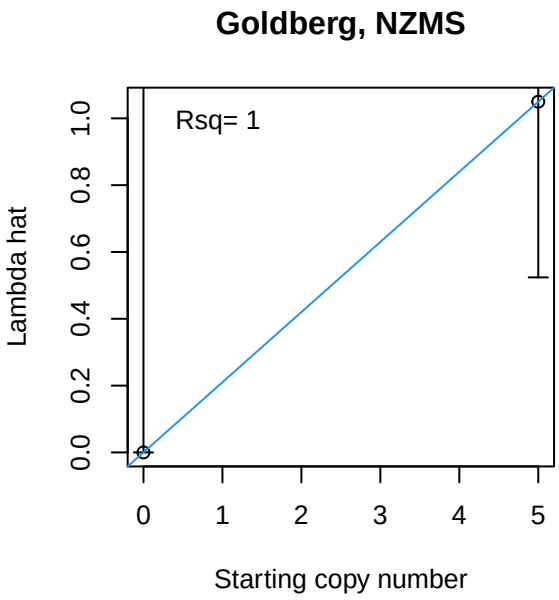
```
##
##
## Too few values for  CID
```

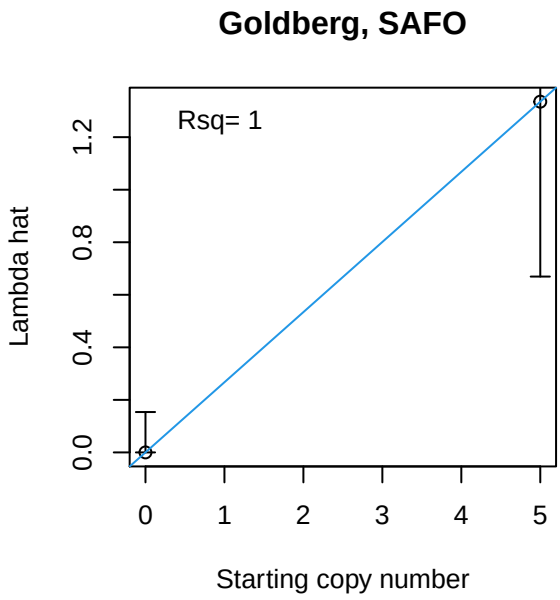

APHIS, D−loop
Rsq= 0.99
Lambda hat
Starting copy number
0.0
0.2
0.4
0.6
0.8
1.0
1.2

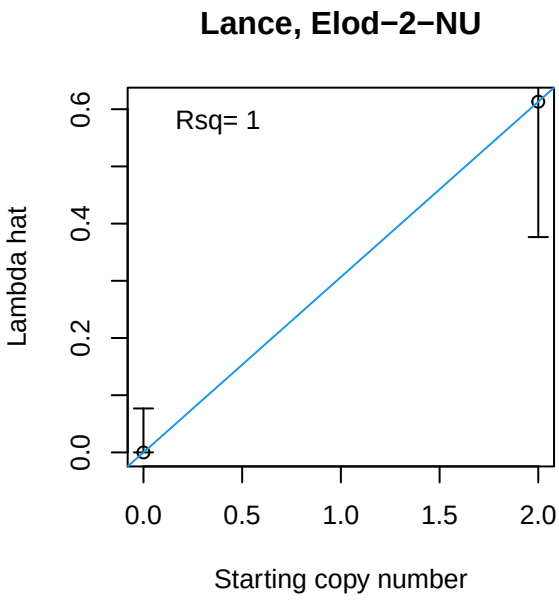

CERC, AC1_CID_1
Rsq= 0.98
Lambda hat
Starting copy number
0.0
0.5
1.0
1.5
0
5
10
15

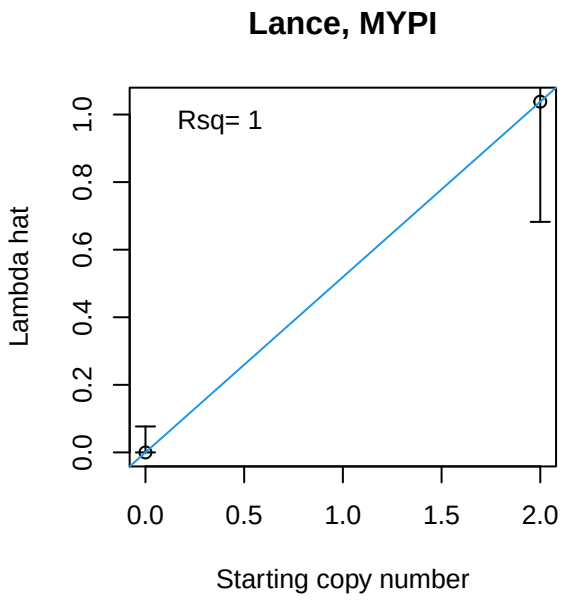

CERC, MYPI−6
Rsq= 0.83
Lambda hat
Starting copy number
0.0
1.0
2.0
3.0
0
50
100
150
200
250

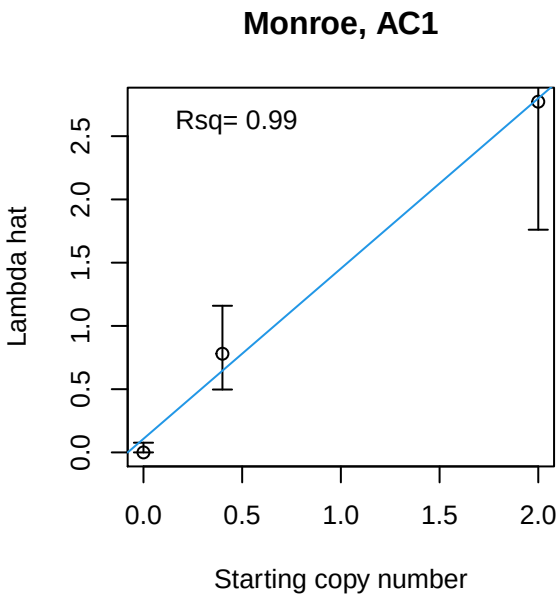

CERC, MYPI−6t
Rsq= 0.83
Lambda hat
Starting copy number
0.0
0.5
1.0
1.5
2.0
2.5
0
2
4
6
8

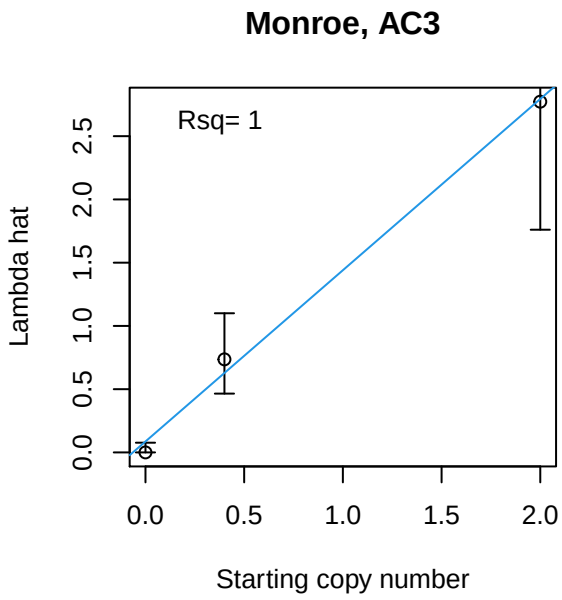

Goldberg, NZMS
Rsq= 1
Lambda hat
0.0
0.2
0.4
0.6
0.8
1.0
0
1
2
3
4
5
Starting copy number


Goldberg, SAFO
Rsq= 1
Lambda hat
0.0
0.4
0.8
1.2
0
1
2
3
4
5
Starting copy number


Lance, Elod−2−NU
Rsq= 1
Lambda hat
0.0
0.2
0.4
0.6
0.0
0.5
1.0
1.5
2.0
Starting copy number


Lance, MYPI
Rsq= 1
Lambda hat
0.0
0.2
0.4
0.6
0.8
1.0
0.0
0.5
1.0
1.5
2.0
Starting copy number


Monroe, AC1
Rsq= 0.99
Lambda hat
Starting copy number
0.0
0.5
1.0
1.5
2.0
2.5


Monroe, AC3
Rsq= 1
Lambda hat
Starting copy number
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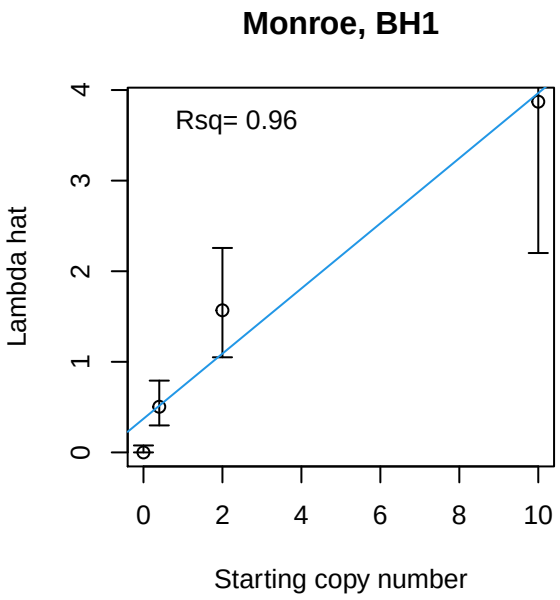
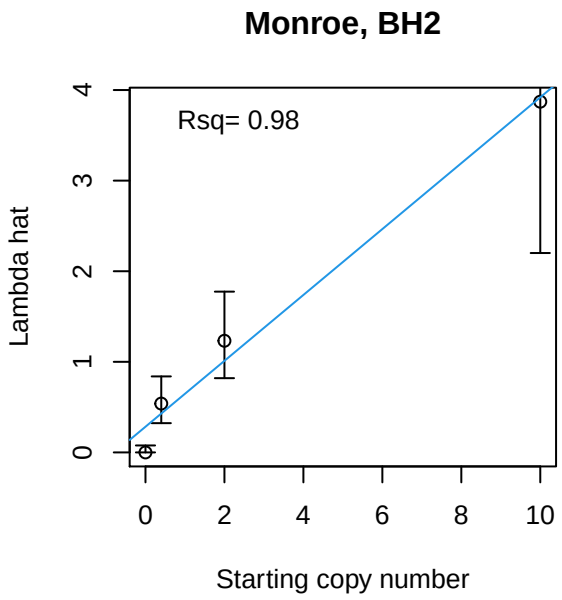
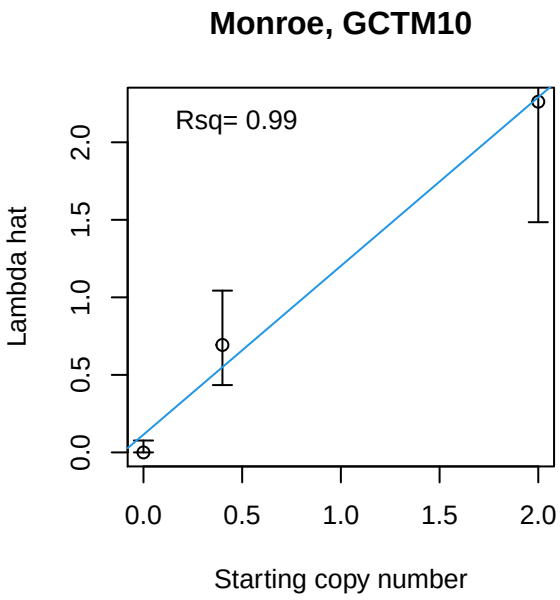
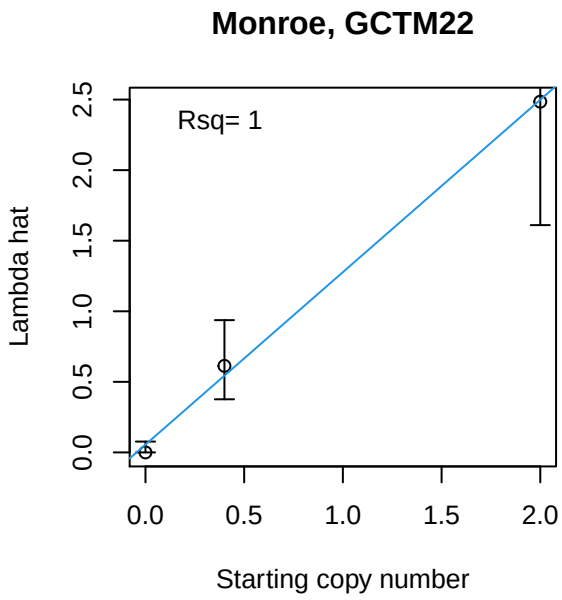
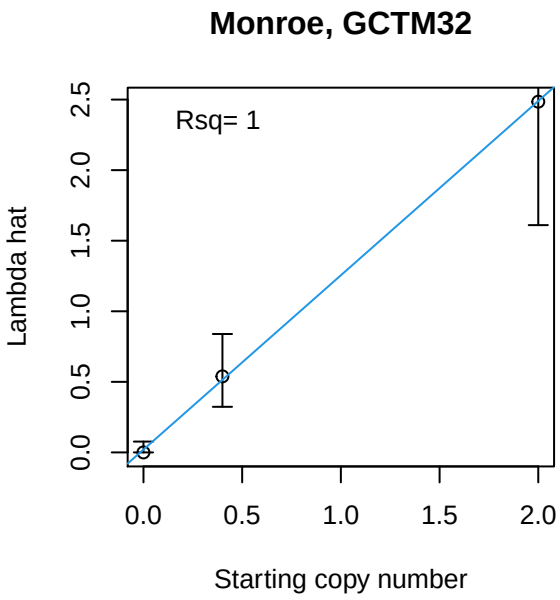
0.5
1.0
1.5
2.0
2.5

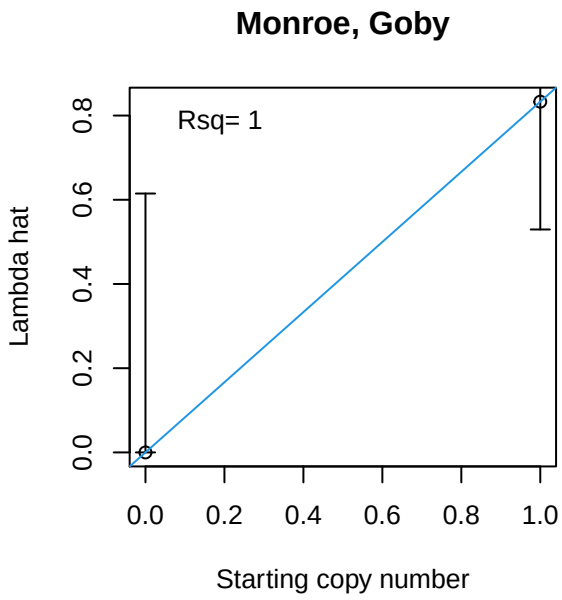

Monroe, BH1
Rsq= 0.96
Lambda hat
Starting copy number
0
1
2
3
4
0
2
4
6
8
10

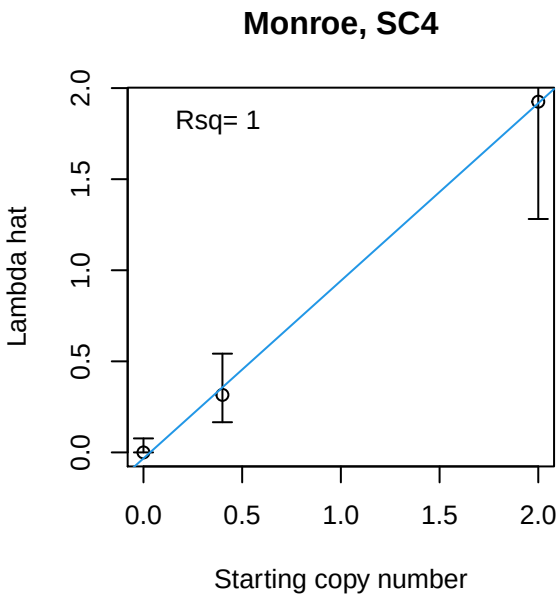

Monroe, BH2
Rsq= 0.98
Lambda hat
Starting copy number
0
1
2
3
4
0
2
4
6
8
10

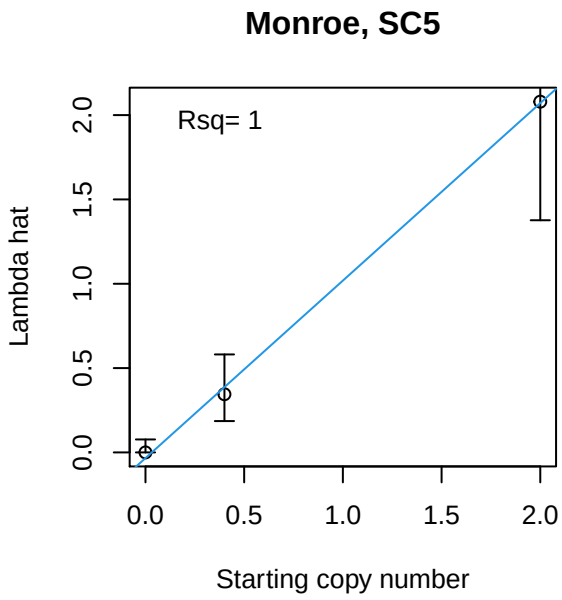

Monroe, GCTM10
Rsq= 0.99
Lambda hat
Starting copy number
0.0
0.5
1.0
1.5
2.0

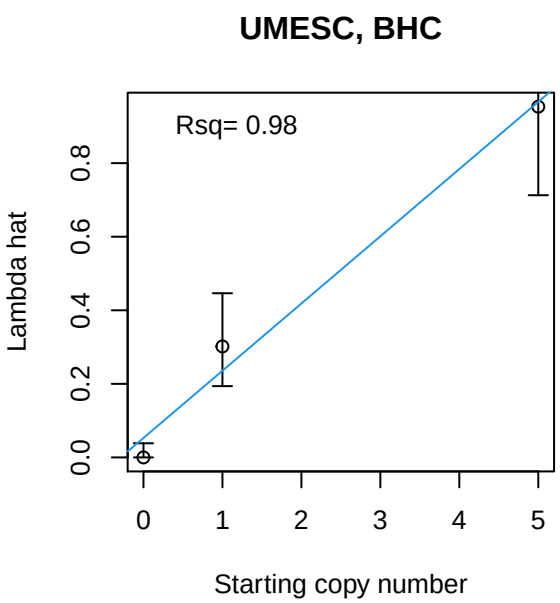

Monroe, GCTM22
Rsq= 1
Lambda hat
Starting copy number
0.0
0.5
1.0
1.5
2.0
2.5

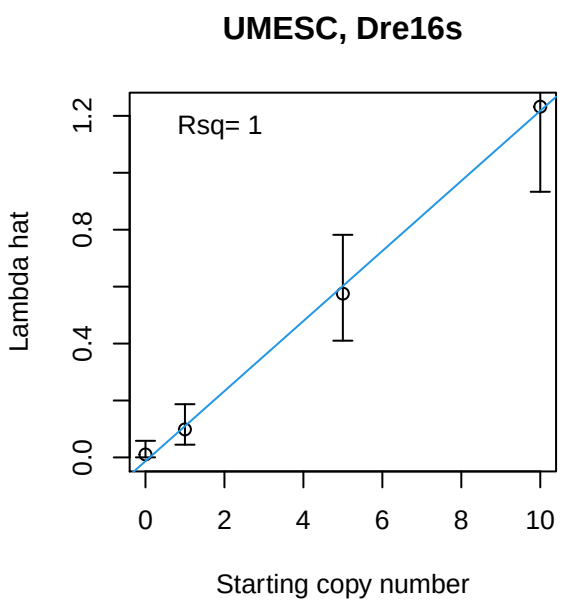

Monroe, GCTM32
Rsq= 1
Lambda hat
Starting copy number
0.0
0.5
1.0
1.5
2.0
2.5

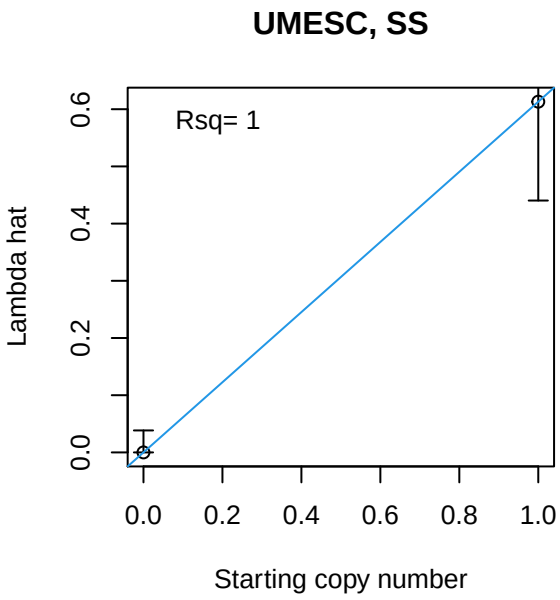

Monroe, Goby
Rsq= 1
Lambda hat
Starting copy number
0.0
0.2
0.4
0.6
0.8
1.0

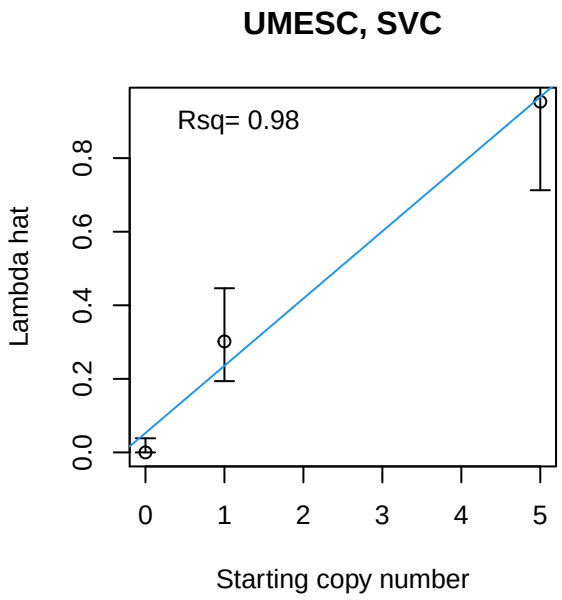

Monroe, SC4
Rsq= 1
Lambda hat
Starting copy number
0.0
0.5
1.0
1.5
2.0

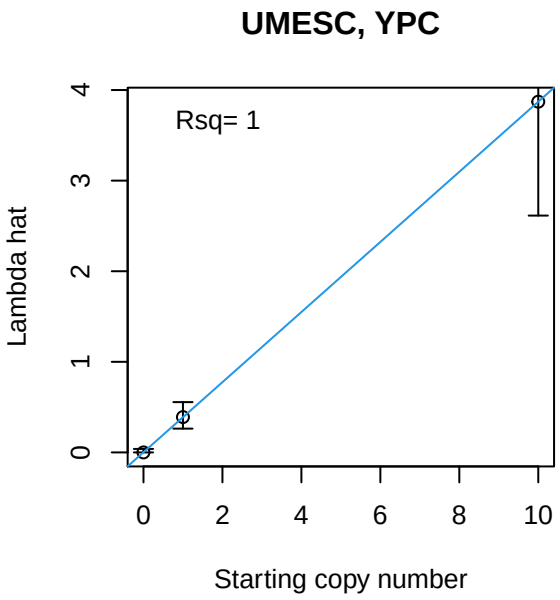

Monroe, SC5
Rsq= 1
Lambda hat
Starting copy number
0.0
0.5
1.0
1.5
2.0

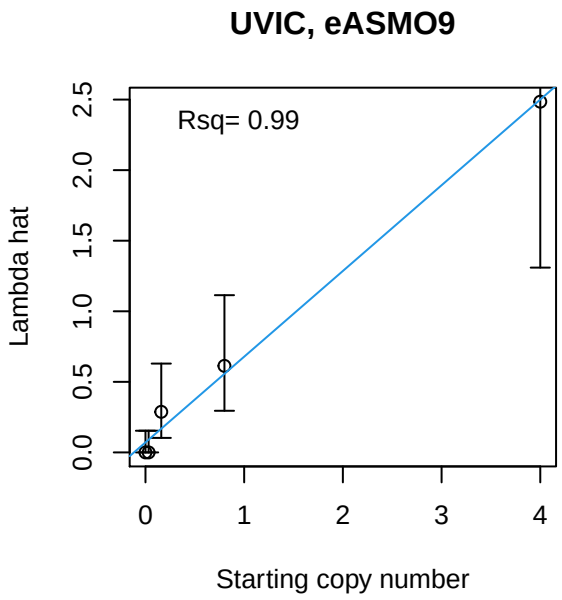

UMESC, BHC
Rsq= 0.98
Lambda hat
Starting copy number
0.0
0.2
0.4
0.6
0.8
0
1
2
3
4
5


UMESC, Dre16s
Rsq= 1
Lambda hat
Starting copy number
0.0
0.4
0.8
1.2
0
2
4
6
8
10


UMESC, SS
Rsq= 1
Lambda hat
Starting copy number
0.0
0.2
0.4
0.6
0.8
1.0


UMESC, SVC
Rsq= 0.98
Lambda hat
Starting copy number
0
1
2
3
4
5


UMESC, YPC
Rsq= 1
Lambda hat
Starting copy number
0
2
4
6
8
10


UVIC, eASMO9
Rsq= 0.99
Lambda hat
Starting copy number
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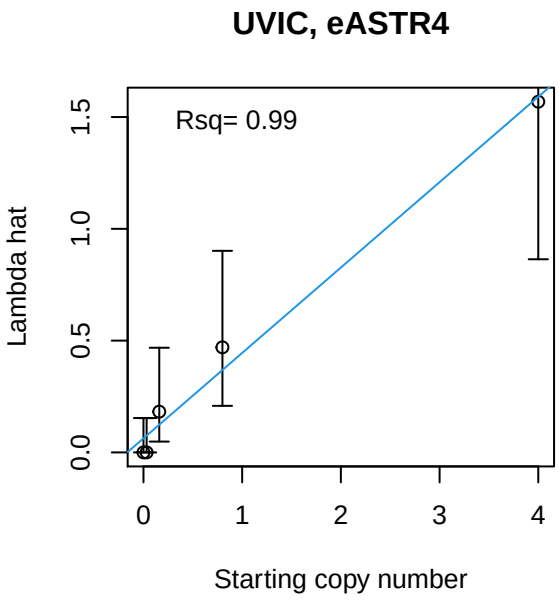
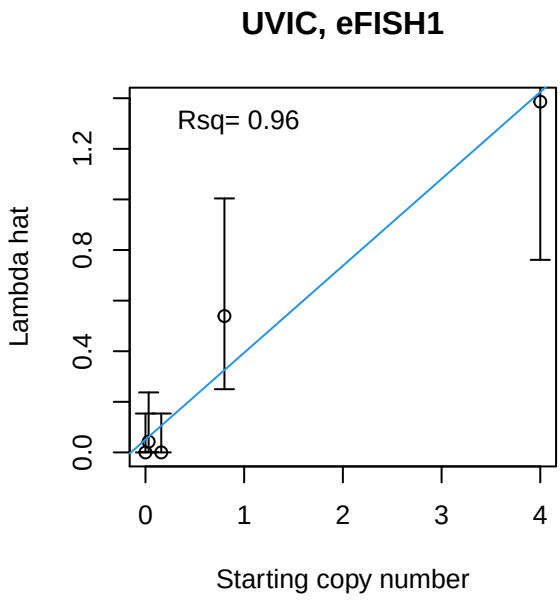
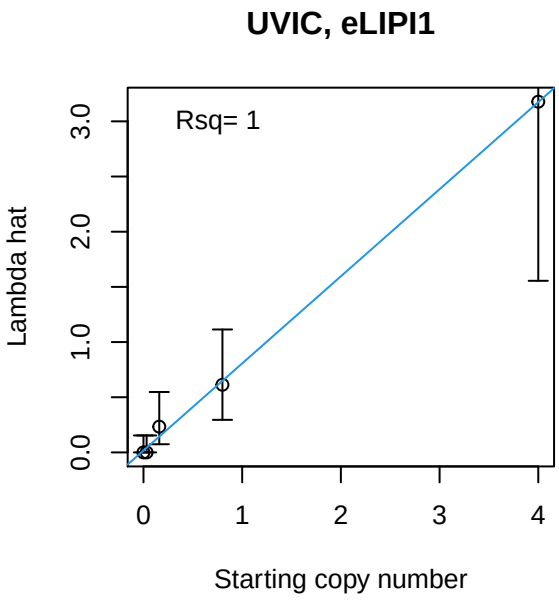
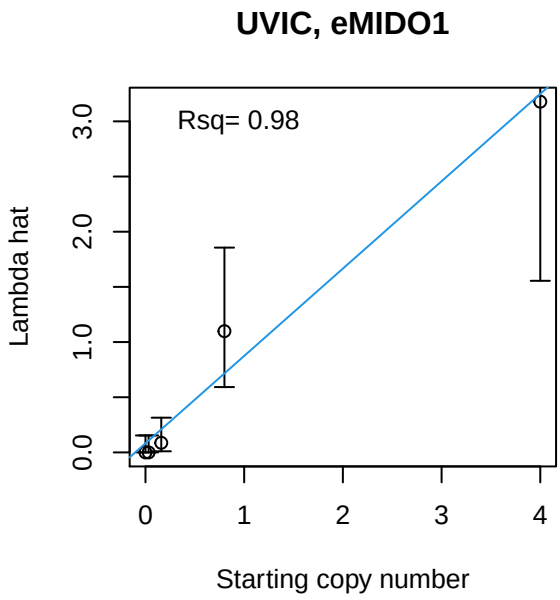
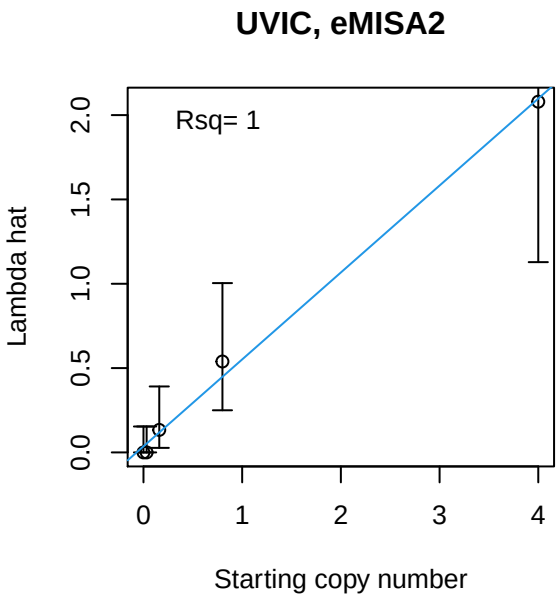
0.5
1.0
1.5
2.0
2.5

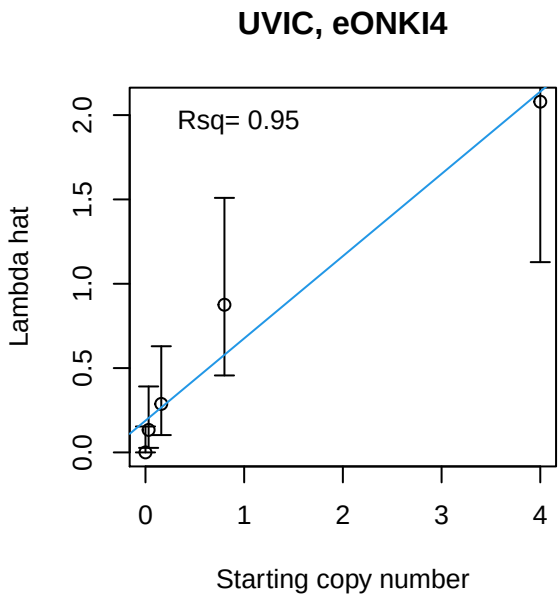

UVIC, eASTR4
Rsq= 0.99
Lambda hat
Starting copy number
0.0
0.5
1.0
1.5
0
1
2
3
4

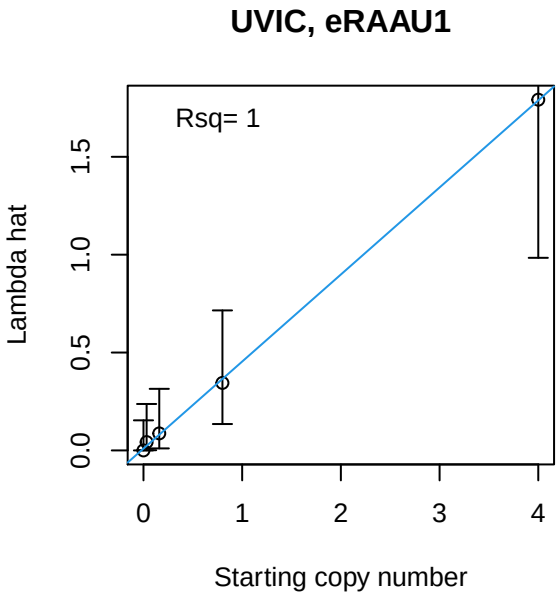

UVIC, eFISH1
Rsq= 0.96
Lambda hat
Starting copy number
0.0
0.4
0.8
1.2
0
1
2
3
4

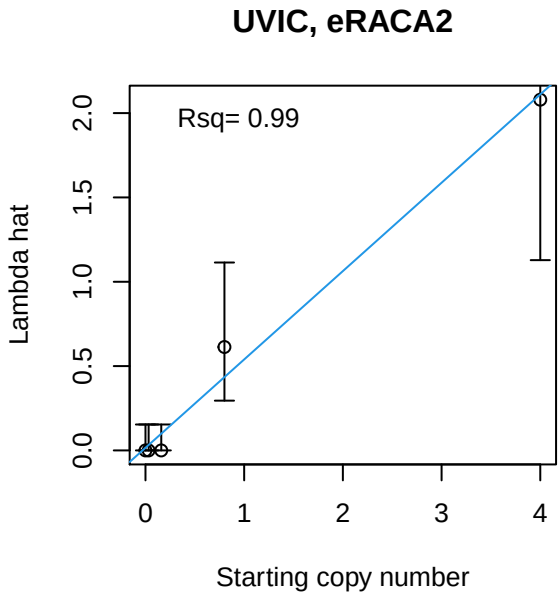

UVIC, eLIPI1
Rsq= 1
Lambda hat
Starting copy number
0.0
1.0
2.0
3.0
0
1
2
3
4

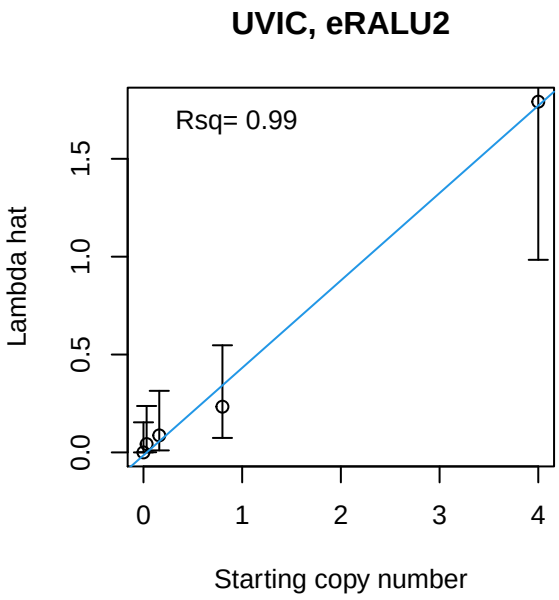

UVIC, eMIDO1
Rsq= 0.98
Lambda hat
Starting copy number
0.0
1.0
2.0
3.0
0
1
2
3
4

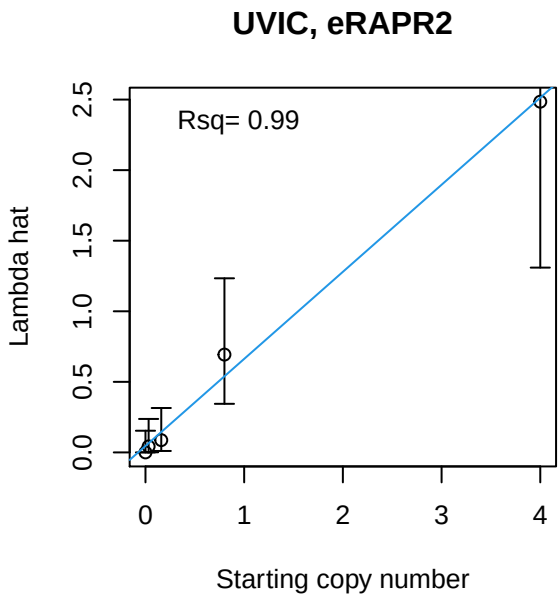

UVIC, eMISA2
Rsq= 1
Lambda hat
0.0
0.5
1.0
1.5
2.0
0
1
2
3
4
Starting copy number

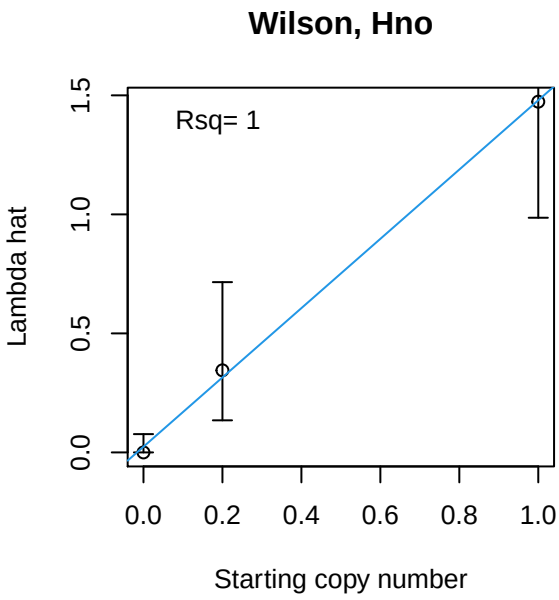

UVIC, eONKI4
Rsq= 0.95
Lambda hat
0.0
0.5
1.0
1.5
2.0
0
1
2
3
4
Starting copy number


UVIC, eRAAU1
Rsq= 1
Lambda hat
0.0
0.5
1.0
1.5
0
1
2
3
4
Starting copy number


UVIC, eRACA2
Rsq= 0.99
Lambda hat
0.0
0.5
1.0
1.5
2.0
0
1
2
3
4
Starting copy number


UVIC, eRALU2
Rsq= 0.99
Lambda hat
Starting copy number
0.0
0.5
1.0
1.5
0
1
2
3
4

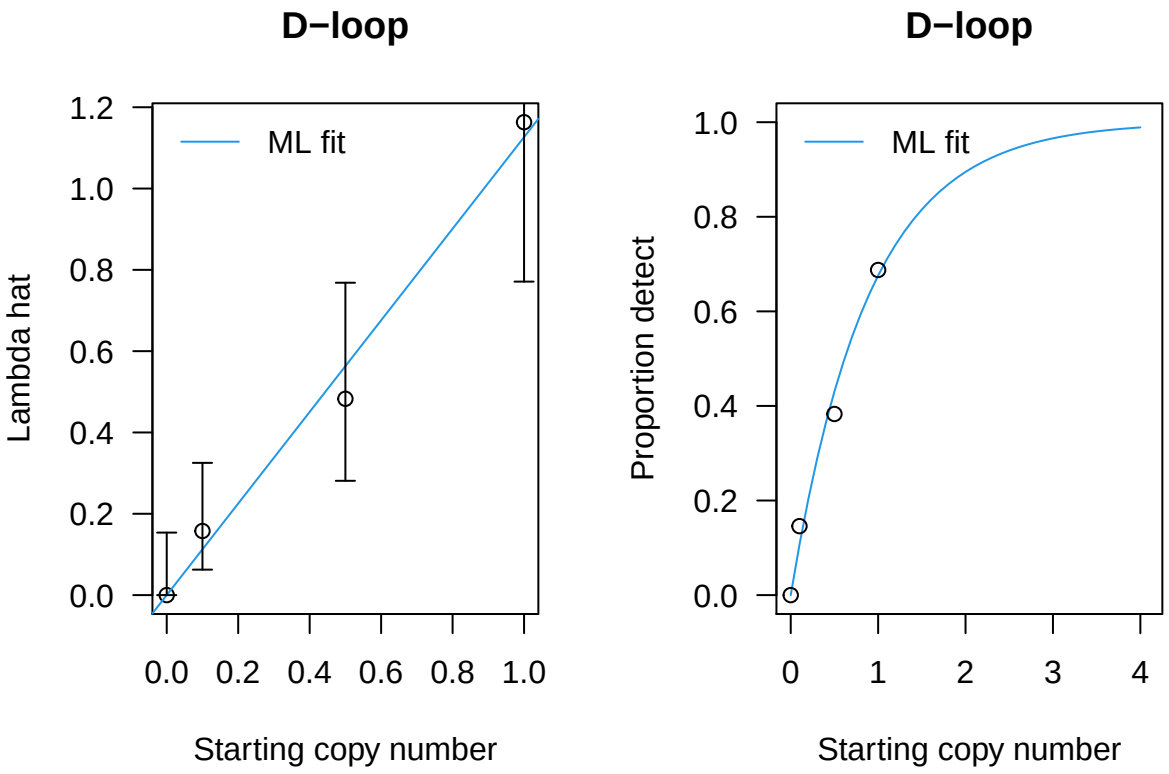

UVIC, eRAPR2
Rsq= 0.99
Lambda hat
Starting copy number
0.0
0.5
1.0
1.5
2.0
2.5
0
1
2
3
4


Wilson, Hno
Rsq= 1
Lambda hat
Starting copy number
0.0
0.5
1.0
1.5
0.0
0.2
0.4
0.6
0.8
1.0

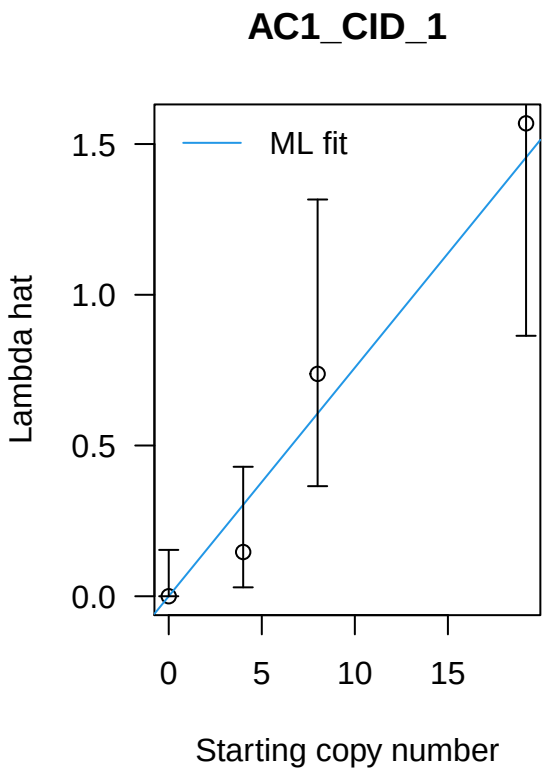
Supplemental Material

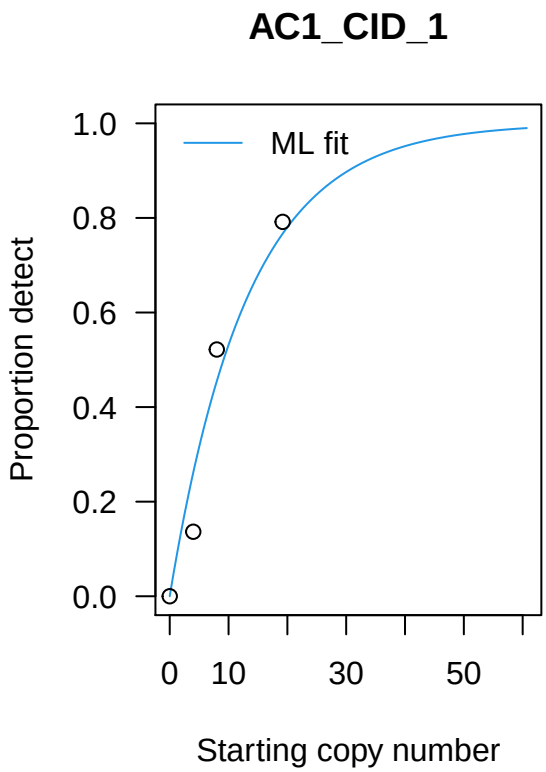
This file contains the outputs from all 29 data sets from Klymus et al. (2019) whose data are summarized in Table 4 of the manuscript. Results from both no intercept and intercept models are presented.

Estimate Poisson models - no intercept model

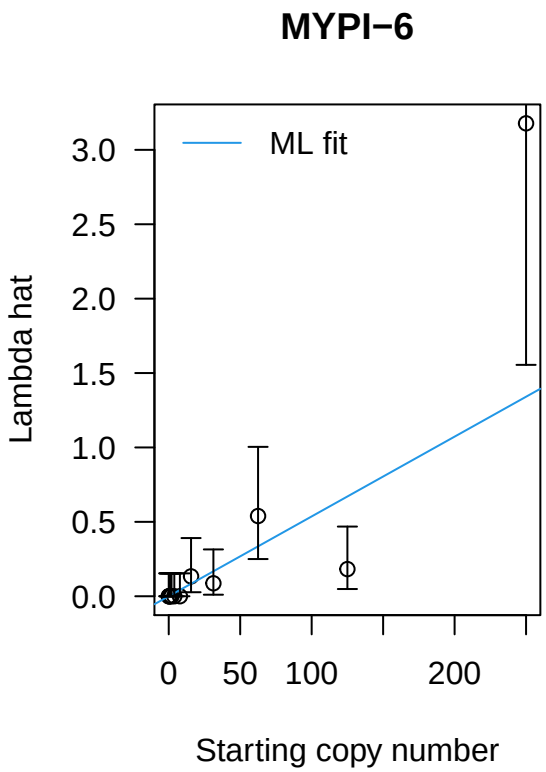


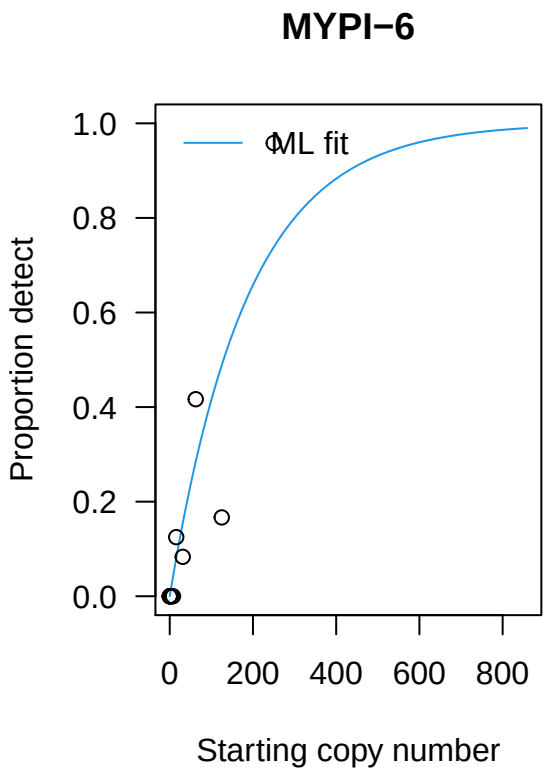

```
##
##
##
##
##  D-loop
## Convergence= 0
##      Estimate Std.Err Z value  Pr(>z)
## beta    1.126   0.153    7.37 1.8e-13 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 1.17757 , df=  3 , p-value= 0.7583889
```



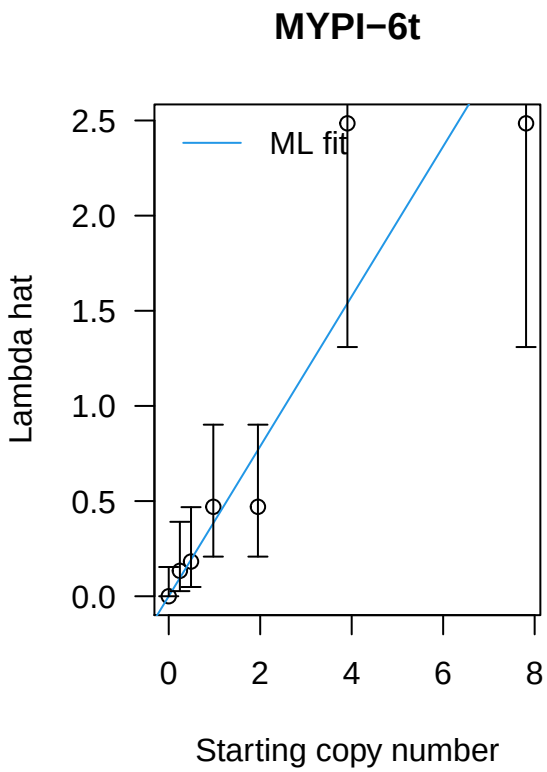


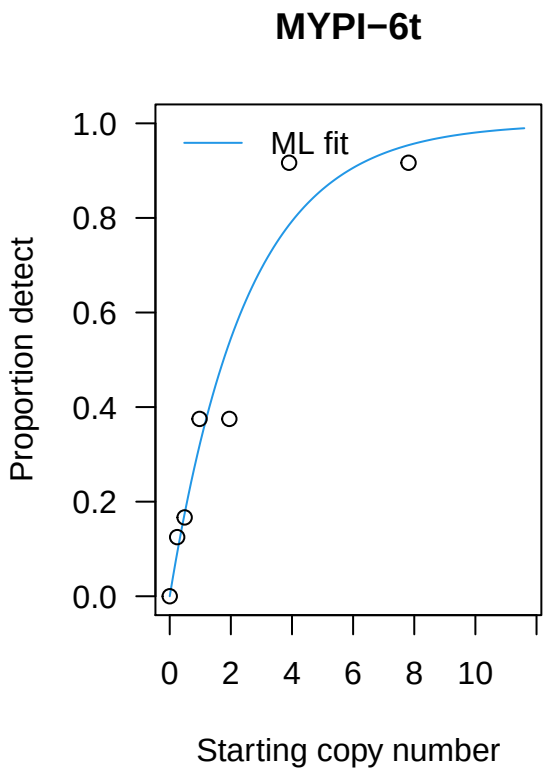

```
##
##
##
##
##  AC1_CID_1
## Convergence= 0
##      Estimate Std.Err Z value  Pr(>z)
## beta   0.0758  0.0137    5.53 3.2e-08 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 2.543745 , df=  3 , p-value= 0.4674373
##
## Too few values for  CID
```



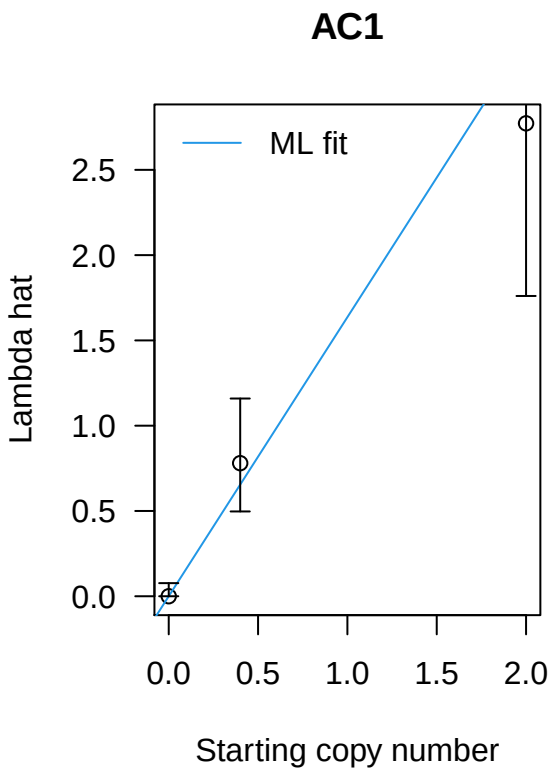


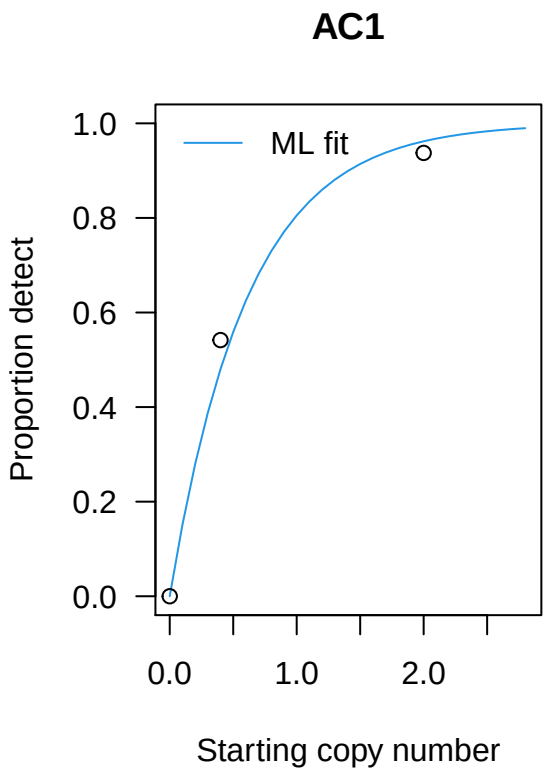

```
##
##
##
##
##  MYPI-6
## Convergence= 0
##      Estimate  Std.Err Z value  Pr(>z)
## beta 0.005363 0.000847    6.33 2.4e-10 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 26.74636 , df=  11 , p-value= 0.005018208
```



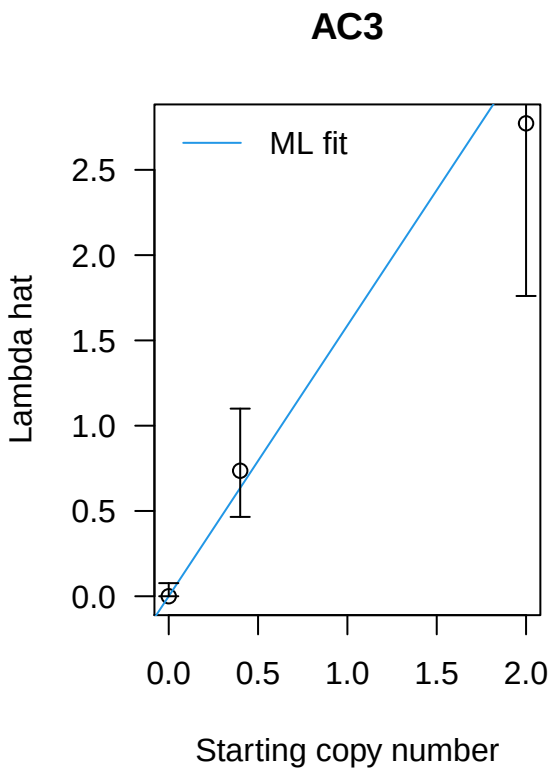


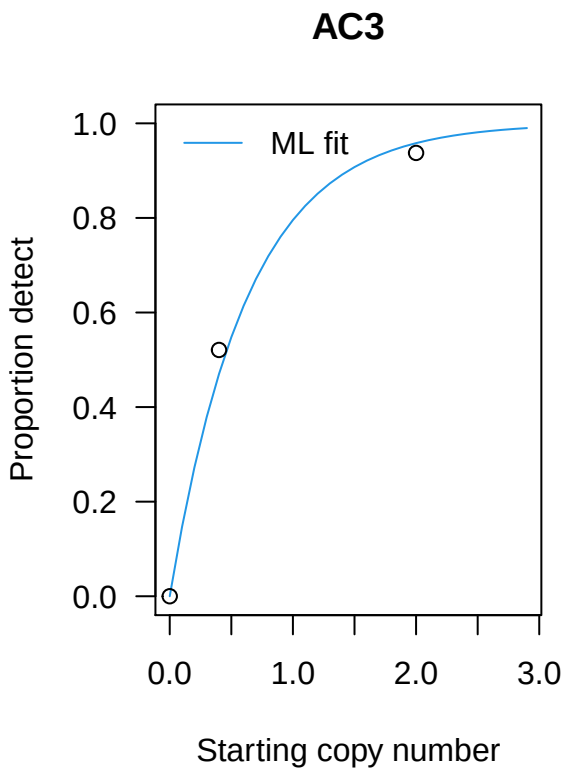

```
##
##
##
##
##  MYPI-6t
## Convergence= 0
##      Estimate Std.Err Z value  Pr(>z)
## beta    0.394   0.054    7.29 3.1e-13 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 6.787625 , df=  6 , p-value= 0.340935
##
## Too few values for  NZMS
## Too few values for  SAFO
## Too few values for  Elod-2-NU
## Too few values for  MYPI
```



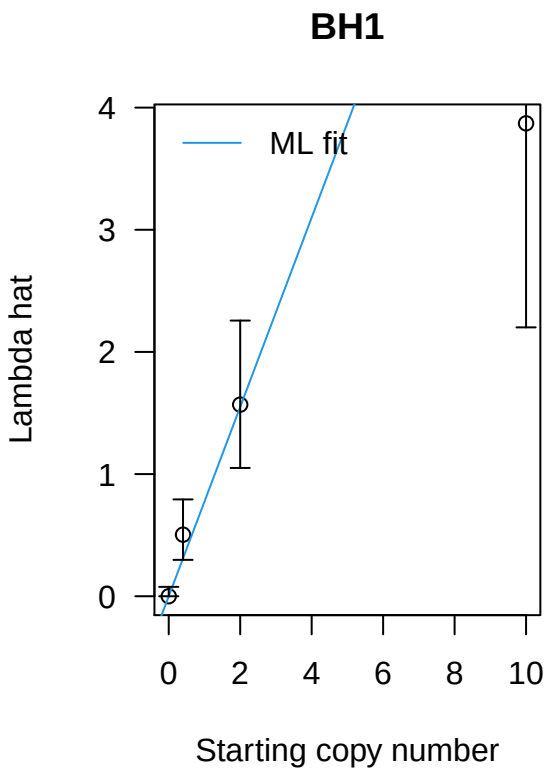


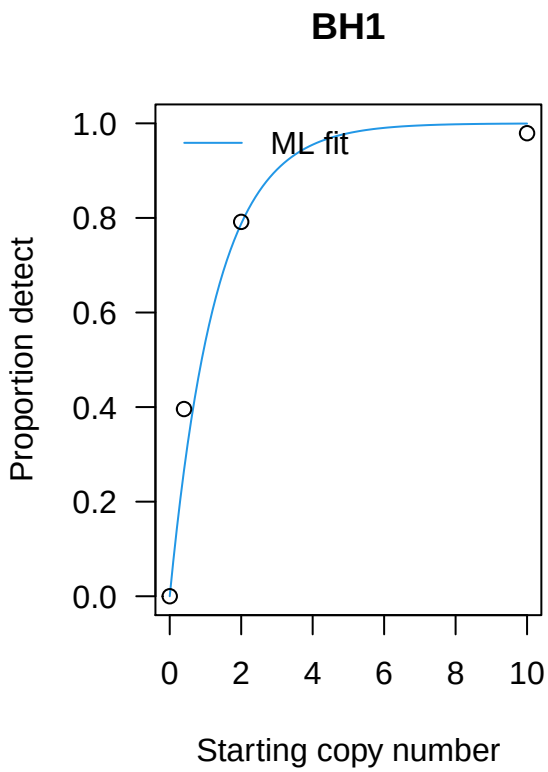

```
##
##
##
##
##  AC1
## Convergence= 0
##      Estimate Std.Err Z value  Pr(>z)
## beta    1.636   0.244     6.7 2.1e-11 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 1.393097 , df=  2 , p-value= 0.4983022
```



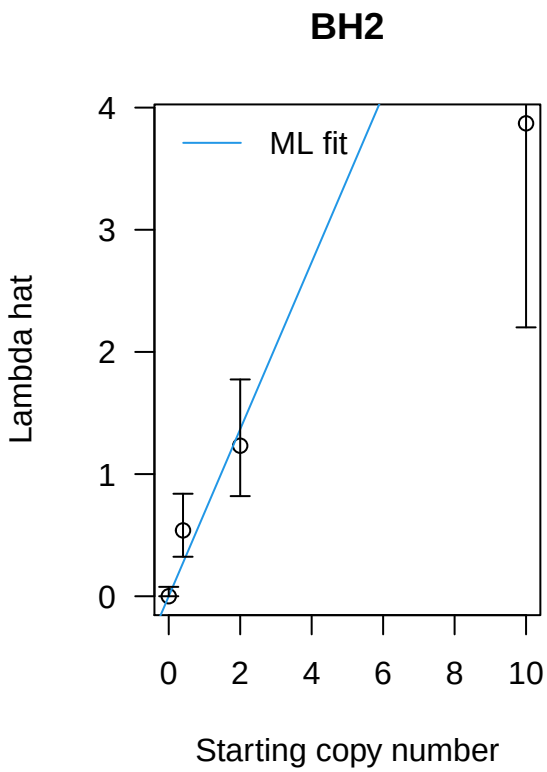


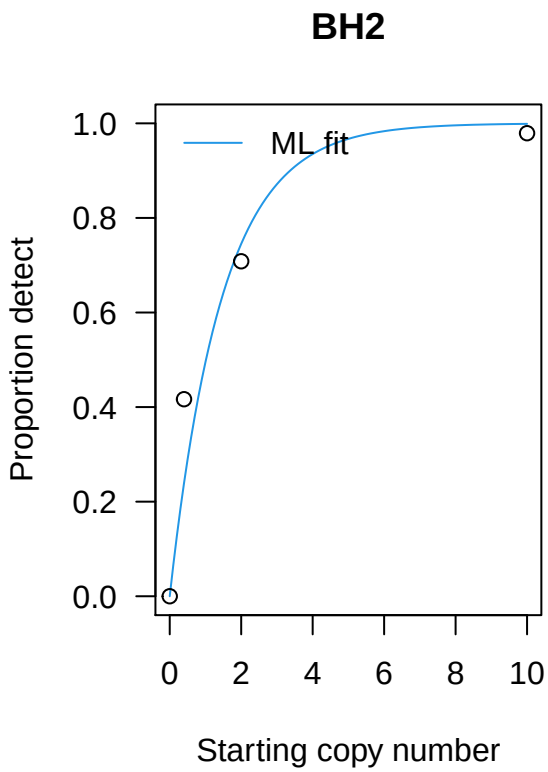

```
##
##
##
##
##  AC3
## Convergence= 0
##      Estimate Std.Err Z value  Pr(>z)
## beta    1.587   0.237     6.7 2.2e-11 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 0.9438297 , df=  2 , p-value= 0.6238066
```



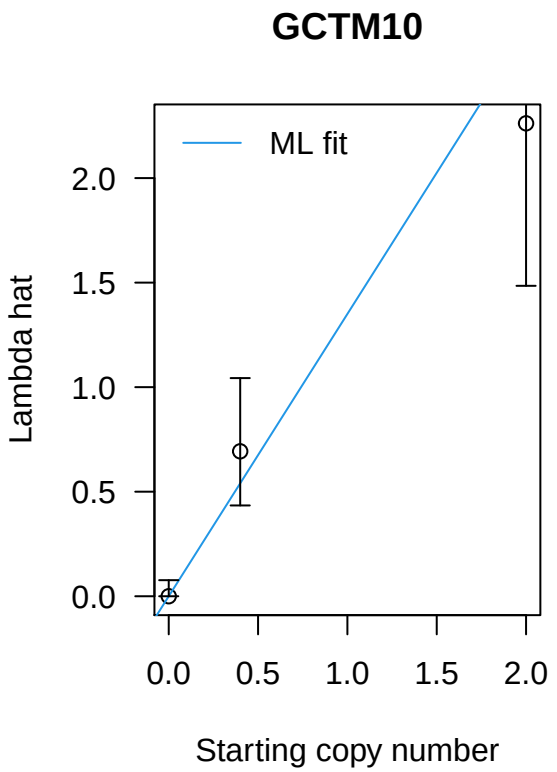


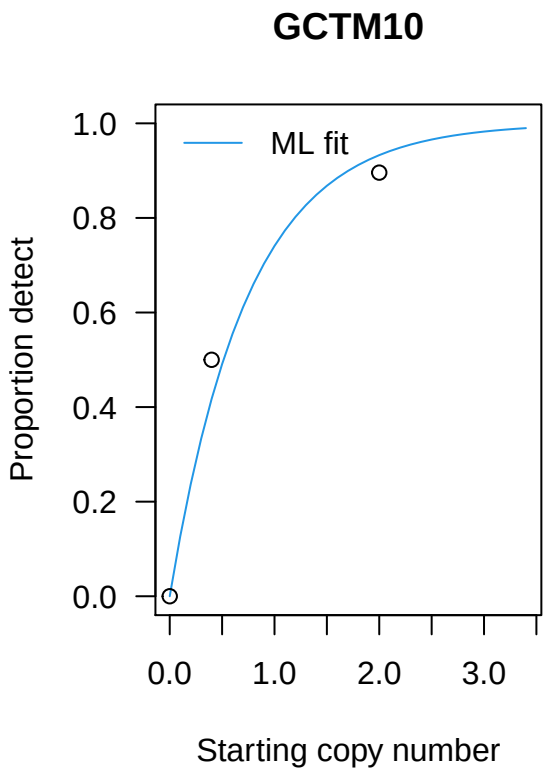

```
##
##
##
##
##  BH1
## Convergence= 0
##      Estimate Std.Err Z value Pr(>z)
## beta    0.775   0.108    7.16  8e-13 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 9.602954 , df=  3 , p-value= 0.02226096
```



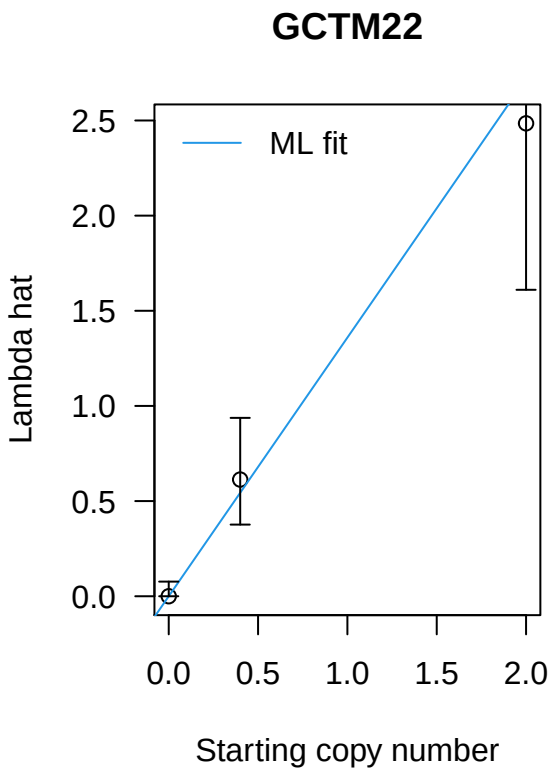


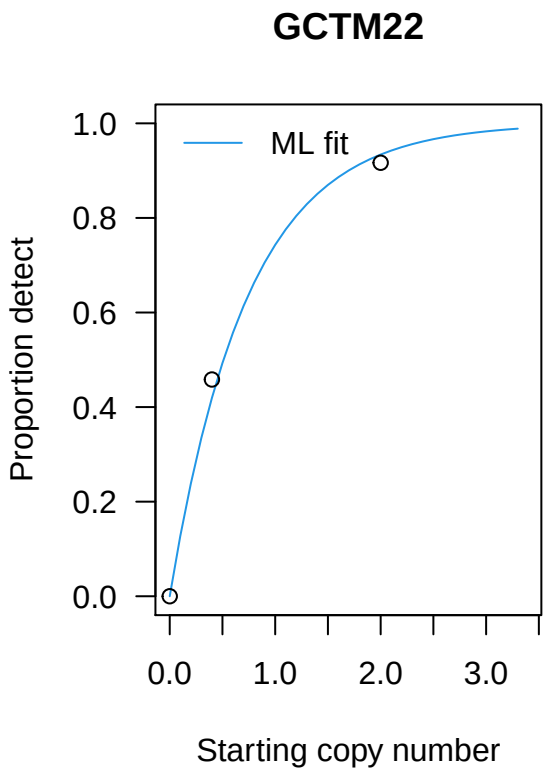

```
##
##
##
##
##  BH2
## Convergence= 0
##      Estimate Std.Err Z value  Pr(>z)
## beta   0.6836  0.0953    7.17 7.5e-13 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 11.70607 , df=  3 , p-value= 0.008460941
```



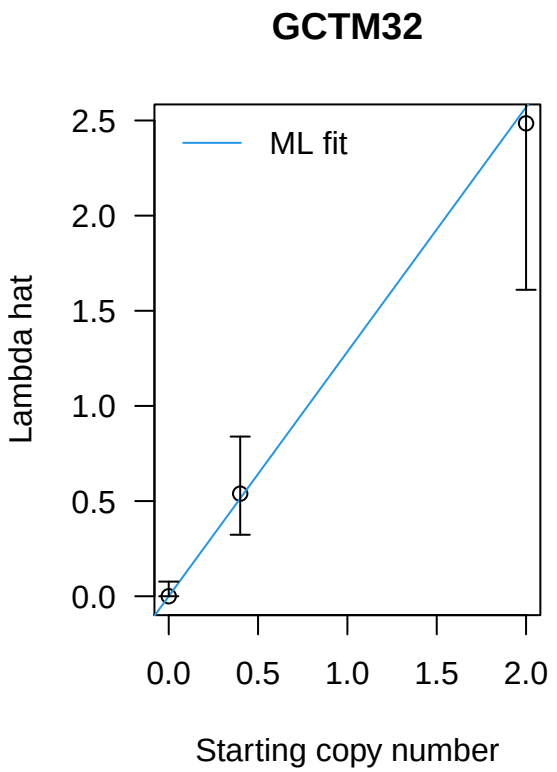


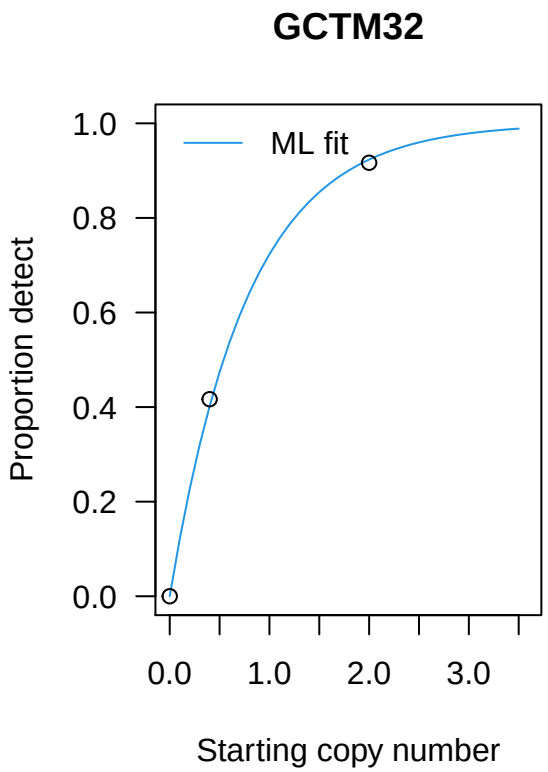

```
##
##
##
##
##  GCTM10
## Convergence= 0
##      Estimate Std.Err Z value  Pr(>z)
## beta    1.350   0.196     6.9 5.1e-12 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 2.23836 , df=  2 , p-value= 0.3265475
```



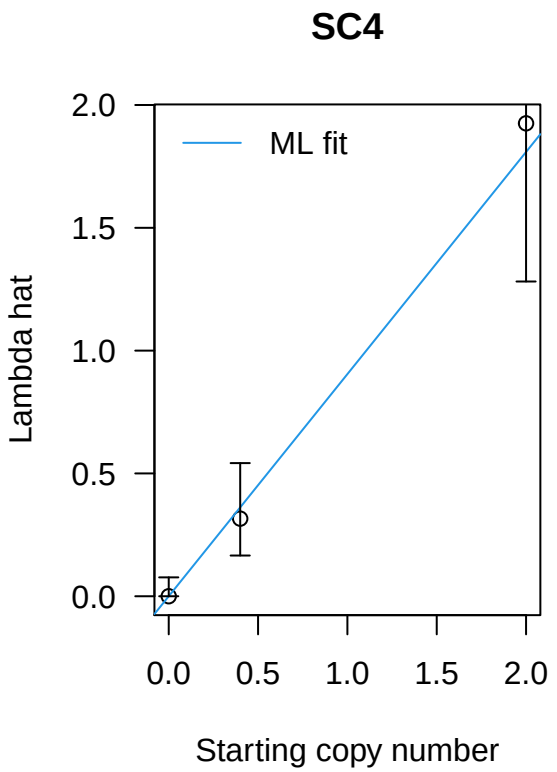


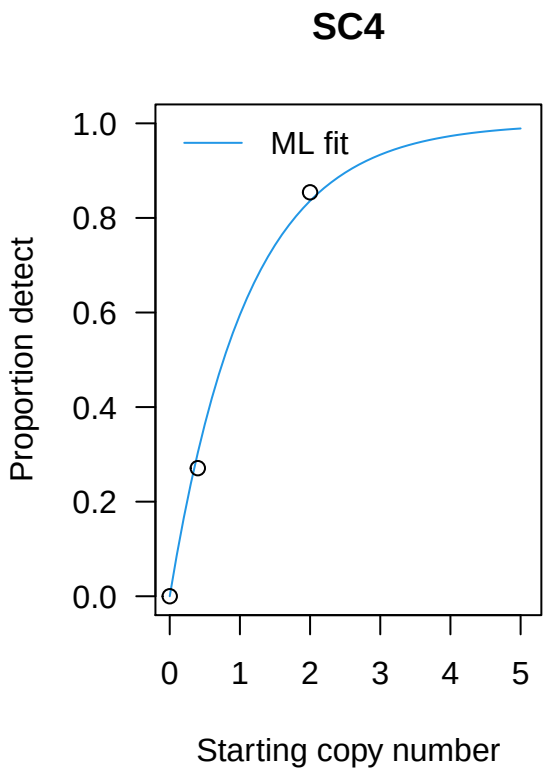

```
##
##
##
##
##  GCTM22
## Convergence= 0
##      Estimate Std.Err Z value  Pr(>z)
## beta     1.36    0.20    6.79 1.1e-11 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 0.5143054 , df=  2 , p-value= 0.7732501
```



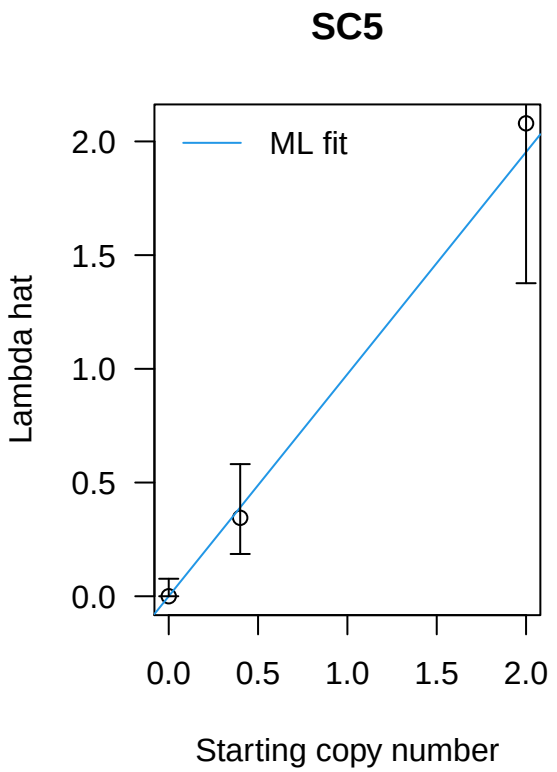


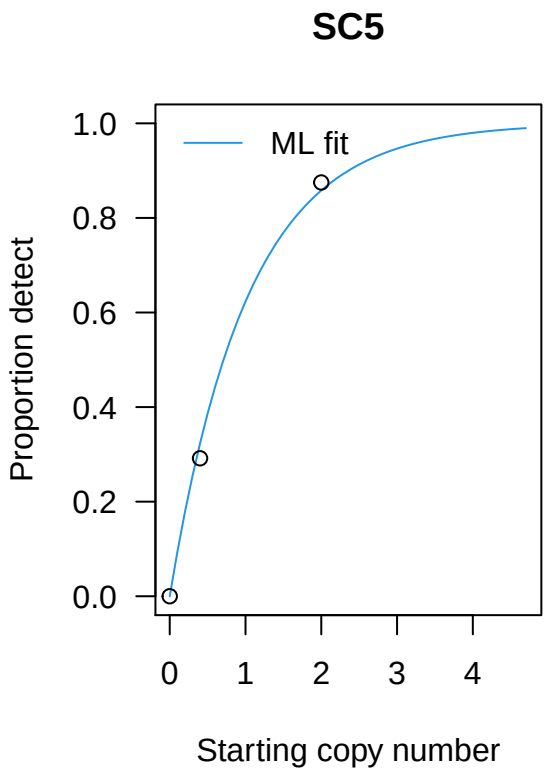

```
##
##
##
##
##  GCTM32
## Convergence= 0
##      Estimate Std.Err Z value  Pr(>z)
## beta     1.28    0.19    6.76 1.4e-11 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 0.07393266 , df=  2 , p-value= 0.9637086
##
## Too few values for  Goby
```



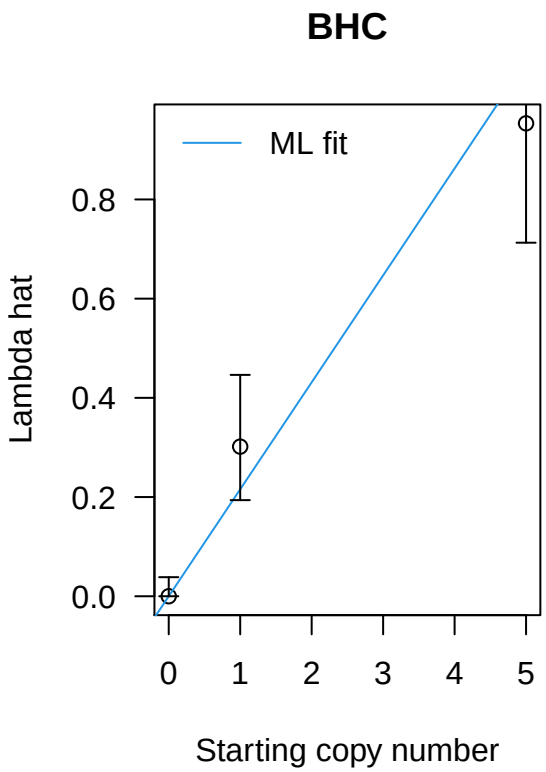


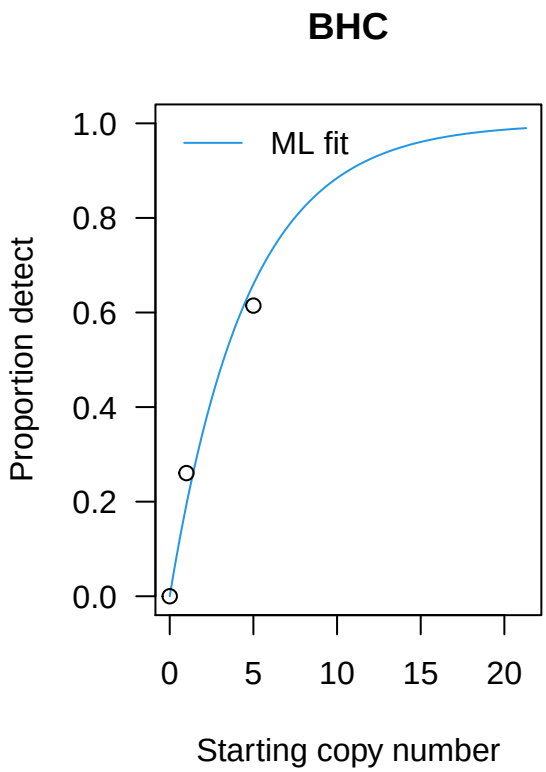

```
##
##
##
##
##  SC4
## Convergence= 0
##      Estimate Std.Err Z value  Pr(>z)
## beta    0.904   0.136    6.66 2.8e-11 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 0.365632 , df=  2 , p-value= 0.8329214
```



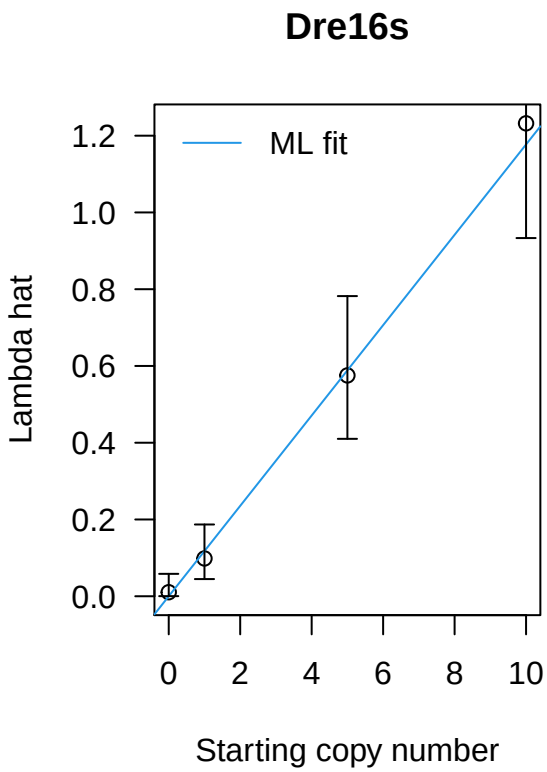


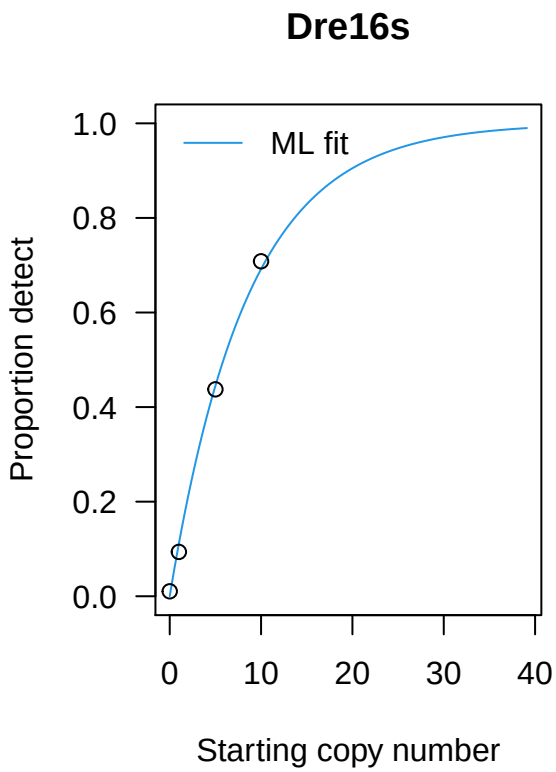

```
##
##
##
##
##  SC5
## Convergence= 0
##      Estimate Std.Err Z value  Pr(>z)
## beta    0.977   0.146    6.68 2.3e-11 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 0.3401212 , df=  2 , p-value= 0.8436137
```



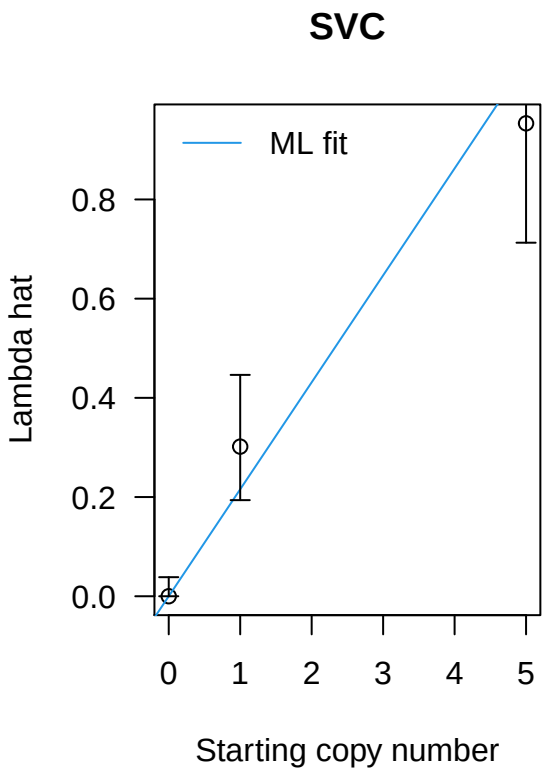


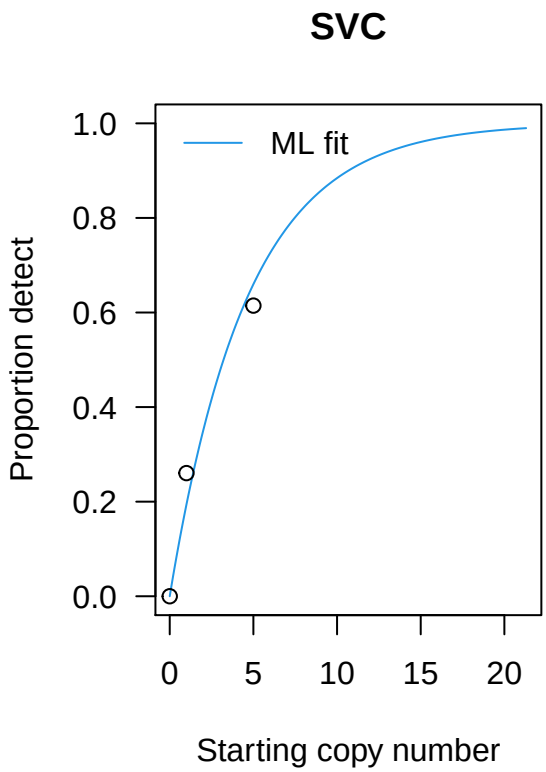

```
##
##
##
##
##  BHC
## Convergence= 0
##      Estimate Std.Err Z value Pr(>z)
## beta   0.2156  0.0243    8.86 <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 3.368959 , df=  2 , p-value= 0.1855409
```



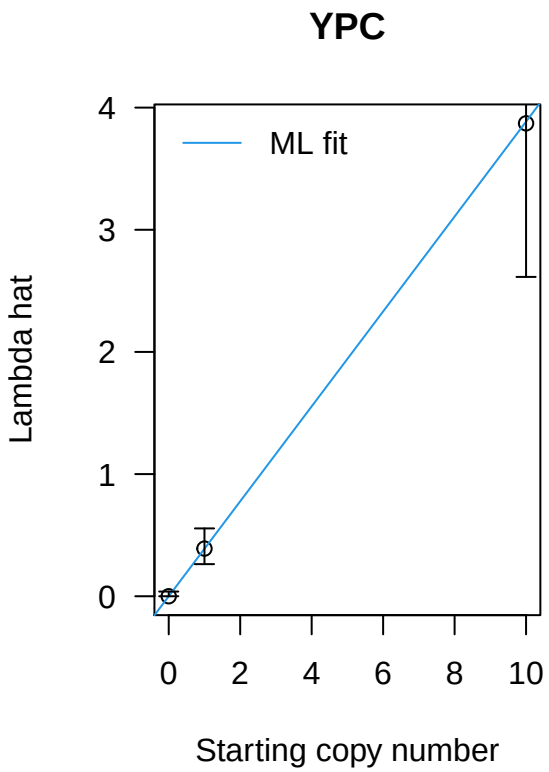


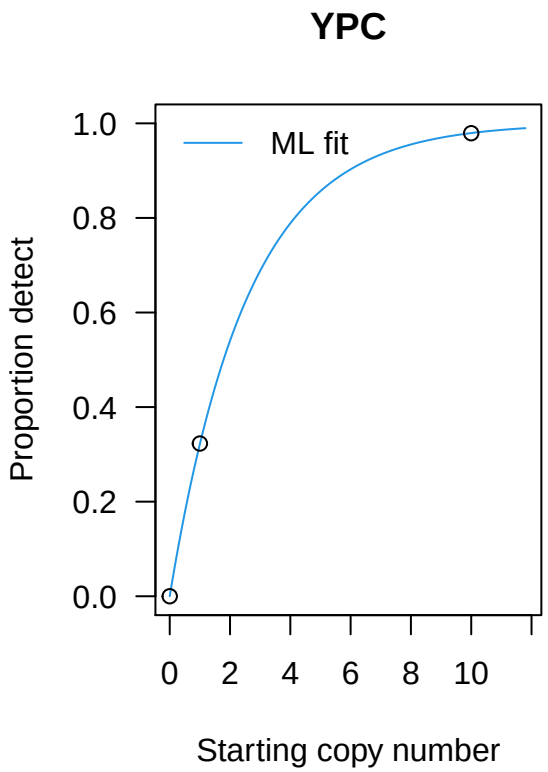

```
##
##
##
##
##  Dre16s
## Convergence= 0
##      Estimate Std.Err Z value Pr(>z)
## beta   0.1177  0.0112    10.5 <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 0.450349 , df=  3 , p-value= 0.929656
##
## Too few values for  SS
```



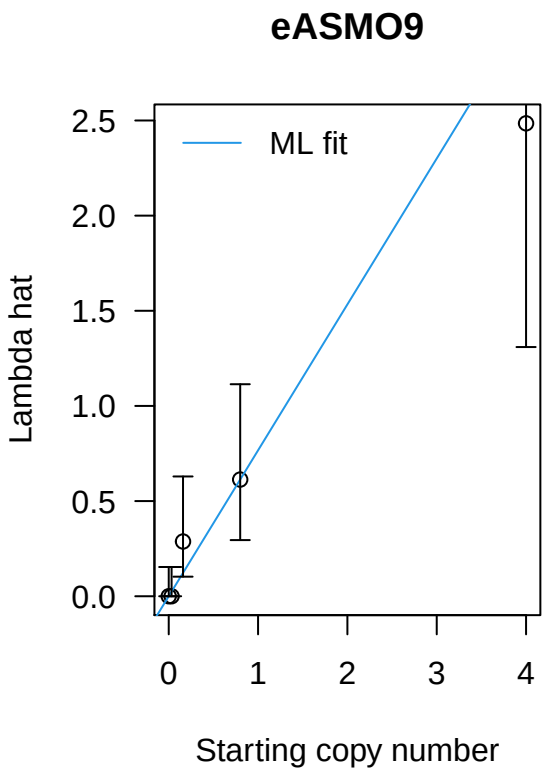


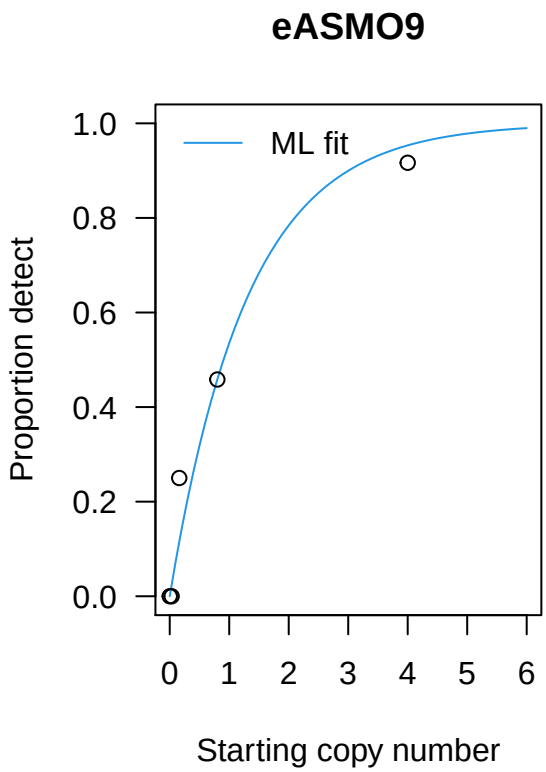

```
##
##
##
##
##  SVC
## Convergence= 0
##      Estimate Std.Err Z value Pr(>z)
## beta   0.2156  0.0243    8.86 <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 3.368959 , df=  2 , p-value= 0.1855409
```



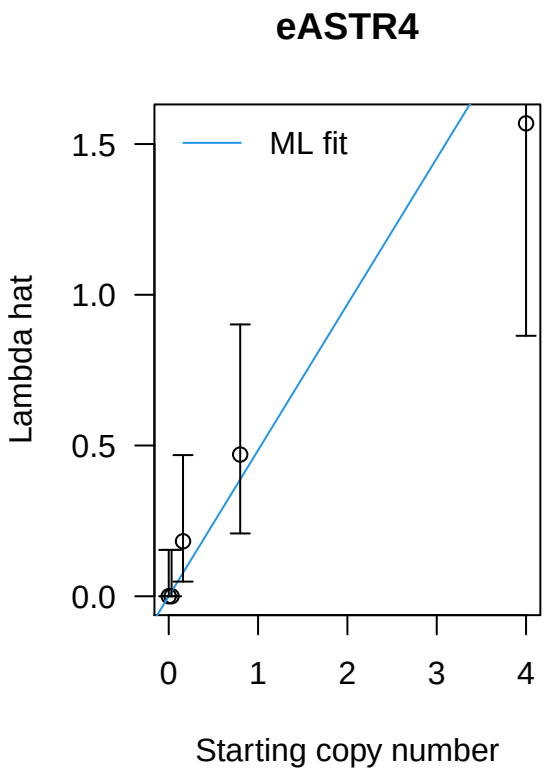


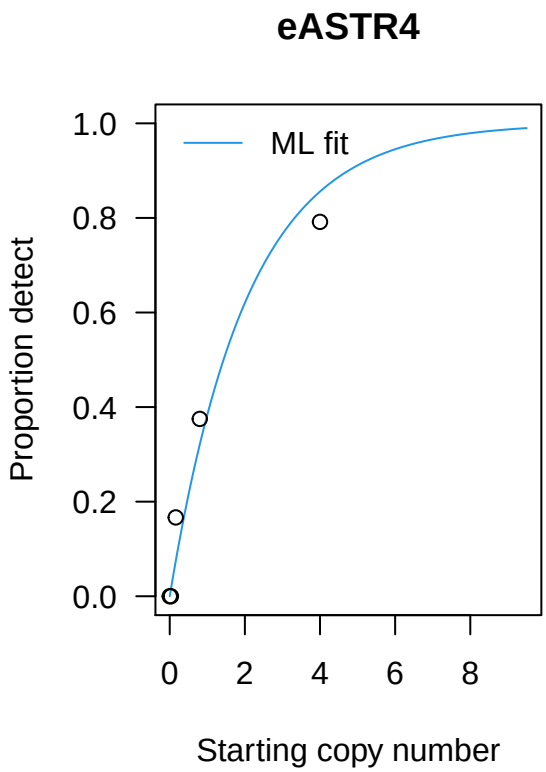

```
##
##
##
##
##  YPC
## Convergence= 0
##      Estimate Std.Err Z value  Pr(>z)
## beta   0.3885  0.0497    7.81 5.7e-15 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 0.0008171872 , df=  2 , p-value= 0.9995915
```



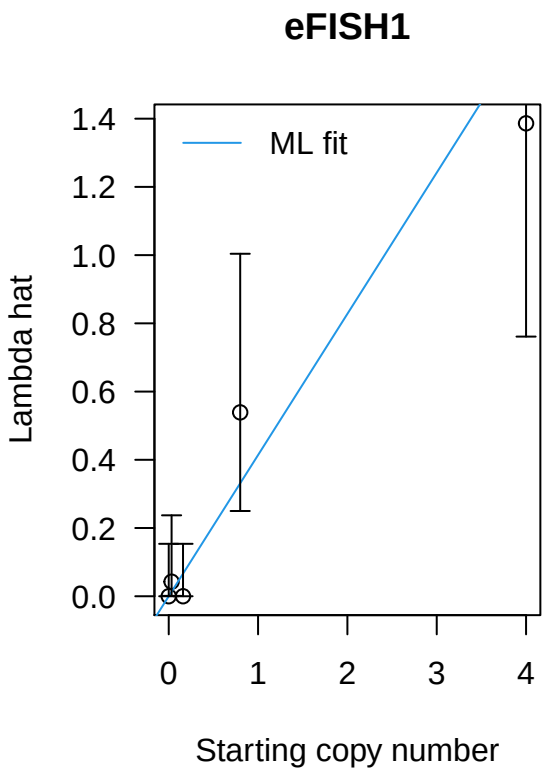


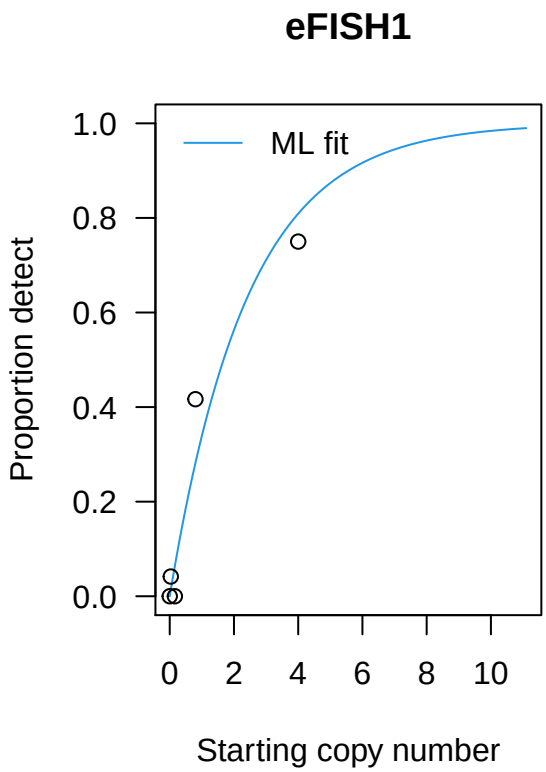

```
##
##
##
##
##  eASMO9
## Convergence= 0
##      Estimate Std.Err Z value  Pr(>z)
## beta    0.767   0.147    5.22 1.8e-07 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 5.106559 , df=  4 , p-value= 0.2765376
```



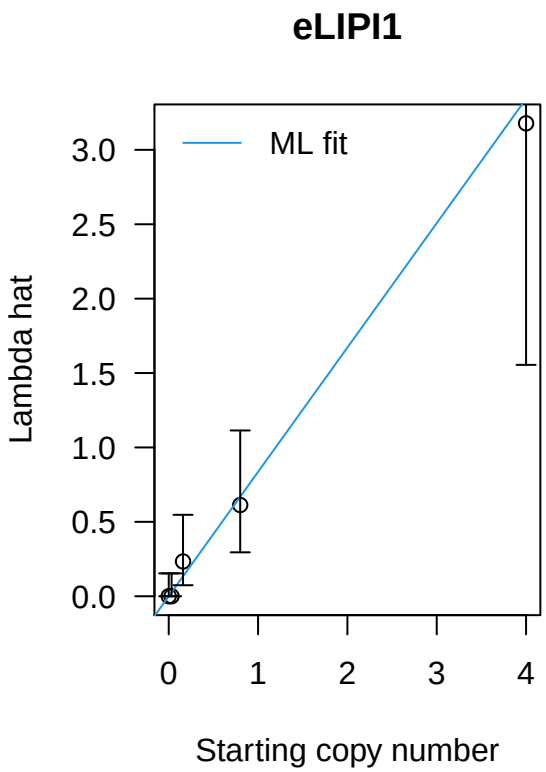


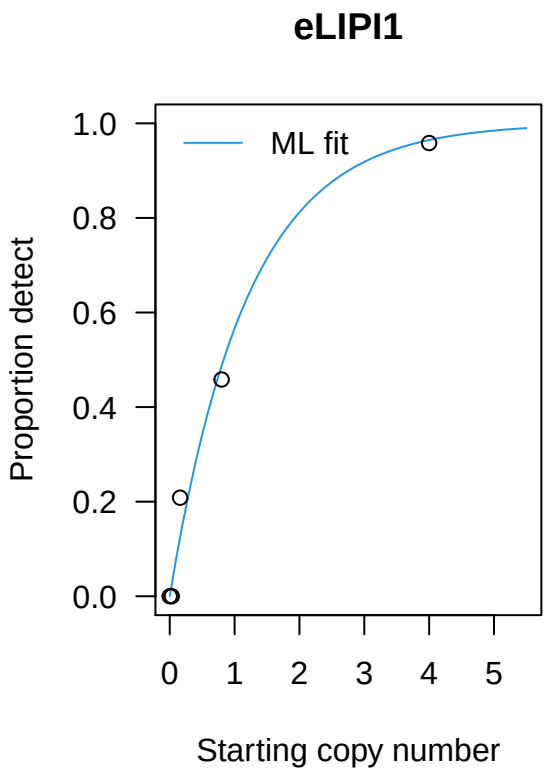

```
##
##
##
##
##  eASTR4
## Convergence= 0
##      Estimate Std.Err Z value  Pr(>z)
## beta   0.4842  0.0933    5.19 2.1e-07 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 4.017301 , df=  4 , p-value= 0.4036694
```



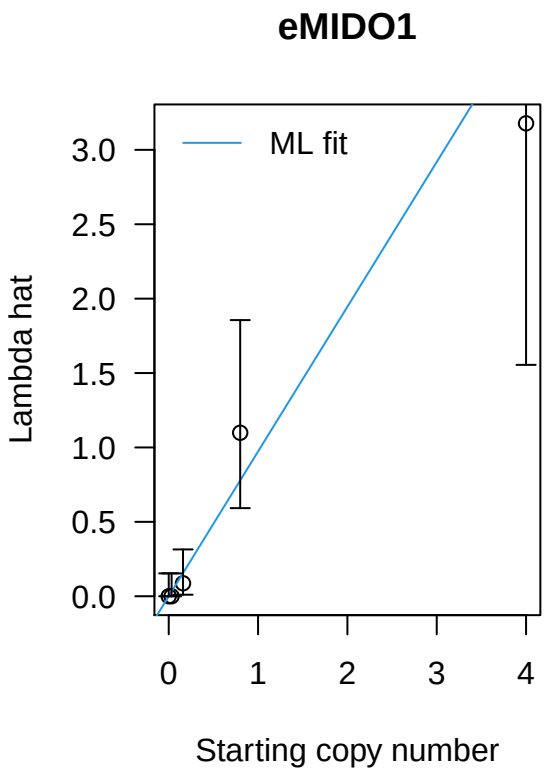


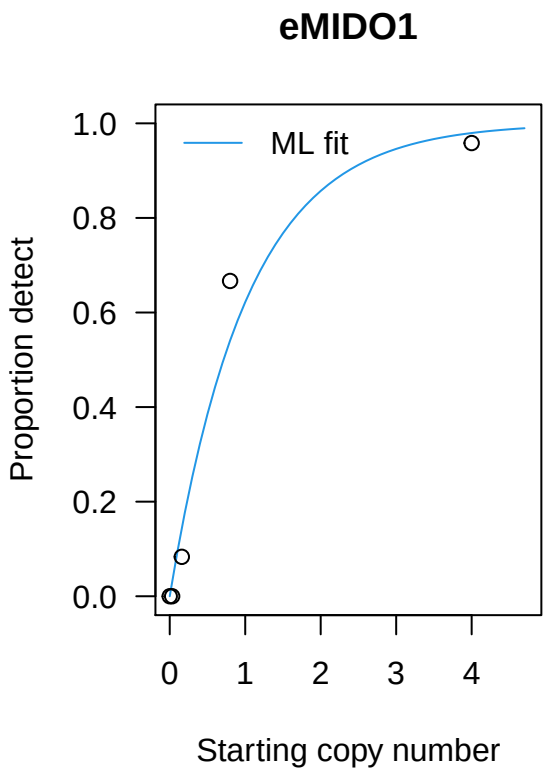

```
##
##
##
##
##  eFISH1
## Convergence= 0
##      Estimate Std.Err Z value  Pr(>z)
## beta   0.4139  0.0823    5.03 4.9e-07 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 6.63572 , df=  4 , p-value= 0.1564373
```



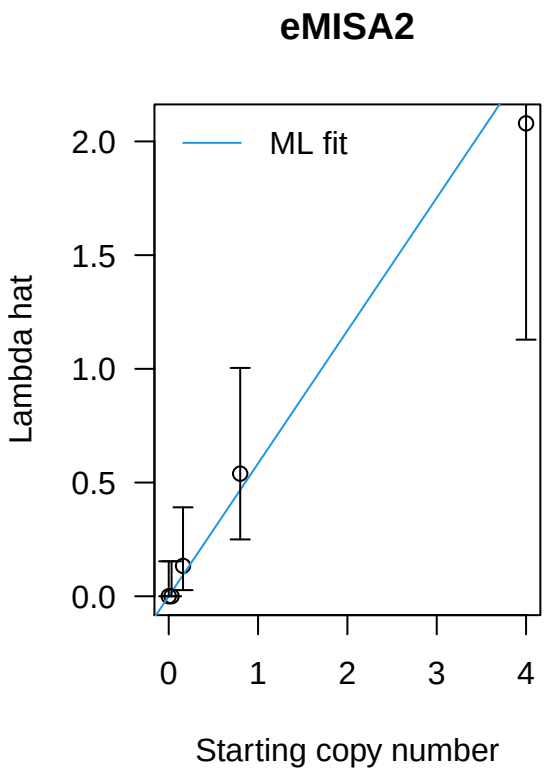


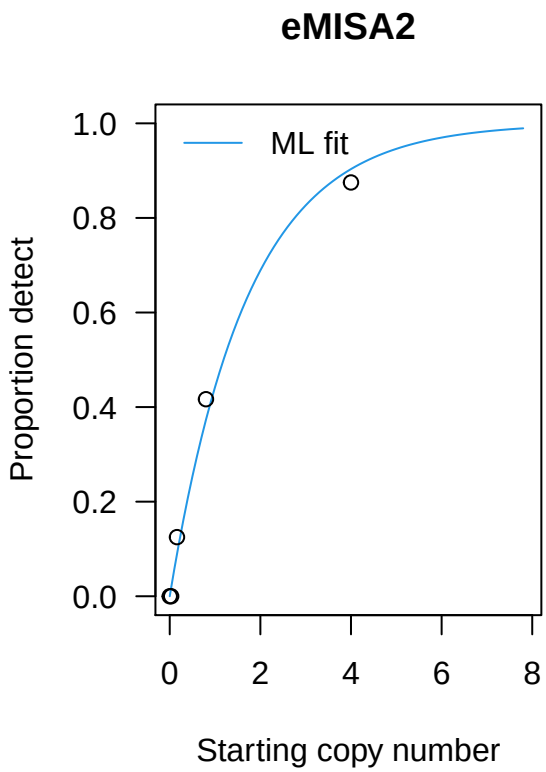

```
##
##
##
##
##  eLIPI1
## Convergence= 0
##      Estimate Std.Err Z value  Pr(>z)
## beta    0.836   0.166    5.03 4.8e-07 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 2.691805 , df=  4 , p-value= 0.6106493
```



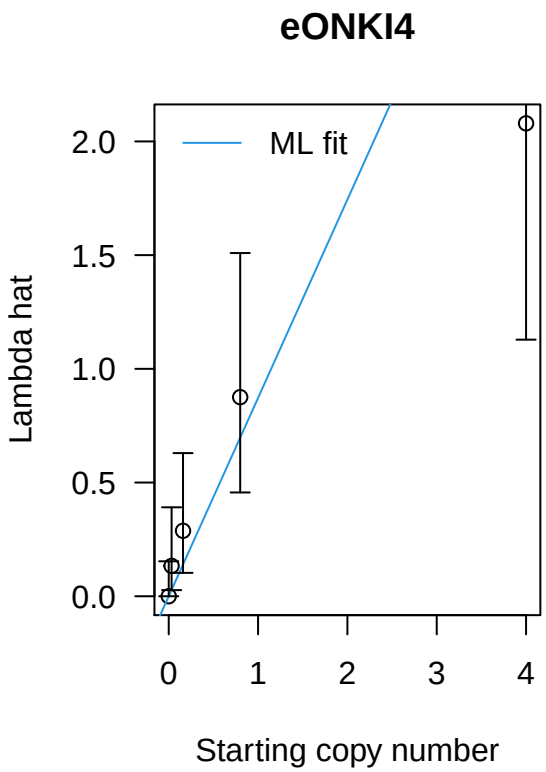


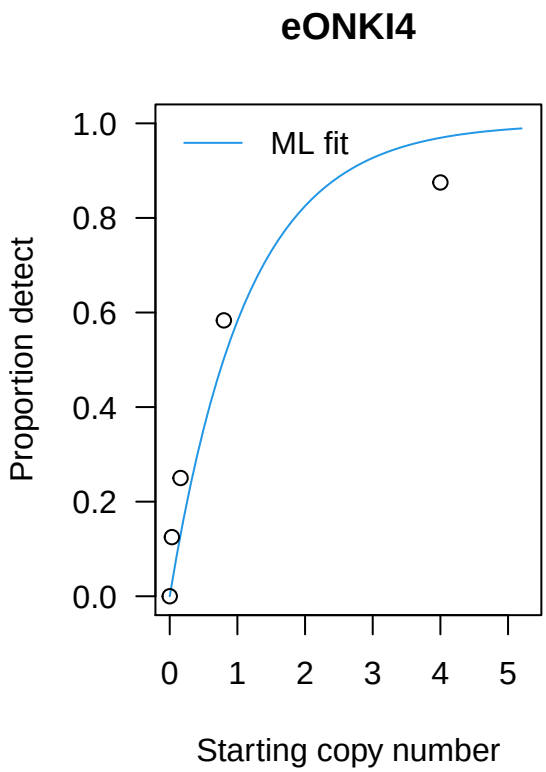

```
##
##
##
##
##  eMIDO1
## Convergence= 0
##      Estimate Std.Err Z value Pr(>z)
## beta    0.973   0.196    4.96  7e-07 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 4.310673 , df=  4 , p-value= 0.3655905
```



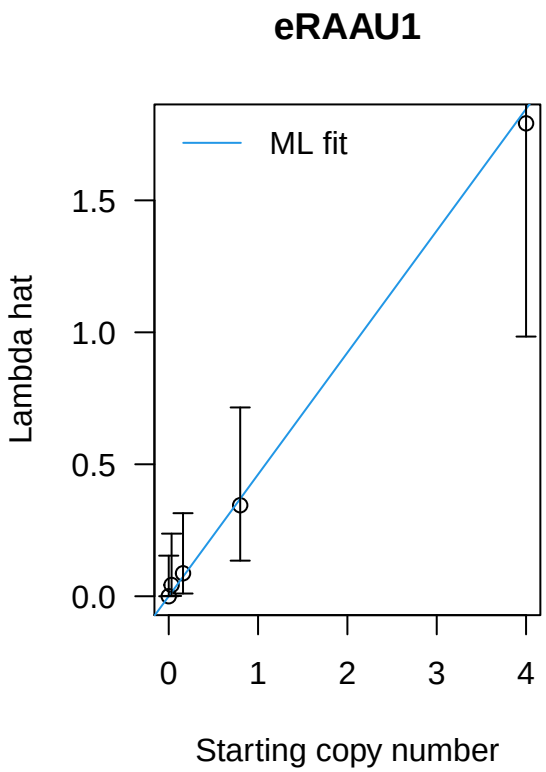


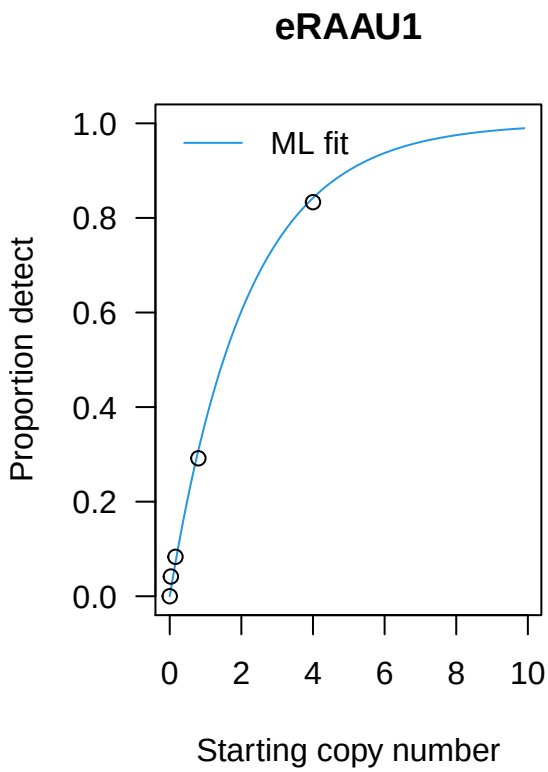

```
##
##
##
##
##  eMISA2
## Convergence= 0
##      Estimate Std.Err Z value  Pr(>z)
## beta    0.584   0.114    5.14 2.8e-07 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 1.631095 , df=  4 , p-value= 0.8031928
```



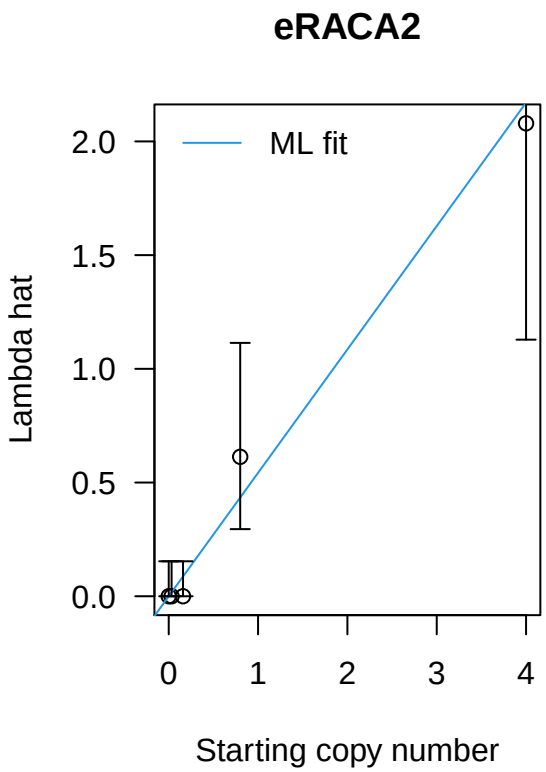


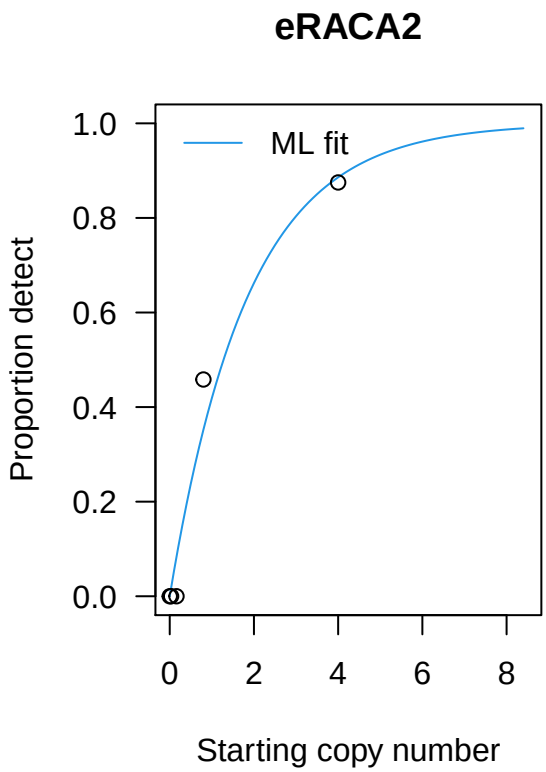

```
##
##
##
##
##  eONKI4
## Convergence= 0
##      Estimate Std.Err Z value  Pr(>z)
## beta    0.872   0.157    5.54 2.9e-08 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 11.91839 , df=  4 , p-value= 0.01796854
```



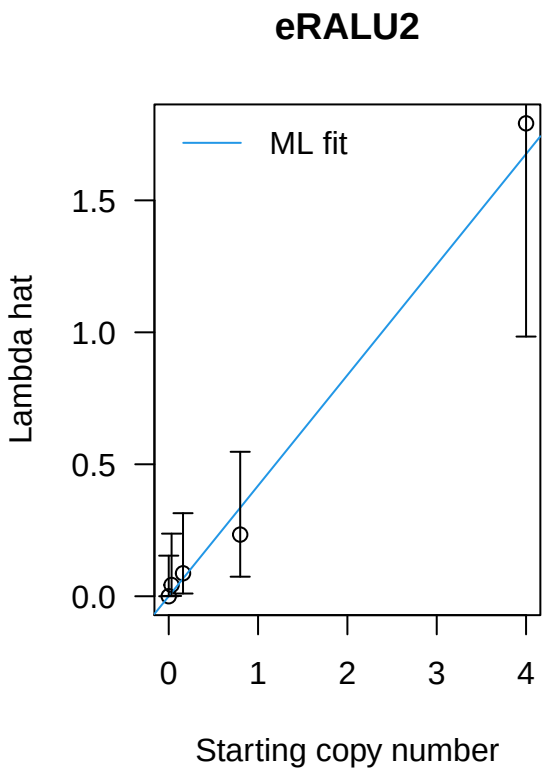


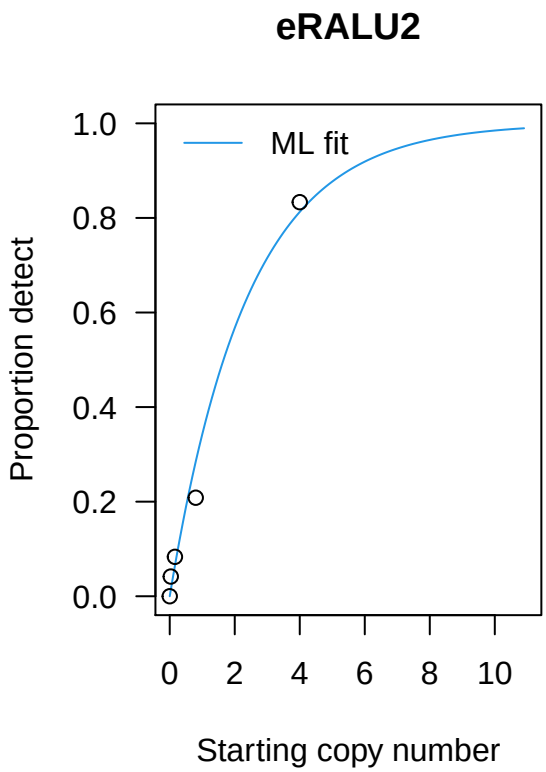

```
##
##
##
##
##  eRAAU1
## Convergence= 0
##      Estimate Std.Err Z value  Pr(>z)
## beta   0.4616  0.0921    5.01 5.5e-07 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 0.9086316 , df=  4 , p-value= 0.9233192
```



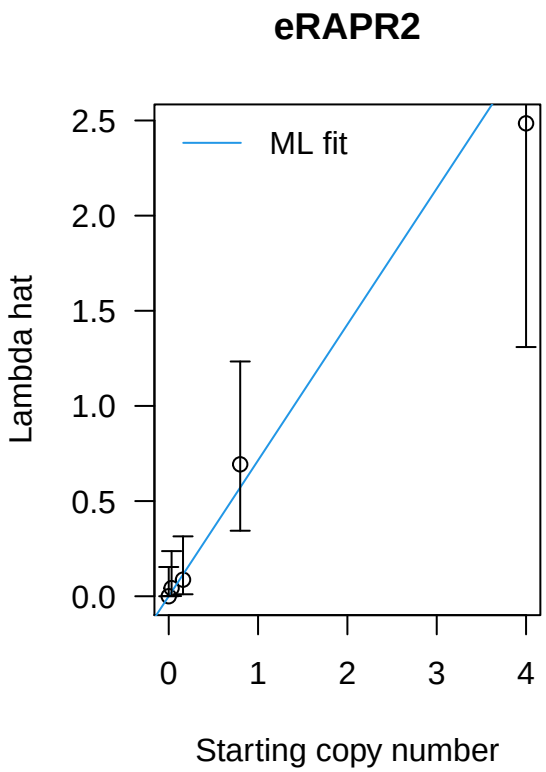


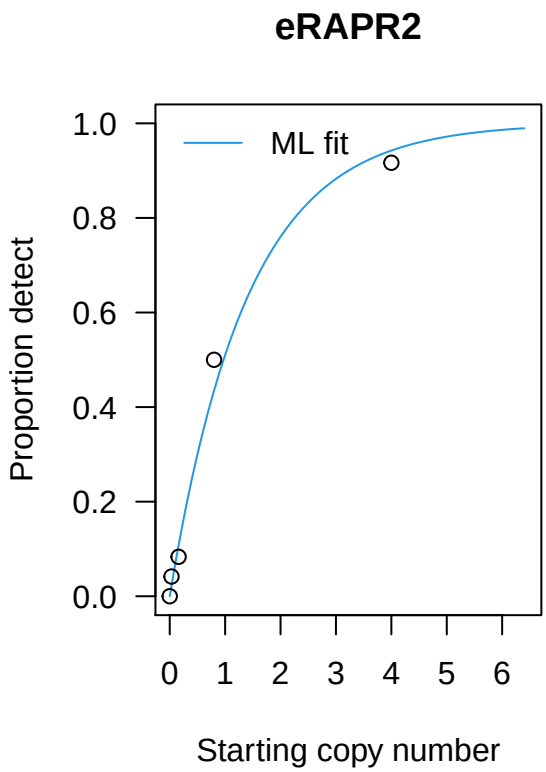

```
##
##
##
##
##  eRACA2
## Convergence= 0
##      Estimate Std.Err Z value  Pr(>z)
## beta    0.543   0.108    5.02 5.1e-07 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 6.171783 , df=  4 , p-value= 0.1866815
```



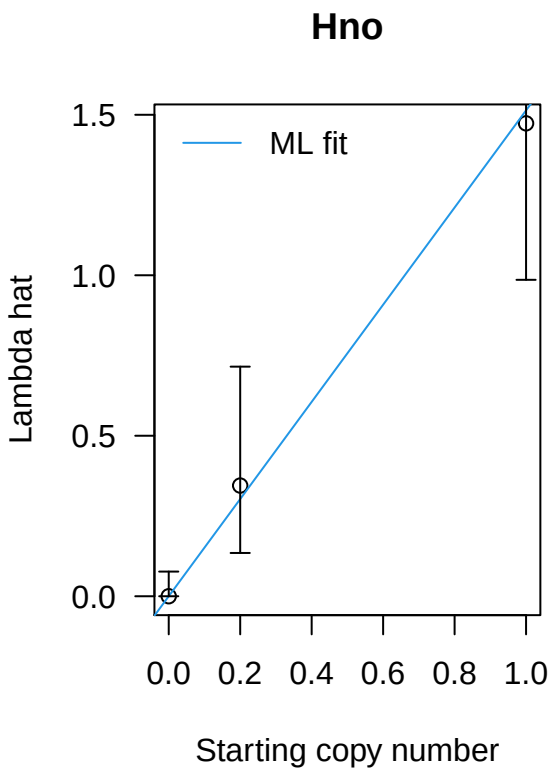


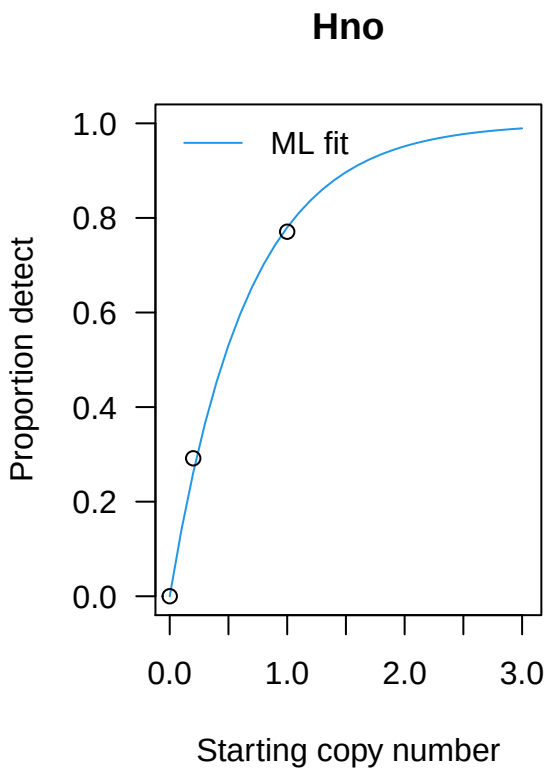

```
##
##
##
##
##  eRALU2
## Convergence= 0
##      Estimate Std.Err Z value Pr(>z)
## beta   0.4189  0.0858    4.89  1e-06 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 1.866509 , df=  4 , p-value= 0.7602944
```






```
##
##
##
##
##  eRAPR2
## Convergence= 0
##      Estimate Std.Err Z value  Pr(>z)
## beta    0.714   0.139    5.13 2.9e-07 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 1.148873 , df=  4 , p-value= 0.8864424
```



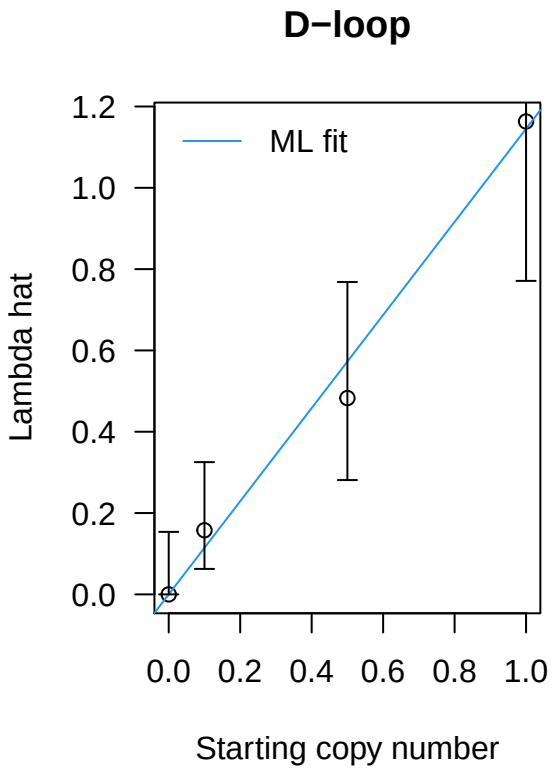


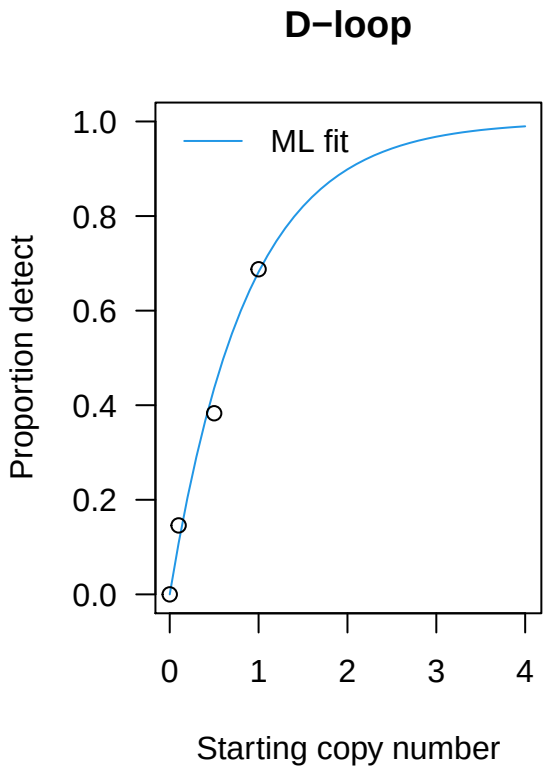

```
##
##
##
##
##  Hno
## Convergence= 0
##      Estimate Std.Err Z value  Pr(>z)
## beta    1.514   0.247    6.13 8.6e-10 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 0.1354573 , df=  2 , p-value= 0.934514
```

Estimate predicted Sq given number detects and technical replicates - no intercept (not shown)

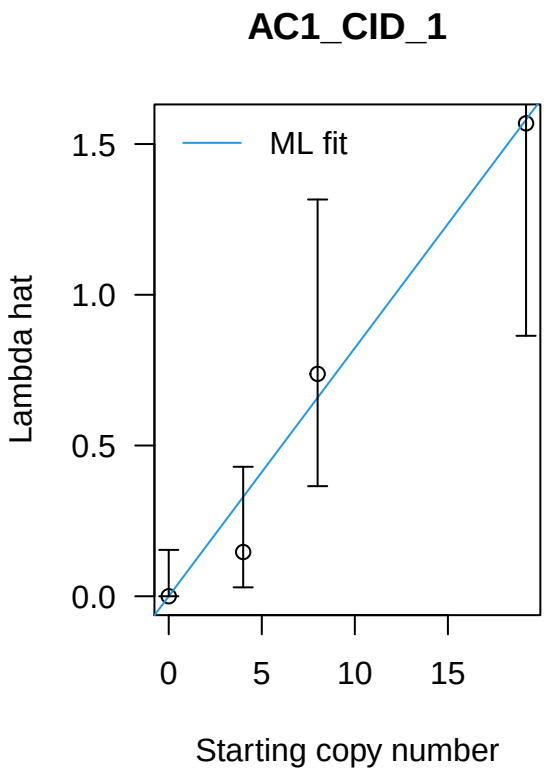
The estimated SQ is easily obtained for given new values of nn0=number of replicates, nd0=number detected and the estimated slope betaS as: Shat <- -(log((nn0 - nd0)/nn0)) / betaS[1] The standard errors are obtained from the Hessian matrix (or via the function CalibS0Or.ddLLik()) .

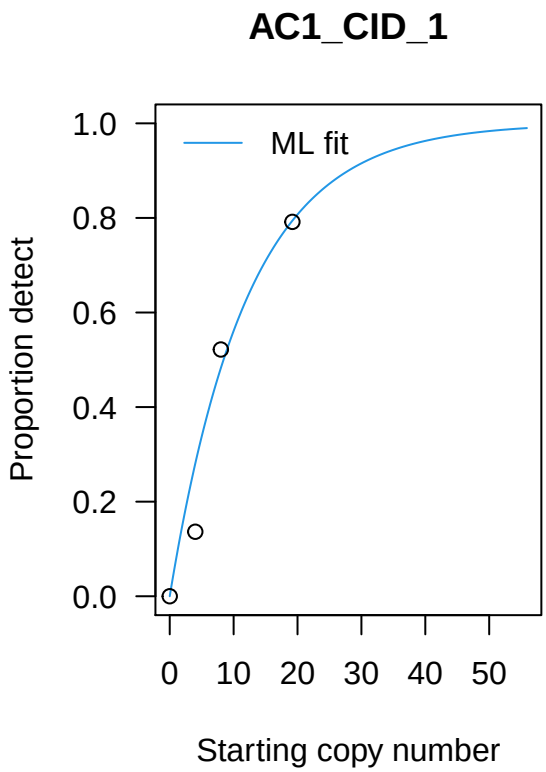
Estimate Poisson models - intercept model



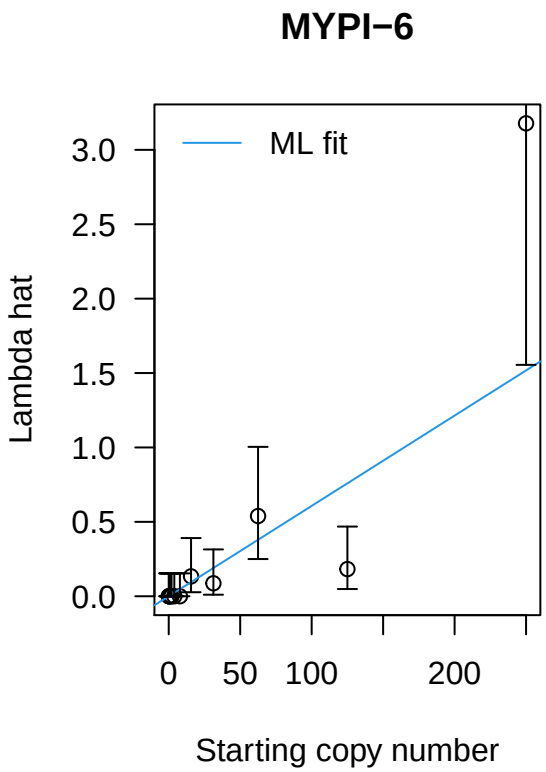


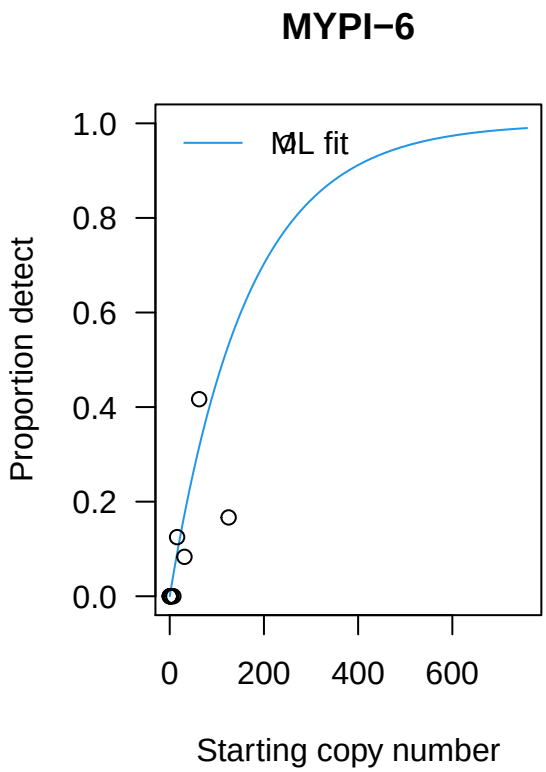

```
##
##
##
##
##  D-loop
## Convergence= 0
##       Estimate  Std.Err Z value Pr(>z)
## alpha 3.33e-09 5.31e-02    0.00      1
## beta  1.15e+00 2.04e-01    5.61  2e-08 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 1.192445 , df=  2 , p-value= 0.5508887
```



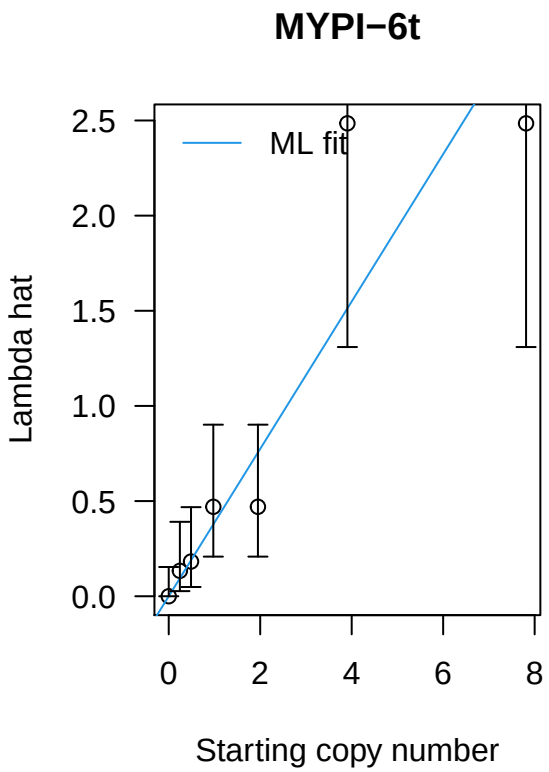


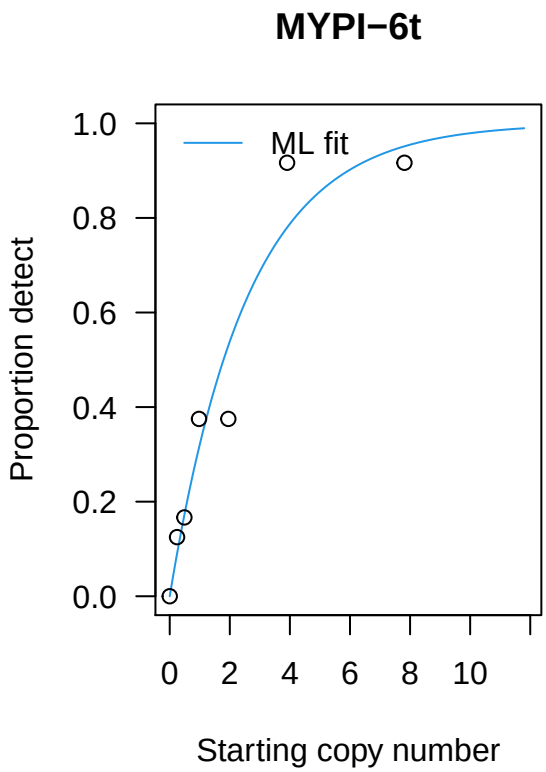

```
##
##
##
##
##  AC1_CID_1
## Convergence= 0
##       Estimate  Std.Err Z value Pr(>z)
## alpha 1.96e-08 2.49e-01    0.00 1.0000
## beta  8.23e-02 2.90e-02    2.84 0.0045 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 2.757685 , df=  2 , p-value= 0.2518699
##
## Too few values for  CID
```



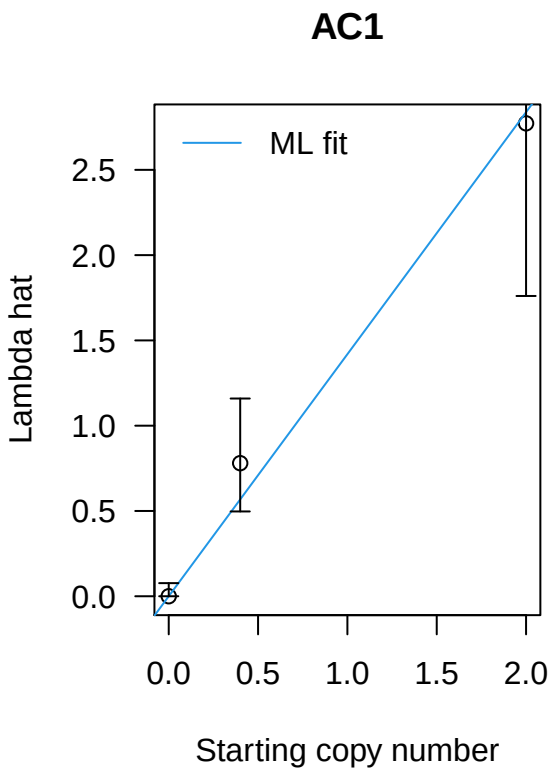


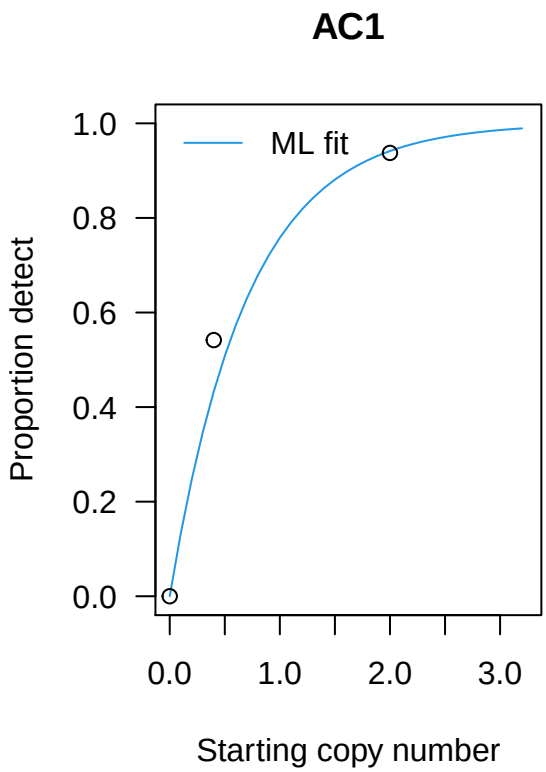

```
##
##
##
##
##  MYPI-6
## Convergence= 0
##       Estimate  Std.Err Z value  Pr(>z)
## alpha 2.78e-11 6.00e-02    0.00       1
## beta  6.07e-03 1.27e-03    4.78 1.7e-06 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 27.35988 , df=  10 , p-value= 0.002283695
```



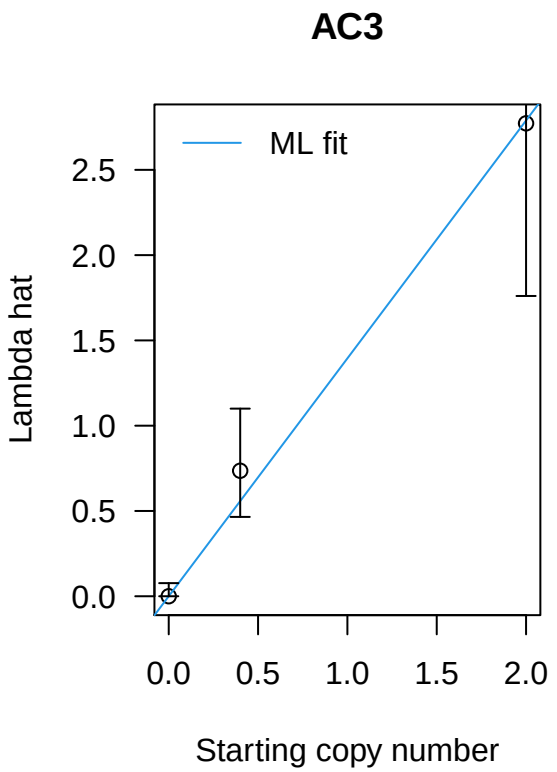


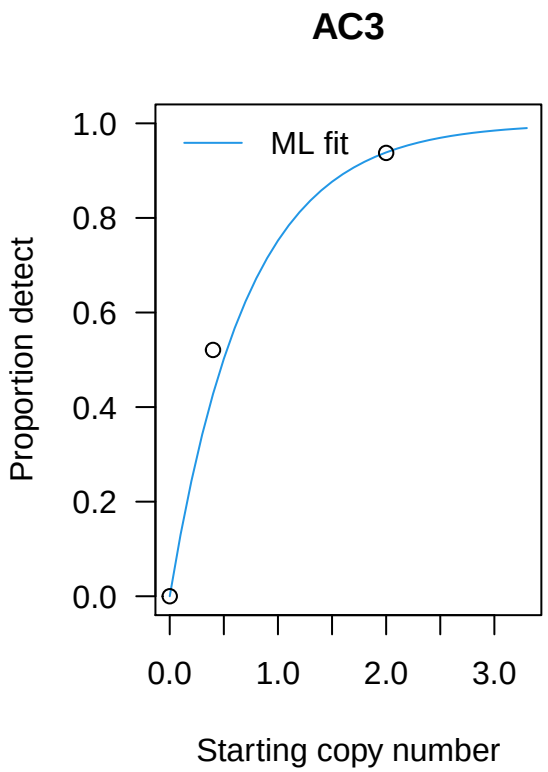

```
##
##
##
##
##  MYPI-6t
## Convergence= 0
##       Estimate  Std.Err Z value  Pr(>z)
## alpha 1.86e-08 5.46e-02    0.00       1
## beta  3.87e-01 6.69e-02    5.79 7.1e-09 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 6.803394 , df=  5 , p-value= 0.2356775
##
## Too few values for  NZMS
## Too few values for  SAFO
## Too few values for  Elod-2-NU
## Too few values for  MYPI
```



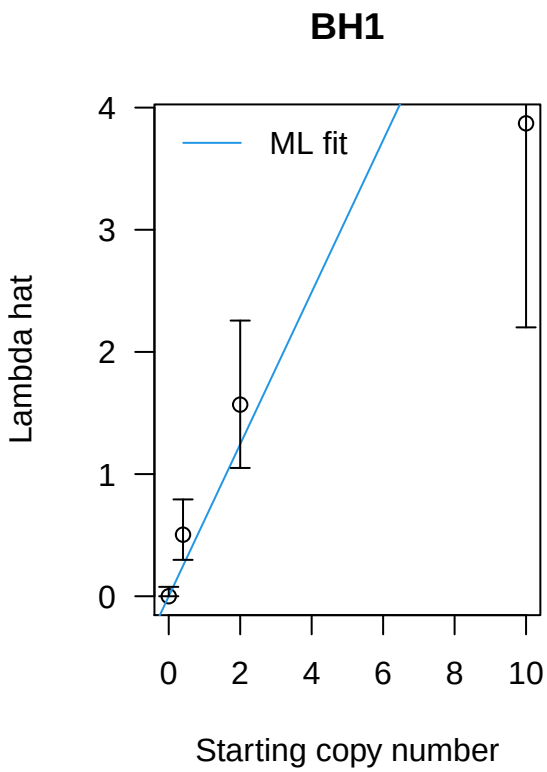


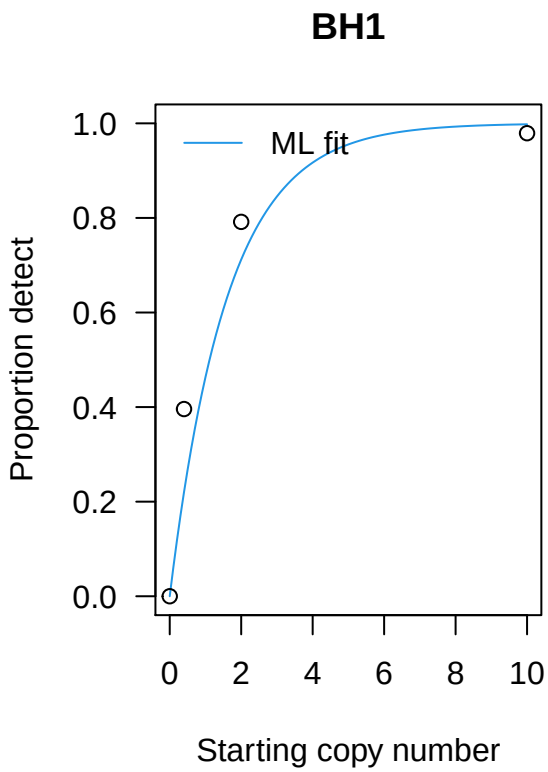

```
##
##
##
##
##  AC1
## Convergence= 0
##       Estimate  Std.Err Z value  Pr(>z)
## alpha 5.57e-10 2.02e-01    0.00 1.00000
## beta  1.42e+00 3.69e-01    3.84 0.00012 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 2.288843 , df=  1 , p-value= 0.1303071
```



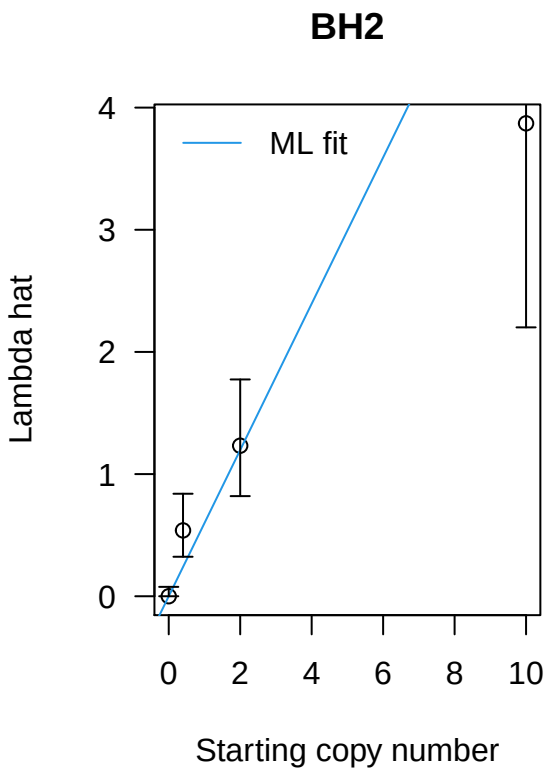


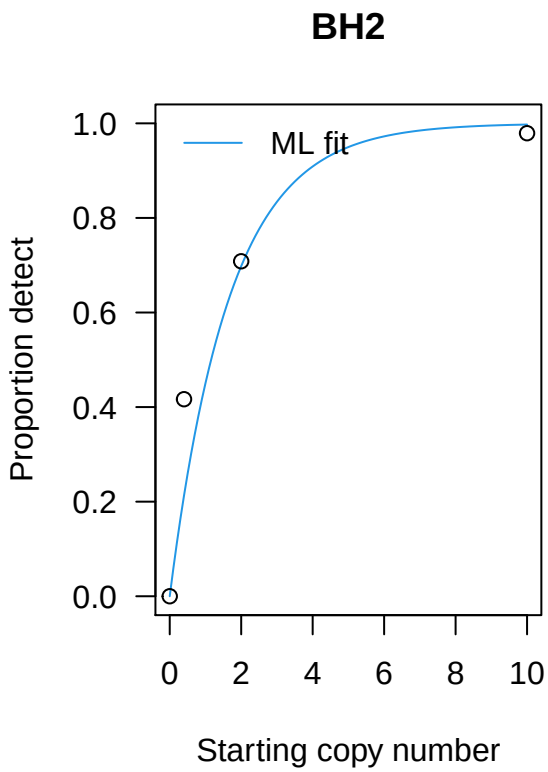

```
##
##
##
##
##  AC3
## Convergence= 0
##       Estimate  Std.Err Z value  Pr(>z)
## alpha 1.83e-08 2.00e-01    0.00 1.00000
## beta  1.39e+00 3.60e-01    3.88 0.00011 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 1.685805 , df=  1 , p-value= 0.1941549
```



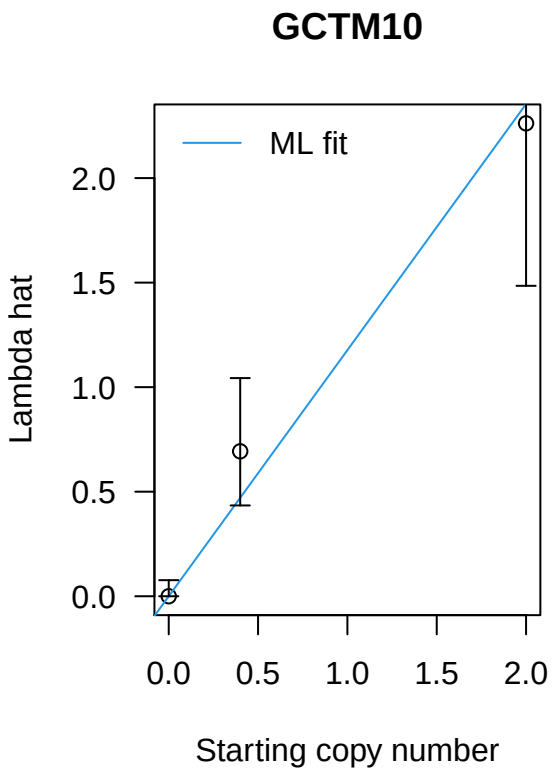


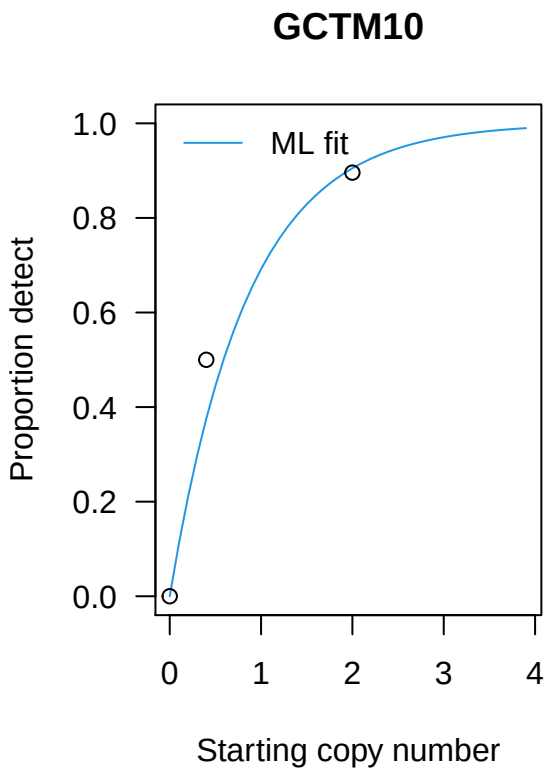

```
##
##
##
##
##  BH1
## Convergence= 0
##       Estimate  Std.Err Z value  Pr(>z)
## alpha 9.77e-09 8.58e-02    0.00       1
## beta  6.22e-01 1.29e-01    4.82 1.4e-06 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 11.96976 , df=  2 , p-value= 0.002516509
```



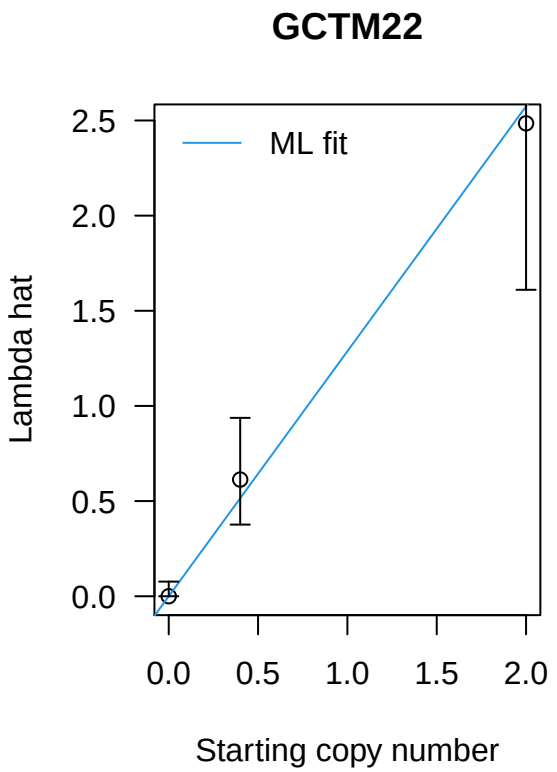


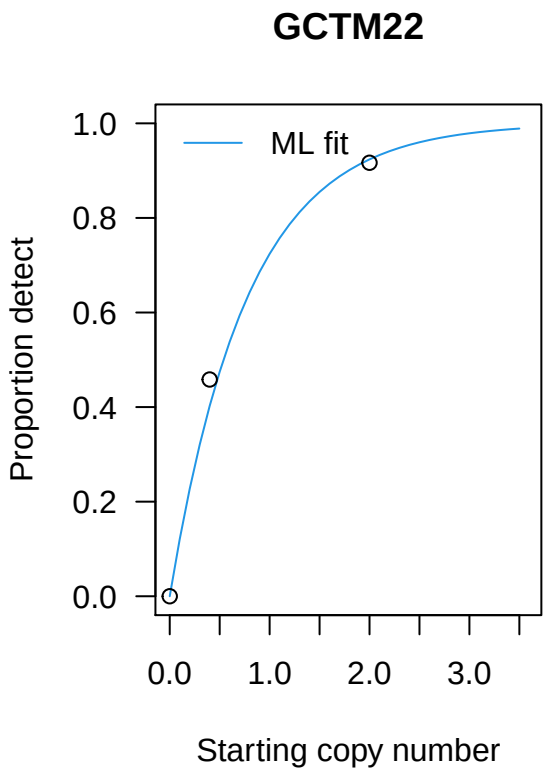

```
##
##
##
##
##  BH2
## Convergence= 0
##       Estimate  Std.Err Z value  Pr(>z)
## alpha 7.41e-09 8.18e-02     0.0       1
## beta  5.99e-01 1.27e-01     4.7 2.6e-06 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 12.58791 , df=  2 , p-value= 0.001847435
```



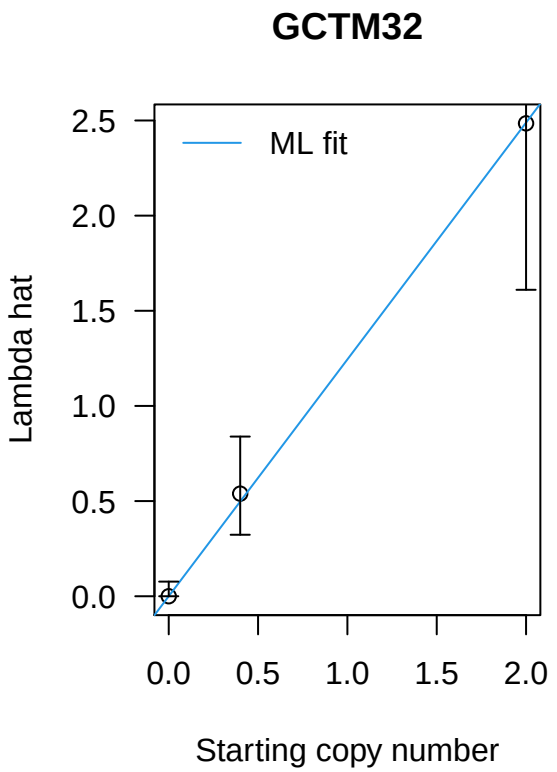


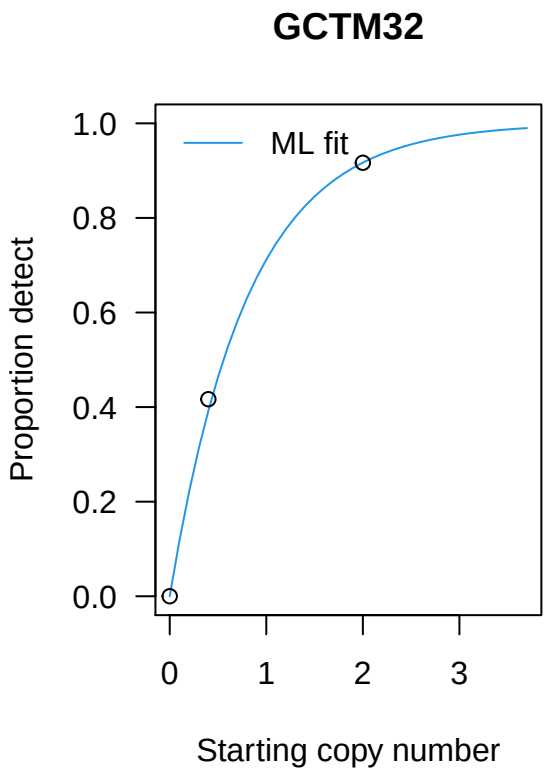

```
##
##
##
##
##  GCTM10
## Convergence= 0
##       Estimate  Std.Err Z value Pr(>z)
## alpha 3.46e-09 1.65e-01    0.00      1
## beta  1.18e+00 2.86e-01    4.11  4e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 3.116952 , df=  1 , p-value= 0.07748159
```



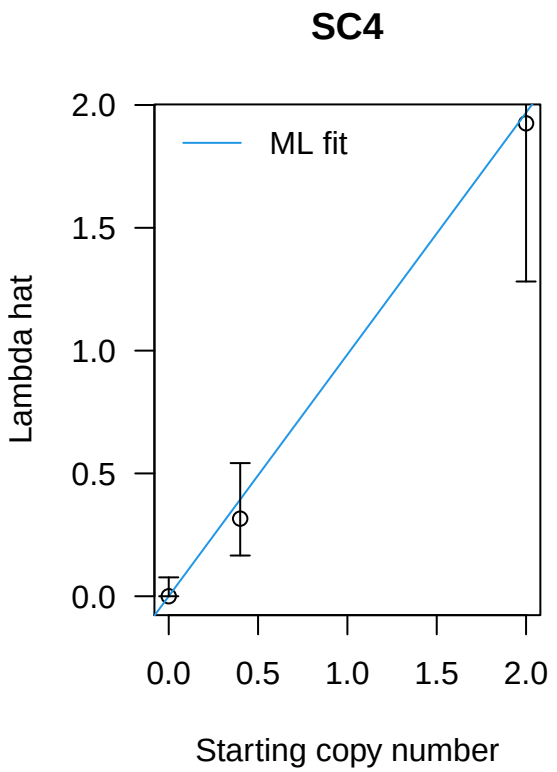


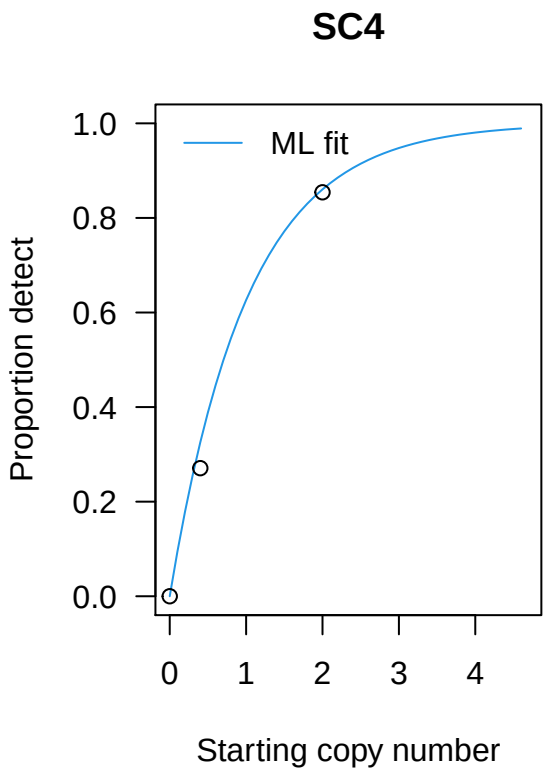

```
##
##
##
##
##  GCTM22
## Convergence= 0
##       Estimate  Std.Err Z value  Pr(>z)
## alpha 1.38e-08 1.88e-01    0.00       1
## beta  1.29e+00 3.23e-01    3.99 6.7e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 0.6489803 , df=  1 , p-value= 0.4204775
```



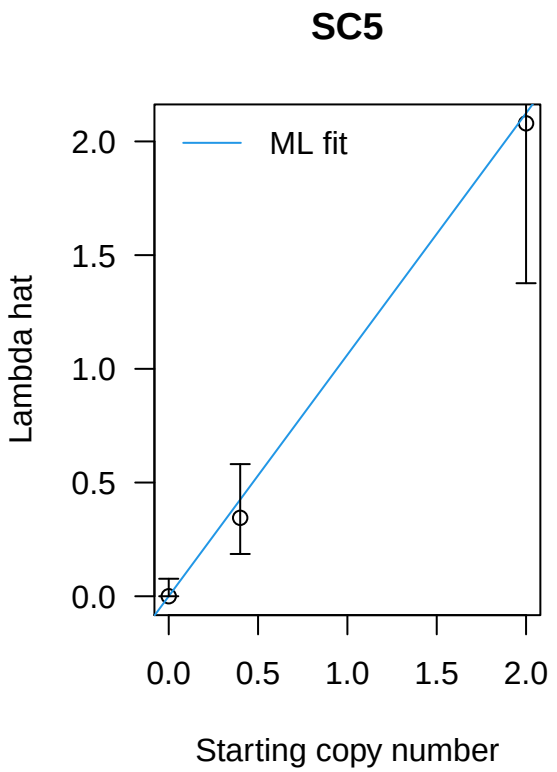


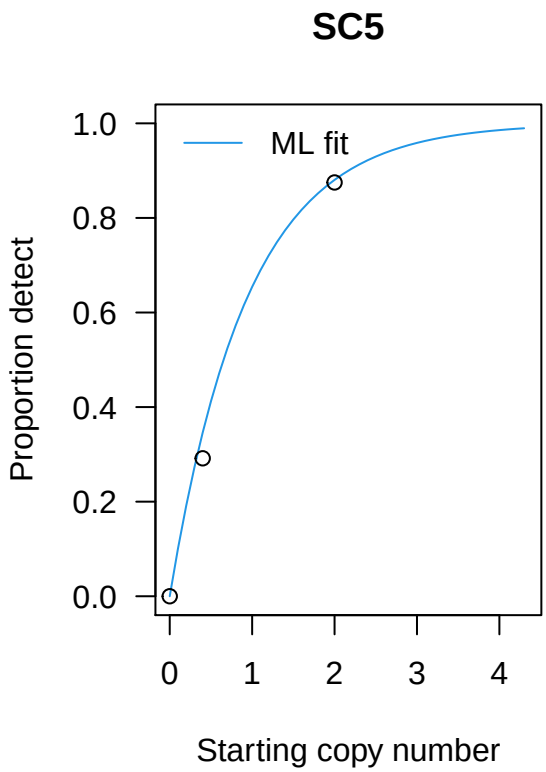

```
##
##
##
##
##  GCTM32
## Convergence= 0
##       Estimate  Std.Err Z value  Pr(>z)
## alpha 1.91e-09 1.85e-01    0.00       1
## beta  1.24e+00 3.08e-01    4.04 5.3e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 0.1206682 , df=  1 , p-value= 0.7283109
##
## Too few values for  Goby
```



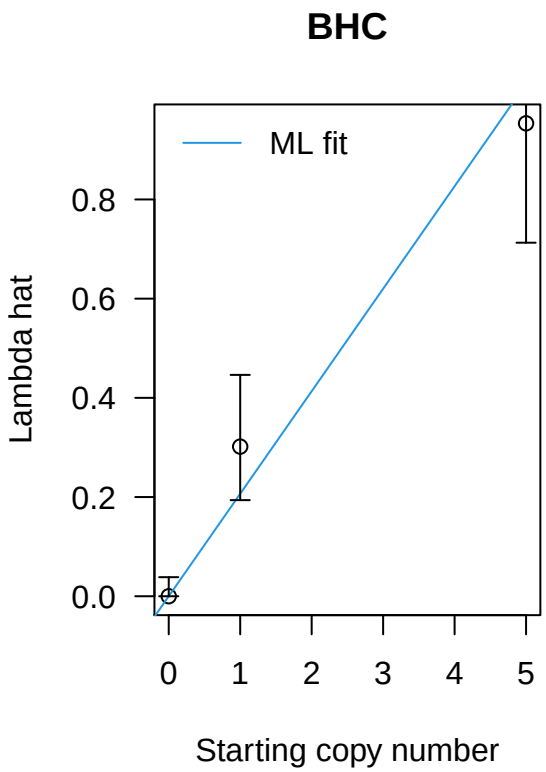


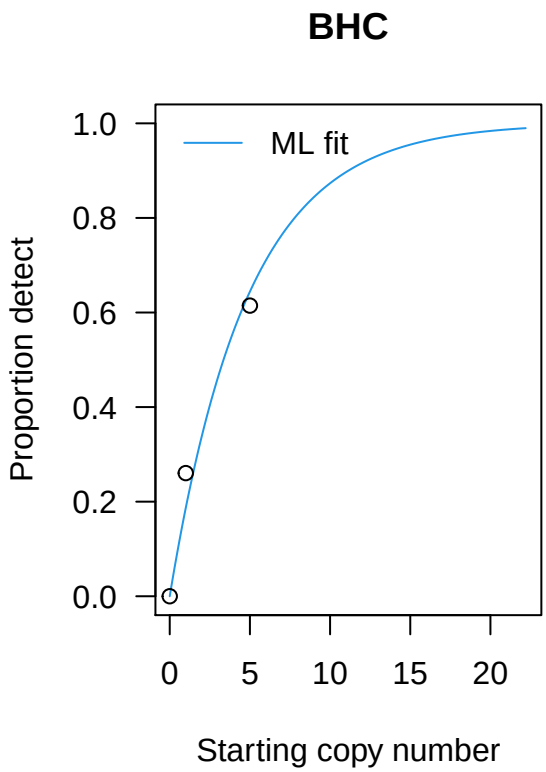

```
##
##
##
##
##  SC4
## Convergence= 0
##       Estimate  Std.Err Z value  Pr(>z)
## alpha 2.54e-10 1.64e-01    0.00       1
## beta  9.84e-01 2.35e-01    4.19 2.8e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 0.6901617 , df=  1 , p-value= 0.4061094
```



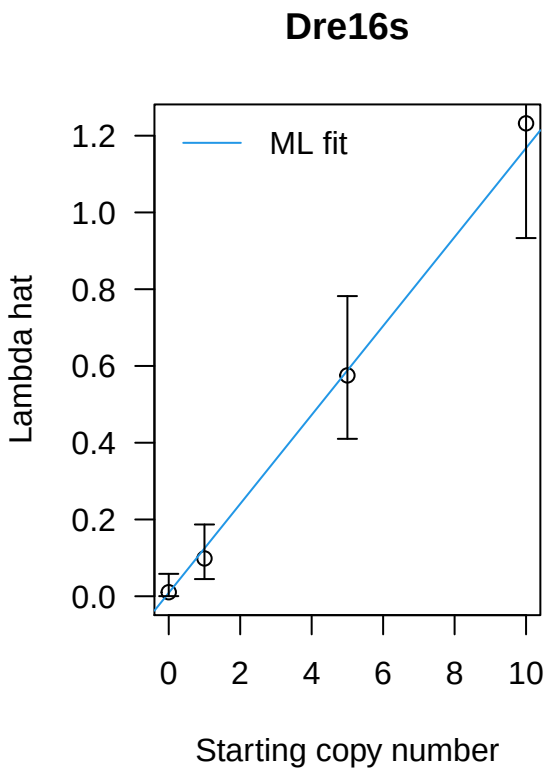


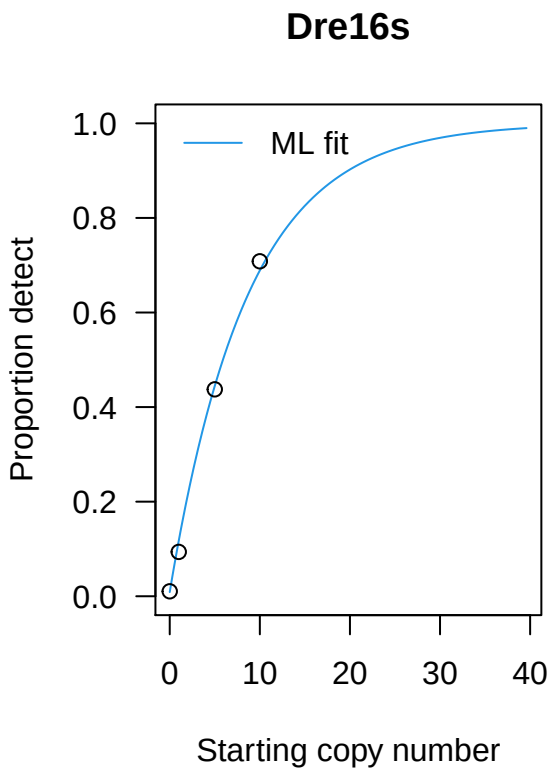

```
##
##
##
##
##  SC5
## Convergence= 0
##       Estimate  Std.Err Z value  Pr(>z)
## alpha 1.46e-09 1.73e-01    0.00       1
## beta  1.06e+00 2.56e-01    4.15 3.3e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 0.6602861 , df=  1 , p-value= 0.416459
```



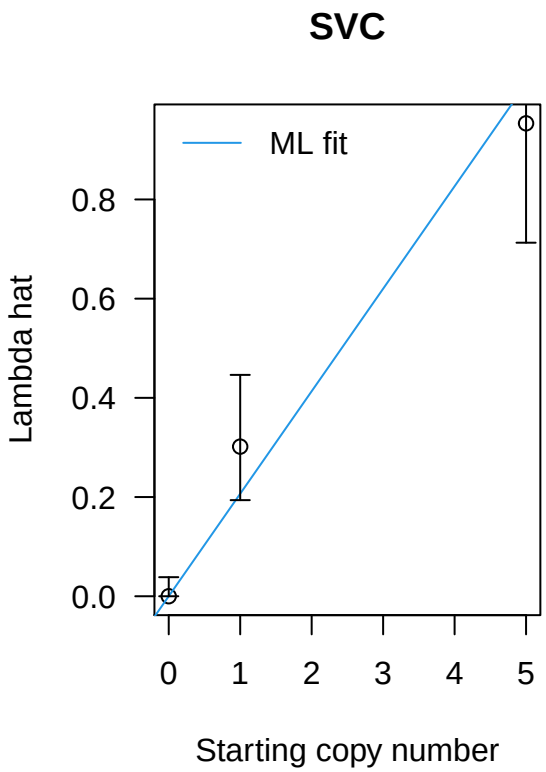


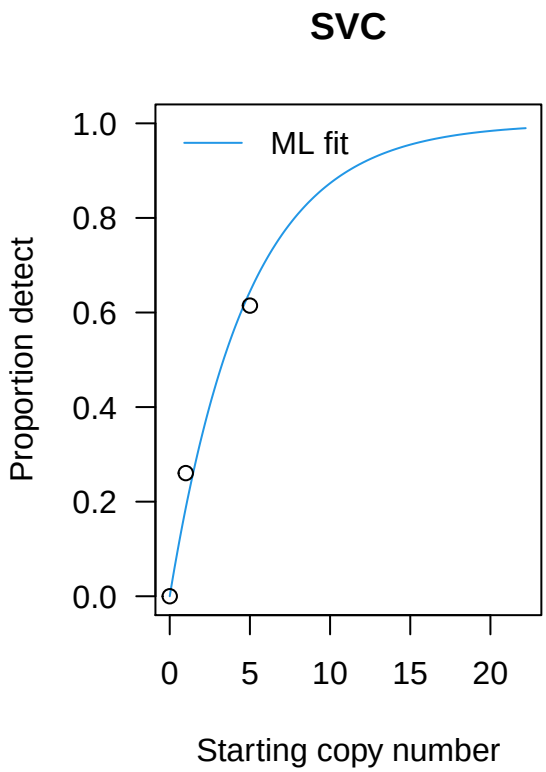

```
##
##
##
##
##  BHC
## Convergence= 0
##       Estimate  Std.Err Z value  Pr(>z)
## alpha 6.31e-09 6.26e-02    0.00       1
## beta  2.07e-01 3.66e-02    5.64 1.7e-08 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 3.509393 , df=  1 , p-value= 0.06102183
```



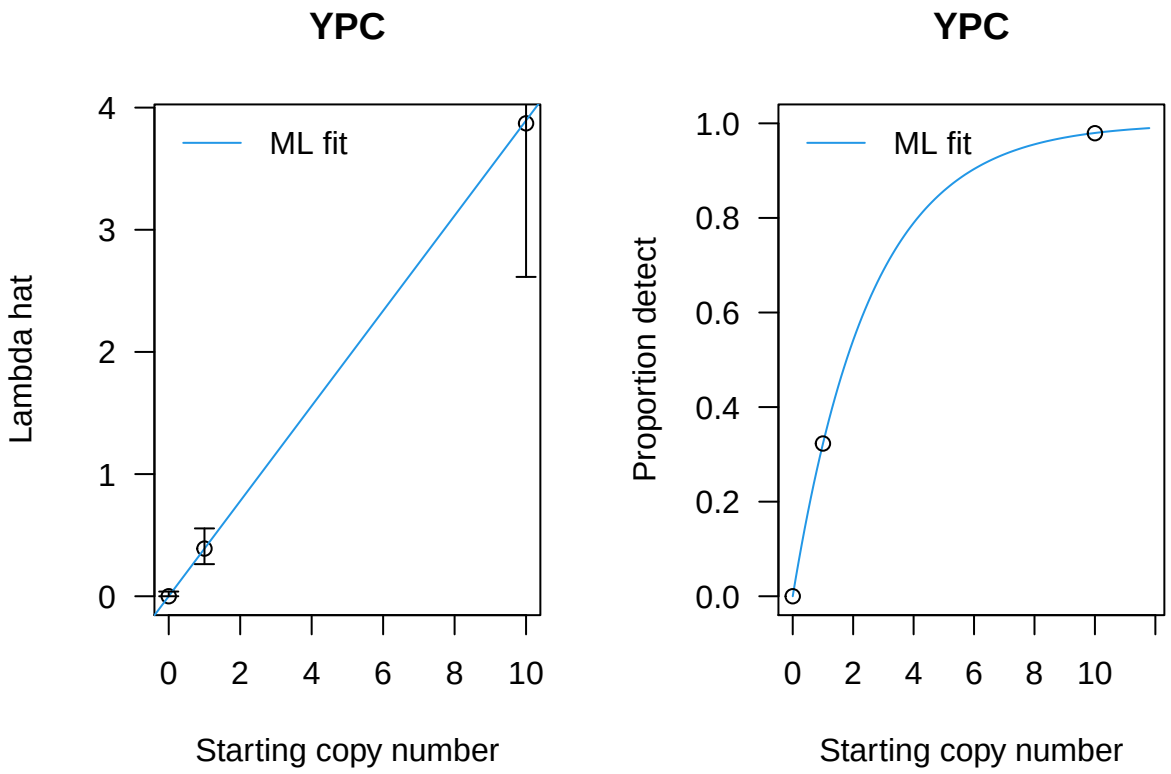



```
##
##
##
##
##  Dre16s
## Convergence= 0
##       Estimate Std.Err Z value Pr(>z)
## alpha  0.00880 0.00861    1.02   0.31
## beta   0.11590 0.01133   10.23 <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 0.7640605 , df=  2 , p-value= 0.6824744
##
## Too few values for  SS
```

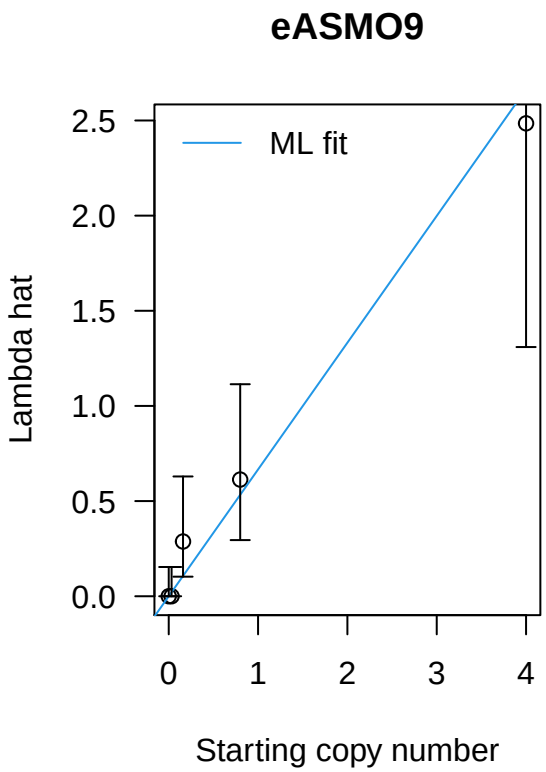


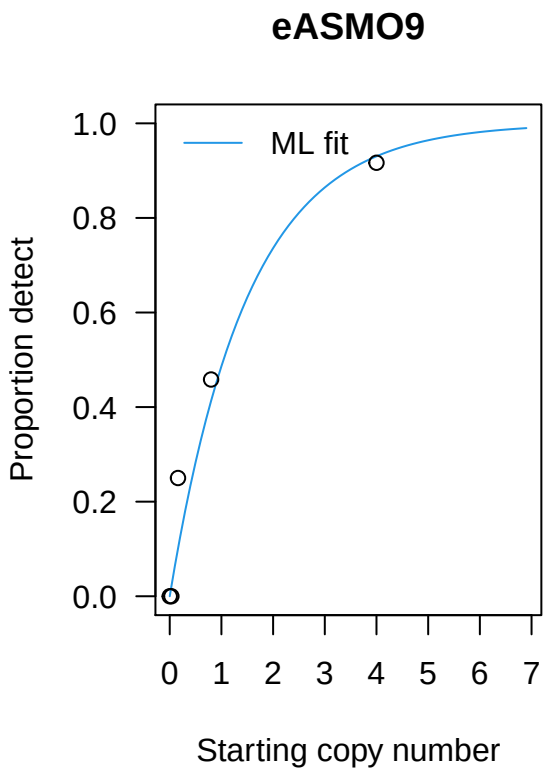



```
##
##
##
##
##  SVC
## Convergence= 0
##       Estimate  Std.Err Z value  Pr(>z)
## alpha 6.31e-09 6.26e-02    0.00       1
## beta  2.07e-01 3.66e-02    5.64 1.7e-08 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 3.509393 , df=  1 , p-value= 0.06102183
```

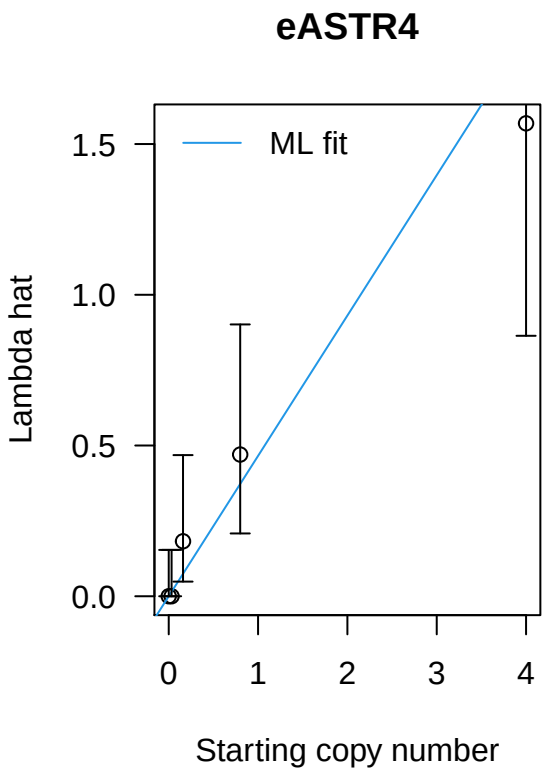


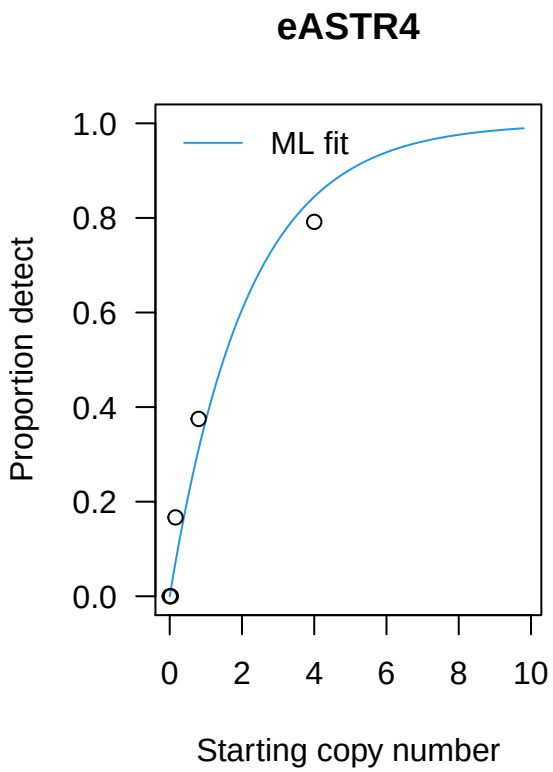

```
##
##
##
##
##  YPC
## Convergence= 0
##       Estimate  Std.Err Z value  Pr(>z)
## alpha 4.99e-09 1.11e-01    0.00       1
## beta  3.89e-01 7.91e-02    4.92 8.5e-07 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 0.001161691 , df=  1 , p-value= 0.9728105
```



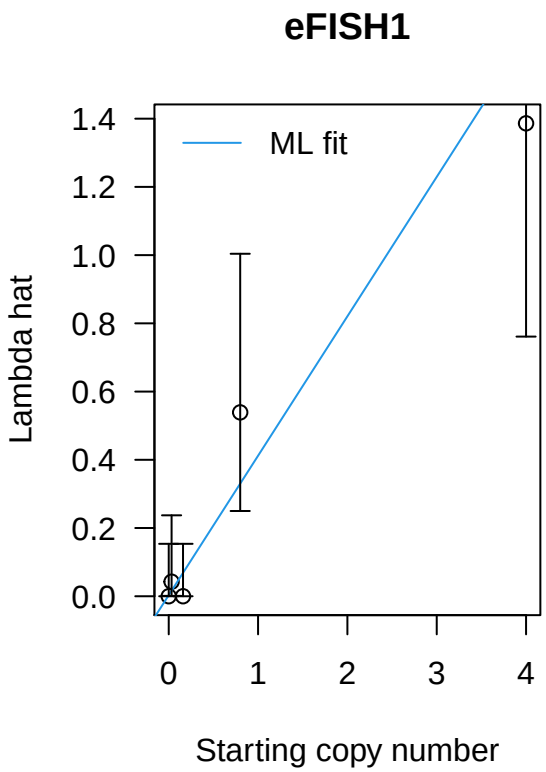


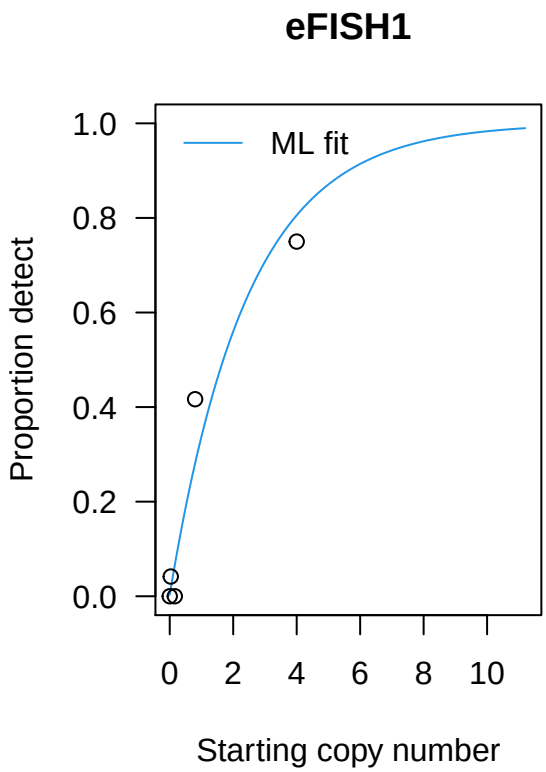

```
##
##
##
##
##  eASMO9
## Convergence= 0
##       Estimate  Std.Err Z value  Pr(>z)
## alpha 2.90e-09 5.40e-02    0.00       1
## beta  6.66e-01 1.58e-01    4.21 2.6e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 5.634289 , df=  3 , p-value= 0.1308237
```



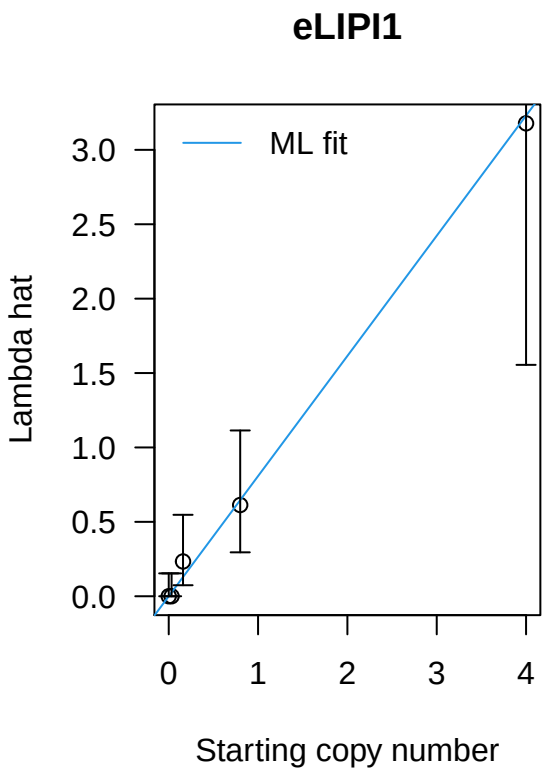


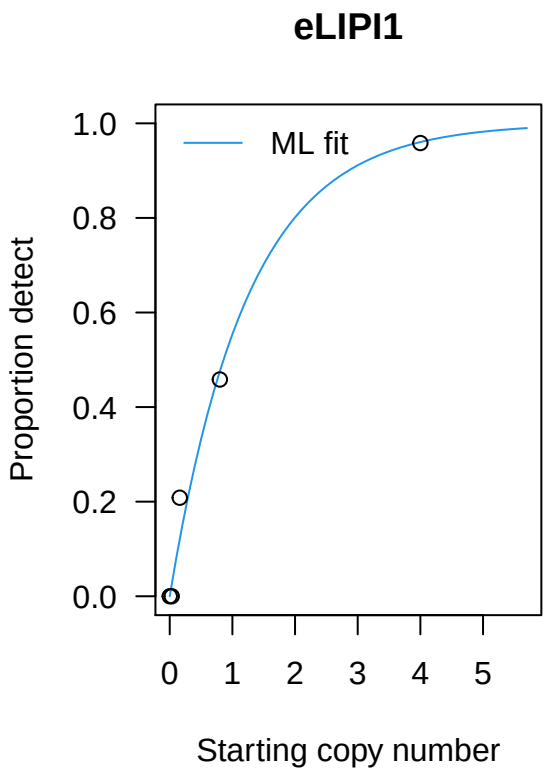

```
##
##
##
##
##  eASTR4
## Convergence= 0
##       Estimate  Std.Err Z value  Pr(>z)
## alpha 2.64e-09 4.38e-02    0.00       1
## beta  4.66e-01 1.10e-01    4.25 2.2e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 4.058386 , df=  3 , p-value= 0.2552281
```



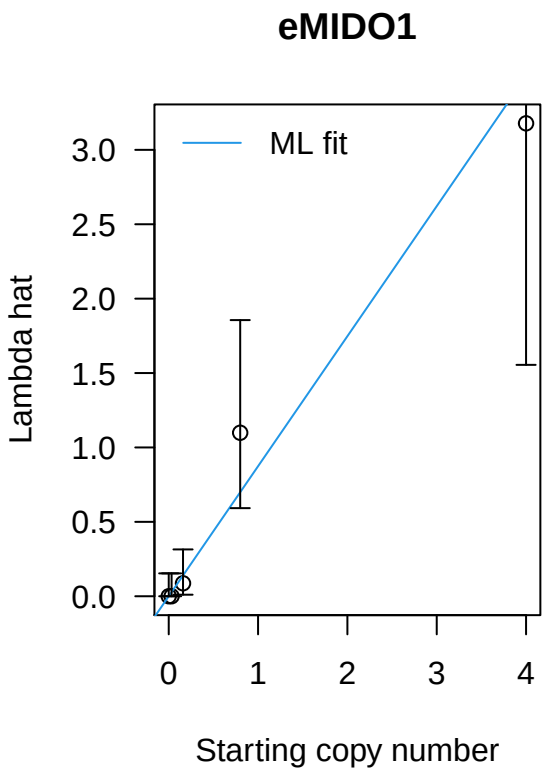


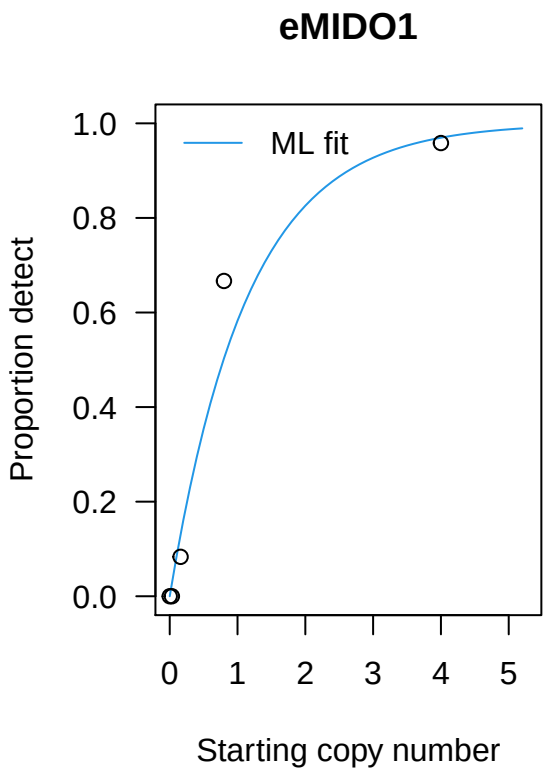

```
##
##
##
##
##  eFISH1
## Convergence= 0
##       Estimate Std.Err Z value  Pr(>z)
## alpha  0.00316 0.01669    0.19    0.85
## beta   0.40873 0.08563    4.77 1.8e-06 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 6.595206 , df=  3 , p-value= 0.08598249
```



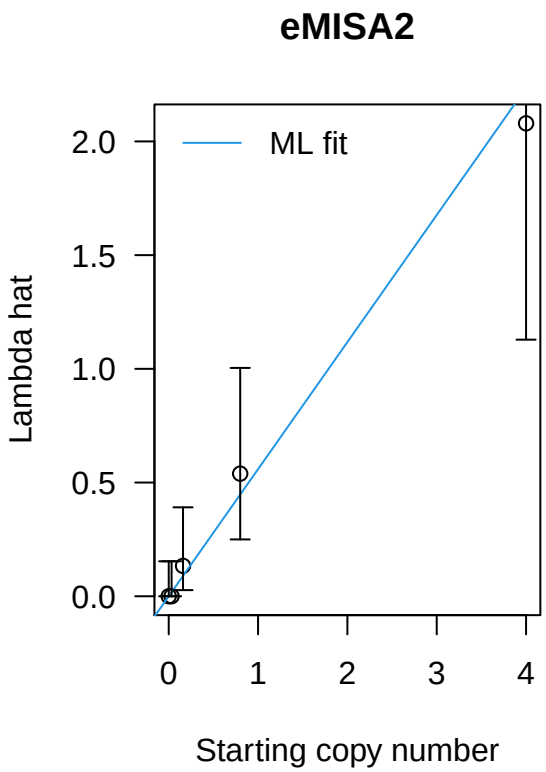


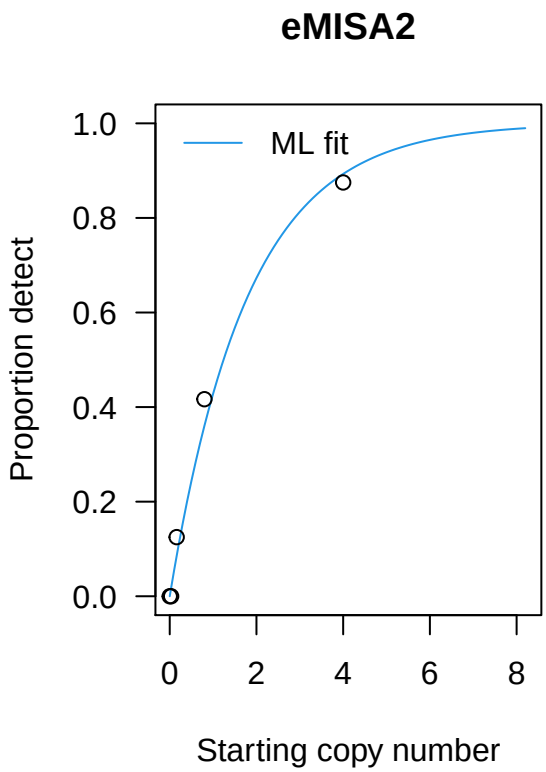

```
##
##
##
##
##  eLIPI1
## Convergence= 0
##       Estimate  Std.Err Z value  Pr(>z)
## alpha 1.03e-09 7.17e-02    0.00       1
## beta  8.08e-01 2.05e-01    3.93 8.4e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 2.721685 , df=  3 , p-value= 0.4365547
```



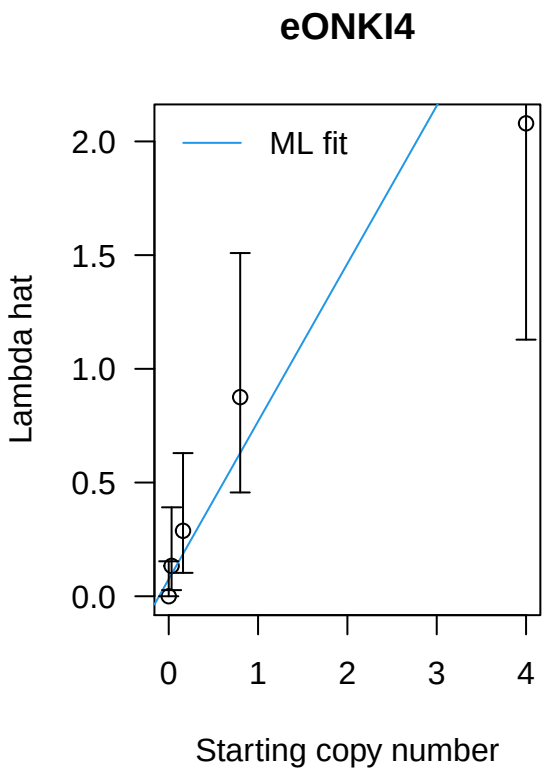


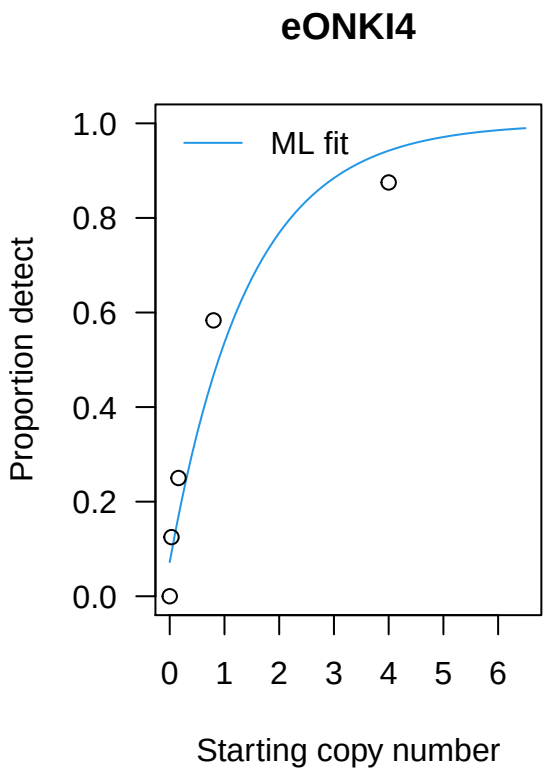

```
##
##
##
##
##  eMIDO1
## Convergence= 0
##       Estimate  Std.Err Z value  Pr(>z)
## alpha 5.97e-10 1.14e-01     0.0       1
## beta  8.74e-01 2.24e-01     3.9 9.7e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 4.59117 , df=  3 , p-value= 0.2043009
```



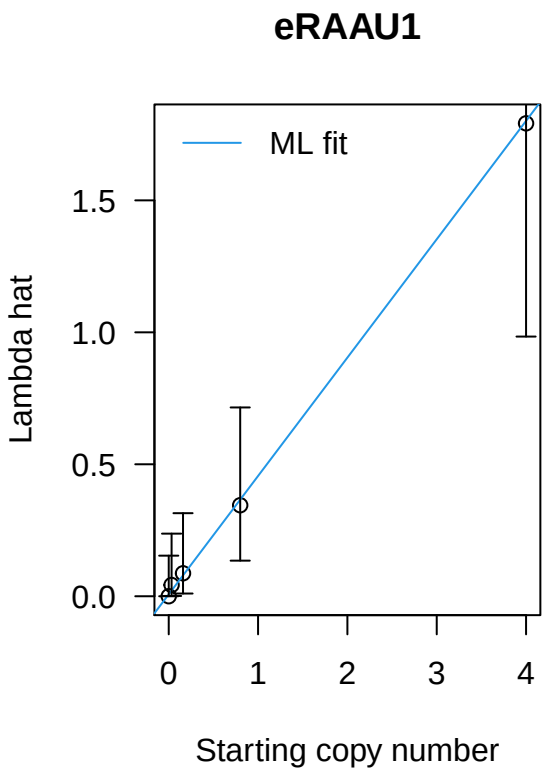


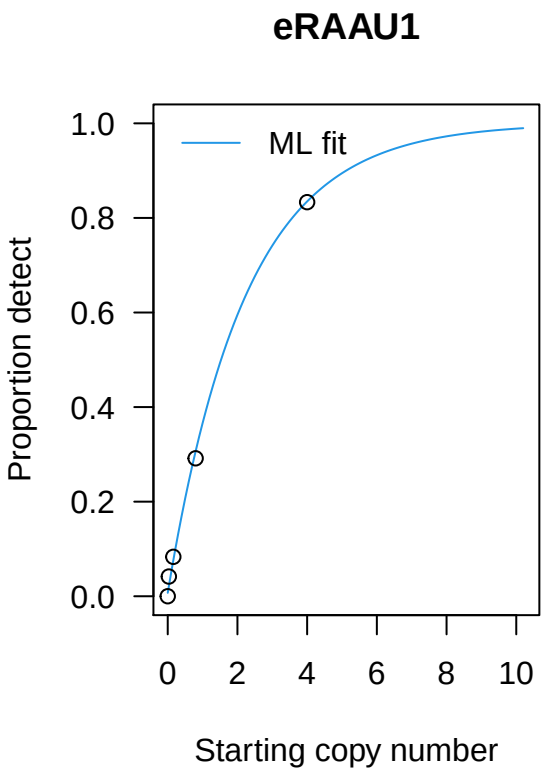

```
##
##
##
##
##  eMISA2
## Convergence= 0
##       Estimate  Std.Err Z value  Pr(>z)
## alpha 5.64e-10 5.92e-02    0.00       1
## beta  5.59e-01 1.32e-01    4.24 2.3e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 1.682044 , df=  3 , p-value= 0.6409331
```



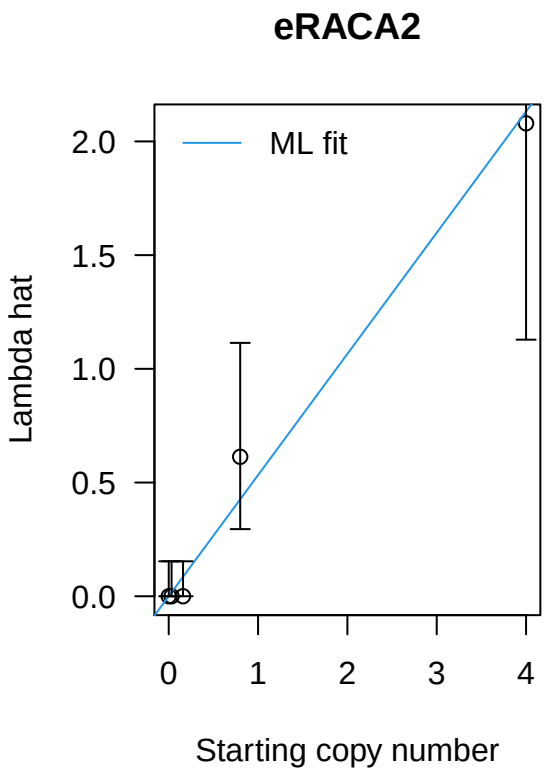


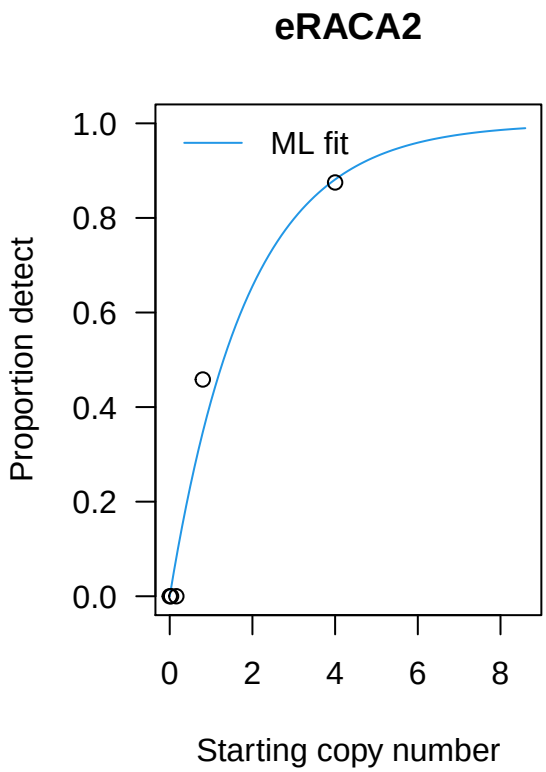

```
##
##
##
##
##  eONKI4
## Convergence= 0
##       Estimate Std.Err Z value  Pr(>z)
## alpha   0.0749  0.0486    1.54    0.12
## beta    0.6939  0.1601    4.33 1.5e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 7.671079 , df=  3 , p-value= 0.05332185
```



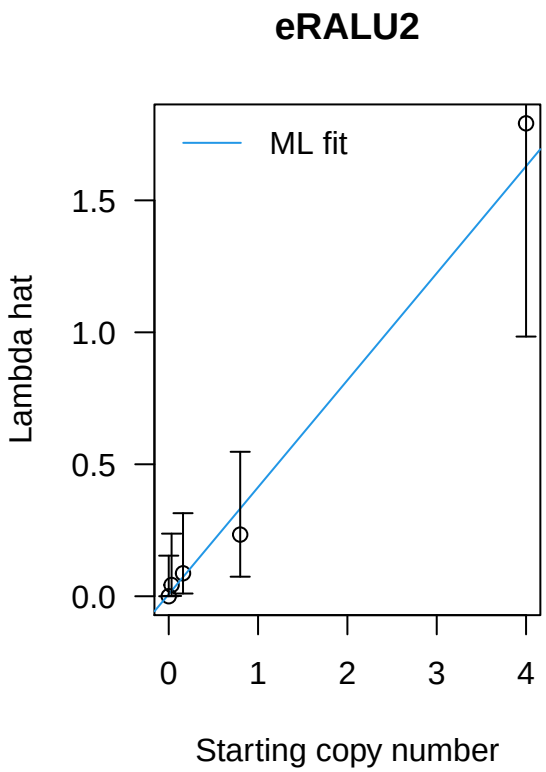


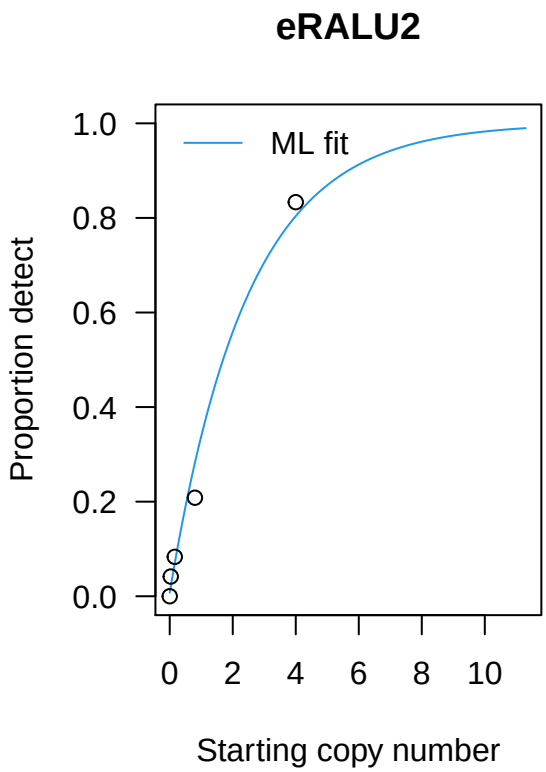

```
##
##
##
##
##  eRAAU1
## Convergence= 0
##       Estimate Std.Err Z value  Pr(>z)
## alpha  0.00722 0.02104    0.34    0.73
## beta   0.44846 0.09693    4.63 3.7e-06 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 0.7629684 , df=  3 , p-value= 0.8583024
```



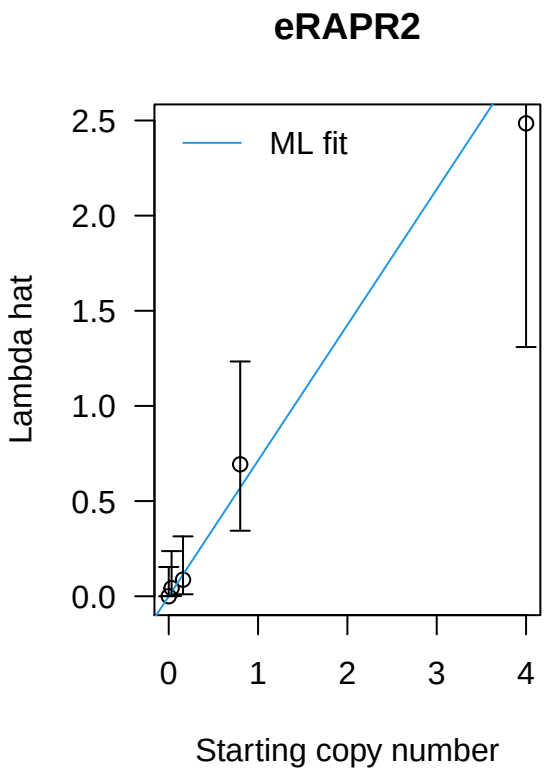


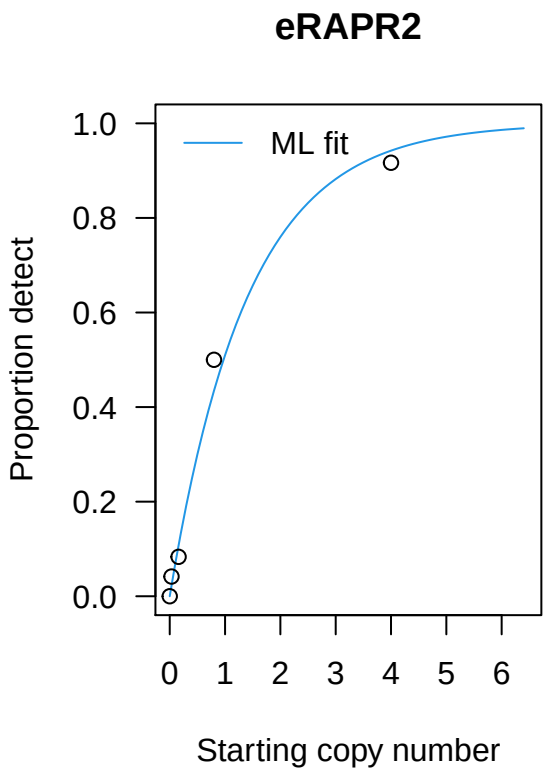

```
##
##
##
##
##  eRACA2
## Convergence= 0
##       Estimate  Std.Err Z value Pr(>z)
## alpha 5.92e-09 2.14e-01    0.00 1.0000
## beta  5.33e-01 1.79e-01    2.98 0.0029 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 6.180137 , df=  3 , p-value= 0.1031676
```



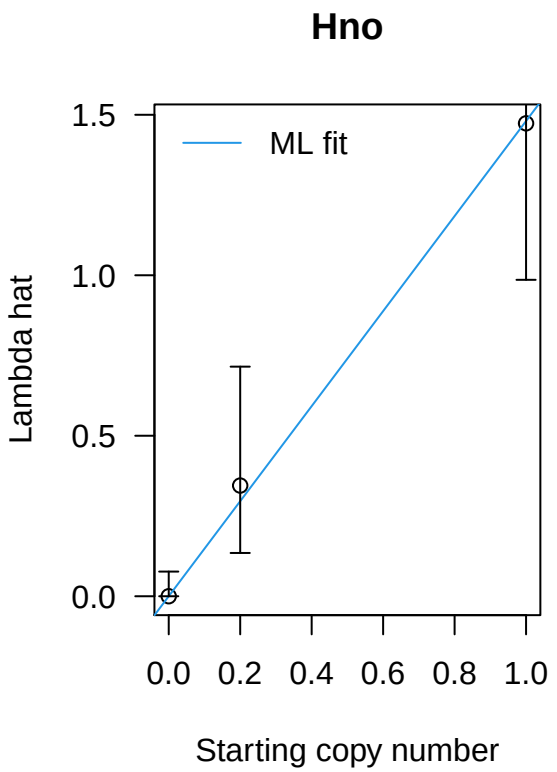


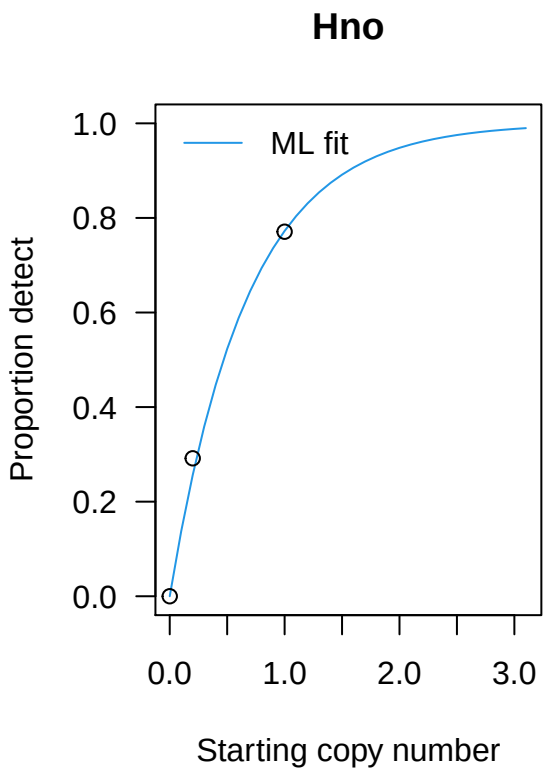

```
##
##
##
##
##  eRALU2
## Convergence= 0
##       Estimate Std.Err Z value  Pr(>z)
## alpha  0.00771 0.02002    0.39     0.7
## beta   0.40536 0.08975    4.52 6.3e-06 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 1.677365 , df=  3 , p-value= 0.6419778
```






```
##
##
##
##
##  eRAPR2
## Convergence= 0
##       Estimate  Std.Err Z value  Pr(>z)
## alpha 6.47e-08 2.33e-02    0.00       1
## beta  7.13e-01 1.49e-01    4.78 1.8e-06 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 1.148937 , df=  3 , p-value= 0.7652756
```






```
##
##
##
##
##  Hno
## Convergence= 0
##       Estimate  Std.Err Z value  Pr(>z)
## alpha 1.15e-09 1.55e-01     0.0       1
## beta  1.48e+00 3.61e-01     4.1 4.2e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 0.1539404 , df=  1 , p-value= 0.6947979
```

Estimate predicted Sq given number detects and technical replicates - intercept model (not shown)

Determine Lc, Ld, Lq - no intercept model

Follows Lavagnini and Magno 2007, Mass Spectrometry Reviews *The notation in the paper was changed from the Lavagnini 2007 paper and is shown in brackets here.*

- Lc (*LOB Limit of blank*) = critical level is the assay signal above which a response is reliably attributed to the presence of analyte
- Ld (*Ld = expected number detects out of NN replicates at concentration LOD*) = signal corresponding to an analyte concentration xd (*=LOD Limit of Detection*) level which may be a priori expected to be recognized
- Lq = quantification limit is a signal with a precision which satisfies an expected value (*=gamma_Q*)

Lc corresponds to a critical response level or a false positive rate, i.e. critical number of detects given NN replicates, above which we would reject the null hypothesis that the concentration/copy number is zero at alpha = alphaLc (*=gamma_{FP}*). It is the critical response level corresponding to the false positive rate of alphaLc. Essentially, the test is positive if the Y~Binomial(m, p) > Lc. The False Positive Rate is P(Y > Lc | S=0).

Ld is computed to correspond to the false negative rate, beta = betaLd (*=gamma_{FN}*) here. It is computed so that the probability of observing a new (unknown concentration) response less than or equal to Lc is less than or equal to betaLd. The probability of observing Lc or less detects if the concentration is xd (*=LOD Limit of Detection*) or more is less than or equal to betaLd. The values of Lc depend on the number of replicates, NN, so xd does as well. Ld is the expected number of detects at values xd and NN replicates. False negative rate Ld computation: P(Y <= Lc | p_xd) <= betaLd, and solve for xd.

Lq is less well defined. The literature suggests using Lq = beta0 + 10 s.e.(beta0), but this uses the normality assumption. Other literature suggests using the "analyte concentration xq (*=LOQ Limit of Quantification*) for which the experimental relative standard deviation of the responses reaches a fixed level (*=gamma_Q*), for example, the level 0.1." Lavagnini and Magno 2007. I interpret the term "relative standard deviation" to mean the coefficient of variation, CV = sd/mean.

In the exercise below, we use the fits from the ML models to estimate the Lc, Ld and Lq, for various values of NN replicates for a new observation, i.e. a new (unknown concentration) response number of detects.

```
## Too few values for  CID
## Too few values for  NZMS
## Too few values for  SAFO
## Too few values for  Elod-2-NU
## Too few values for  MYPI
## Too few values for  Goby
## Too few values for  SS
```

Determine Lc, Ld, Lq - intercept model

```
## Too few values for  CID
## Too few values for  NZMS
## Too few values for  SAFO
## Too few values for  Elod-2-NU
## Too few values for  MYPI
## Too few values for  Goby
## Too few values for  SS
```

Estimates, Lc, Ld, Lq for a given number of technical reps NN[NNi]

Choose the model (intercept versus no intercept) with the best LLR test fit.

```
## Limits for best choice model for N= 8
```

	InterModel	alpha	aSE	beta	bSE	Lc	SdLow	Sd	SdUp	SqLow	Sq	SqUp
D-loop	0	0.00	0.00	1.13	0.15	0	0.26	0.33	0.45	0.99	1.26	1.71
AC1_CID_1	0	0.00	0.00	0.08	0.01	0	3.65	4.94	7.65	13.80	18.69	28.95
MYPI-6	0	0.00	0.00	0.01	0.00	0	53.32	69.83	101.12	201.79	264.24	382.66
MYPI-6t	0	0.00	0.00	0.39	0.05	0	0.75	0.95	1.30	2.83	3.60	4.92
AC1	0	0.00	0.00	1.64	0.24	0	0.18	0.23	0.32	0.67	0.87	1.22
AC3	0	0.00	0.00	1.59	0.24	0	0.18	0.24	0.33	0.69	0.89	1.26
BH1	0	0.00	0.00	0.77	0.11	0	0.38	0.48	0.67	1.44	1.83	2.52
BH2	0	0.00	0.00	0.68	0.10	0	0.43	0.55	0.75	1.63	2.07	2.85
GCTM10	0	0.00	0.00	1.35	0.20	0	0.22	0.28	0.39	0.82	1.05	1.47
GCTM22	0	0.00	0.00	1.36	0.20	0	0.21	0.28	0.39	0.81	1.04	1.47
GCTM32	0	0.00	0.00	1.28	0.19	0	0.23	0.29	0.41	0.85	1.10	1.55
SC4	0	0.00	0.00	0.90	0.14	0	0.32	0.41	0.59	1.21	1.57	2.22
SC5	0	0.00	0.00	0.98	0.15	0	0.30	0.38	0.54	1.12	1.45	2.05
BHC	0	0.00	0.00	0.22	0.02	0	1.42	1.74	2.23	5.38	6.57	8.44
Dre16s	0	0.00	0.00	0.12	0.01	0	2.68	3.18	3.91	10.15	12.04	14.80
SVC	0	0.00	0.00	0.22	0.02	0	1.42	1.74	2.23	5.38	6.57	8.44
YPC	0	0.00	0.00	0.39	0.05	0	0.77	0.96	1.29	2.92	3.65	4.87
eASM09	0	0.00	0.00	0.77	0.15	0	0.36	0.49	0.78	1.34	1.85	2.52
eASTR4	0	0.00	0.00	0.48	0.09	0	0.56	0.77	1.24	2.12	2.93	3.98
eFISH1	0	0.00	0.00	0.41	0.08	0	0.65	0.90	1.48	2.46	3.42	4.64
eLIPI1	0	0.00	0.00	0.84	0.17	0	0.32	0.45	0.73	1.22	1.70	2.31
eMIDO1	0	0.00	0.00	0.97	0.20	0	0.28	0.38	0.64	1.04	1.46	1.98
eMISA2	0	0.00	0.00	0.58	0.11	0	0.46	0.64	1.04	1.76	2.43	3.30
eONKI4	1	0.07	0.05	0.69	0.16	2	0.74	1.21	2.41	1.24	1.93	3.01
eRAAU1	0	0.00	0.00	0.46	0.09	0	0.58	0.81	1.33	2.21	3.07	4.26
eRACA2	0	0.00	0.00	0.54	0.11	0	0.50	0.69	1.13	1.88	2.61	3.62
eRALU2	0	0.00	0.00	0.42	0.09	0	0.64	0.89	1.49	2.41	3.38	4.74
eRAPR2	0	0.00	0.00	0.71	0.14	0	0.38	0.52	0.85	1.44	1.98	2.72
Hno	0	0.00	0.00	1.51	0.25	0	0.19	0.25	0.36	0.71	0.94	1.25

```
## eASMO9      2.96
## eASTR4      4.70
## eFISH1      5.61
## eLIPI1      2.78
## eMIDO1      2.41
## eMISA2      3.92
## eONKI4      3.73
## eRAAU1      5.04
## eRACA2      4.28
## eRALU2      5.65
## eRAPR2      3.21
## Hno         1.38
```

Tables and Graphs for the manuscript

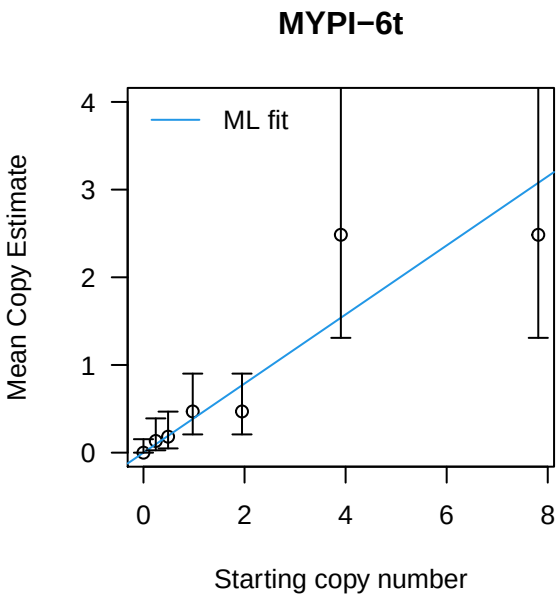
Include MYPI-6t and eASMO9, eFISH1, eONKI4 in paper.
Index into uTargets (5, 25, 27, 31)

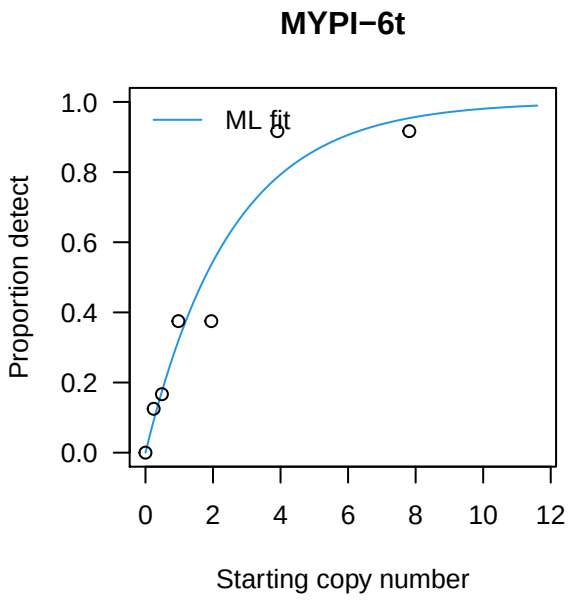
```
##
##
##               alpha   aSE   beta   bSE   Lc   LODL    LOD    LODU    LOQL     LOQ    LOQU   K.LOD8
## ----------  ------  ----  -----  ----  ---  -----  -----  ------  ------  ------  ------  -------
## AC1            0.0     0    1.6   0.2    0    0.2    0.2     0.3     0.7     0.9     1.2      0.2
## AC1_CID_1      0.0     0    0.1   0.0    0    3.6    4.9     7.7    13.8    18.7    29.0      5.6
## AC3            0.0     0    1.6   0.2    0    0.2    0.2     0.3     0.7     0.9     1.3      0.2
## BH1            0.0     0    0.8   0.1    0    0.4    0.5     0.7     1.4     1.8     2.5      0.3
## BH2            0.0     0    0.7   0.1    0    0.4    0.5     0.8     1.6     2.1     2.9      0.2
## BHC            0.0     0    0.2   0.0    0    1.4    1.7     2.2     5.4     6.6     8.4      1.9
## D-loop         0.0     0    1.1   0.2    0    0.3    0.3     0.5     1.0     1.3     1.7      0.3
## Dre16s         0.0     0    0.1   0.0    0    2.7    3.2     3.9    10.1    12.0    14.8      3.4
## eASMO9         0.0     0    0.8   0.1    0    0.4    0.5     0.8     1.3     1.8     3.0      0.3
## eASTR4         0.0     0    0.5   0.1    0    0.6    0.8     1.2     2.1     2.9     4.7      0.5
## eFISH1         0.0     0    0.4   0.1    0    0.7    0.9     1.5     2.5     3.4     5.6      0.6
## eLIPI1         0.0     0    0.8   0.2    0    0.3    0.4     0.7     1.2     1.7     2.8      0.3
## eMIDO1         0.0     0    1.0   0.2    0    0.3    0.4     0.6     1.0     1.5     2.4      0.4
## eMISA2         0.0     0    0.6   0.1    0    0.5    0.6     1.0     1.8     2.4     3.9      0.5
## eONKI4         0.1     0    0.7   0.2    2    0.7    1.2     2.4     1.2     1.9     3.7      0.2
## eRAAU1         0.0     0    0.5   0.1    0    0.6    0.8     1.3     2.2     3.1     5.0      0.8
## eRACA2         0.0     0    0.5   0.1    0    0.5    0.7     1.1     1.9     2.6     4.3      0.6
## eRALU2         0.0     0    0.4   0.1    0    0.6    0.9     1.5     2.4     3.4     5.6      0.7
## eRAPR2         0.0     0    0.7   0.1    0    0.4    0.5     0.8     1.4     2.0     3.2      0.4
## GCTM10         0.0     0    1.4   0.2    0    0.2    0.3     0.4     0.8     1.0     1.5      0.2
## GCTM22         0.0     0    1.4   0.2    0    0.2    0.3     0.4     0.8     1.0     1.5      0.2
## GCTM32         0.0     0    1.3   0.2    0    0.2    0.3     0.4     0.9     1.1     1.6      0.3
## Hno            0.0     0    1.5   0.2    0    0.2    0.2     0.4     0.7     0.9     1.4      0.2
## MYPI-6         0.0     0    0.0   0.0    0   53.3   69.8   101.1   201.8   264.2   382.7     78.0
## MYPI-6t        0.0     0    0.4   0.1    0    0.7    1.0     1.3     2.8     3.6     4.9      1.0
## SC4            0.0     0    0.9   0.1    0    0.3    0.4     0.6     1.2     1.6     2.2      0.4
## SC5            0.0     0    1.0   0.1    0    0.3    0.4     0.5     1.1     1.5     2.1      0.4
## SVC            0.0     0    0.2   0.0    0    1.4    1.7     2.2     5.4     6.6     8.4      1.9
## YPC            0.0     0    0.4   0.0    0    0.8    1.0     1.3     2.9     3.6     4.9      1.0
```

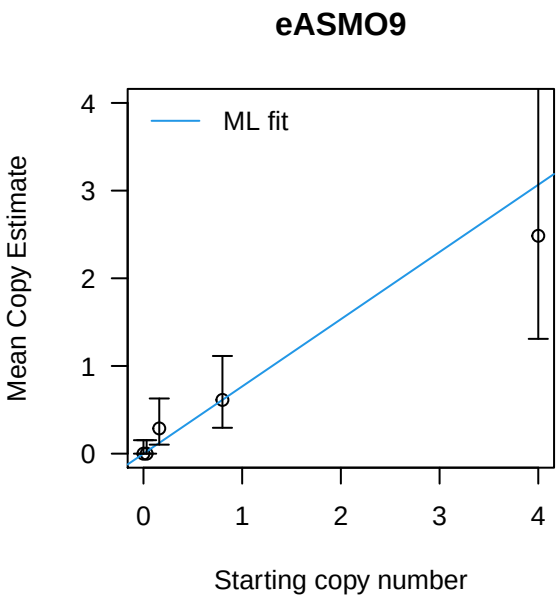
```
##
##
## Table: MYPI-6t
##
##      S   num.detect    n   p.tilde   lambda.tilde
## ------  -----------  ---  --------  -------------
##  0.000            0   24     0.000          0.000
##  0.244            3   24     0.125          0.134
##  0.488            4   24     0.167          0.182
##  0.975            9   24     0.375          0.470
##  1.950            9   24     0.375          0.470
##  3.906           22   24     0.917          2.485
##  7.812           22   24     0.917          2.485
##
##
```

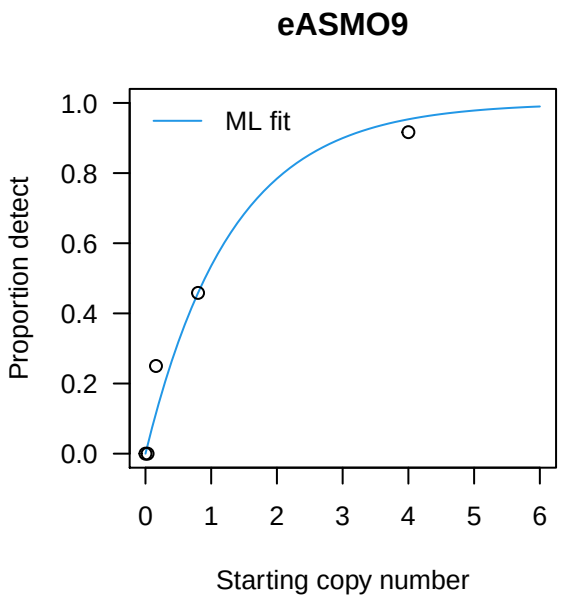
```
## Table: eASMO9
##
##      S   num.detect    n   p.tilde   lambda.tilde
## ------  -----------  ---  --------  -------------
##  0.000            0   24     0.000          0.000
##  0.032            0   24     0.000          0.000
##  0.160            6   24     0.250          0.288
##  0.800           11   24     0.458          0.613
##  4.000           22   24     0.917          2.485
##
##
## Table: eFISH1
##
##      S   num.detect    n   p.tilde   lambda.tilde
## ------  -----------  ---  --------  -------------
##  0.000            0   24     0.000          0.000
##  0.032            1   24     0.042          0.043
##  0.160            0   24     0.000          0.000
##  0.800           10   24     0.417          0.539
##  4.000           18   24     0.750          1.386
##
##
## Table: eONKI4
##
##      S   num.detect    n   p.tilde   lambda.tilde
## ------  -----------  ---  --------  -------------
##  0.000            0   24     0.000          0.000
##  0.032            3   24     0.125          0.134
##  0.160            6   24     0.250          0.288
##  0.800           14   24     0.583          0.875
##  4.000           21   24     0.875          2.079
##
##  MYPI-6t
## Convergence= 0
##      Estimate Std.Err Z value  Pr(>z)
## beta    0.394   0.054    7.29 3.1e-13 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 6.787625 , df=  6 , p-value= 0.340935
##
##
##  eASMO9
## Convergence= 0
##      Estimate Std.Err Z value  Pr(>z)
## beta    0.767   0.147    5.22 1.8e-07 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 5.106559 , df=  4 , p-value= 0.2765376
##
##
##  eFISH1
## Convergence= 0
```

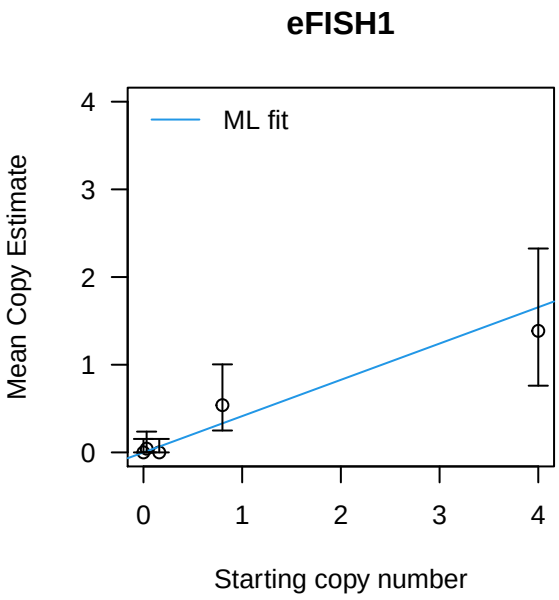
```
##      Estimate Std.Err Z value  Pr(>z)
## beta   0.4139  0.0823    5.03 4.9e-07 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 6.63572 , df=  4 , p-value= 0.1564373
##
##
##  eONKI4
## Convergence= 0
##       Estimate Std.Err Z value  Pr(>z)
## alpha   0.0749  0.0486    1.54    0.12
## beta    0.6939  0.1601    4.33 1.5e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## LLR test stat= 7.671079 , df=  3 , p-value= 0.05332185
```

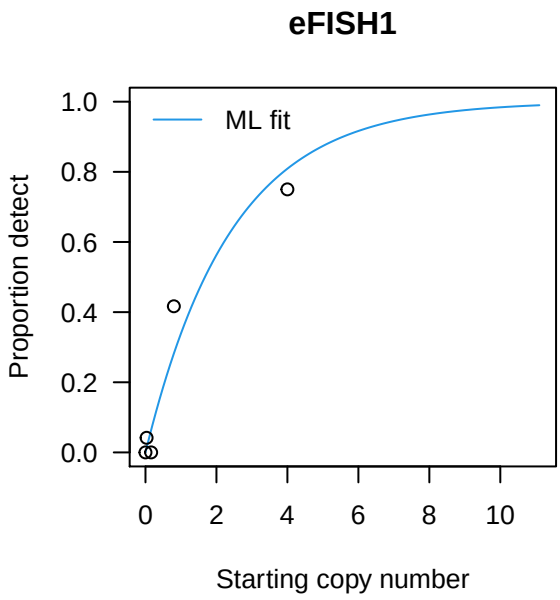









eFISH1
ML fit
Mean Copy Estimate
Starting copy number
0
1
2
3
4


eFISH1
ML fit
Proportion detect
Starting copy number
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0.4
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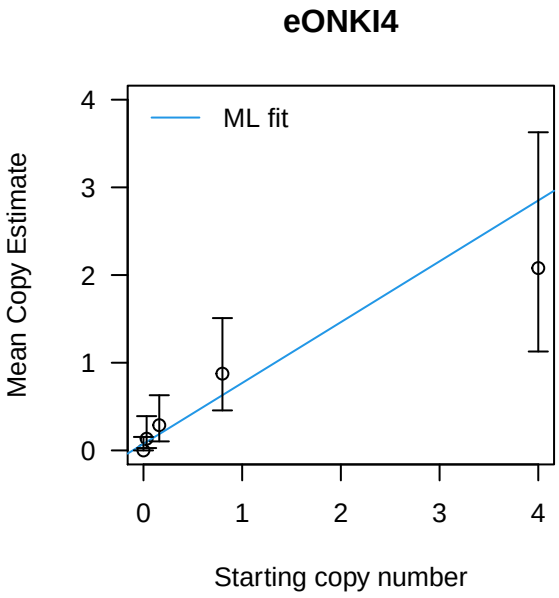
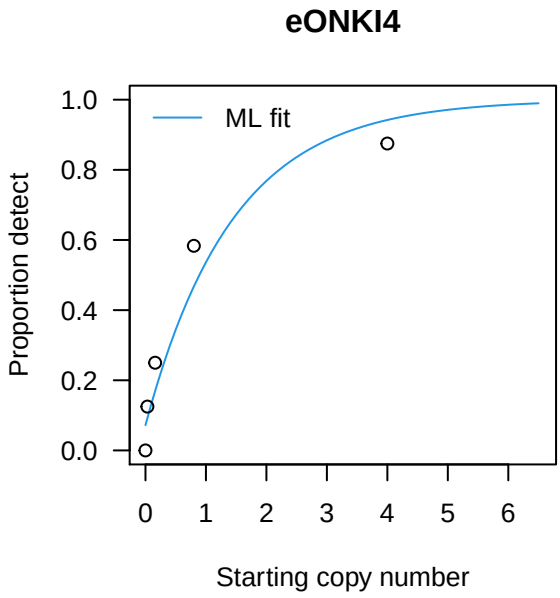
1.0
0
2
4
6
8
10


eONKI4
ML fit
Mean Copy Estimate
Starting copy number
0
1
2
3
4


eONKI4
ML fit
Proportion detect
Starting copy number
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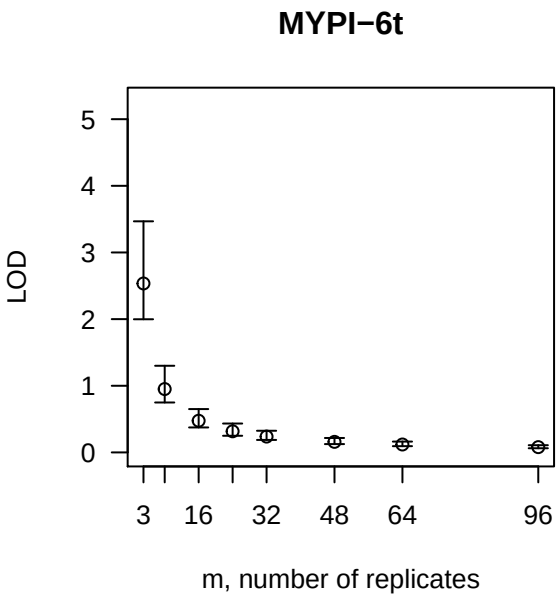
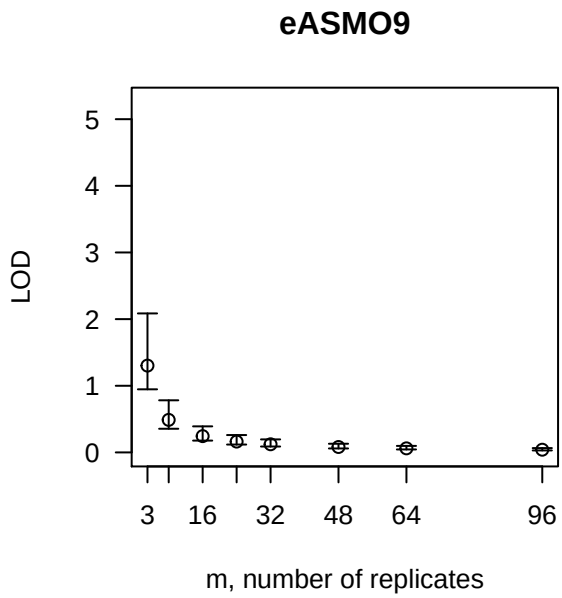
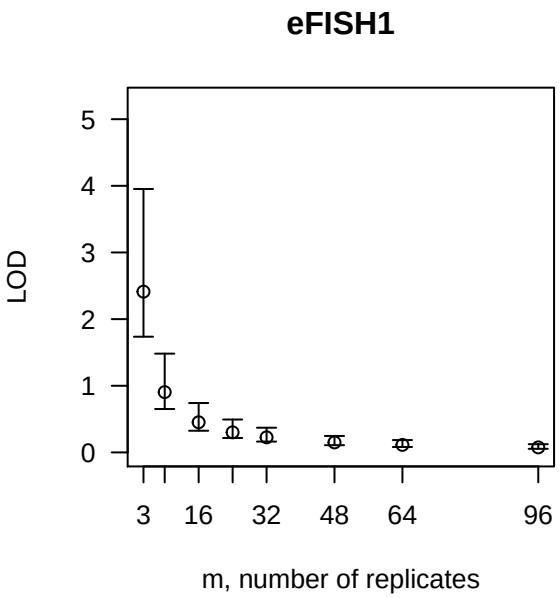
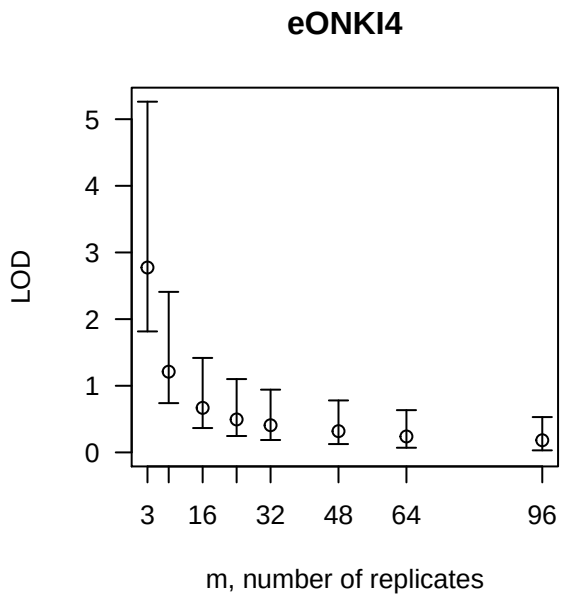
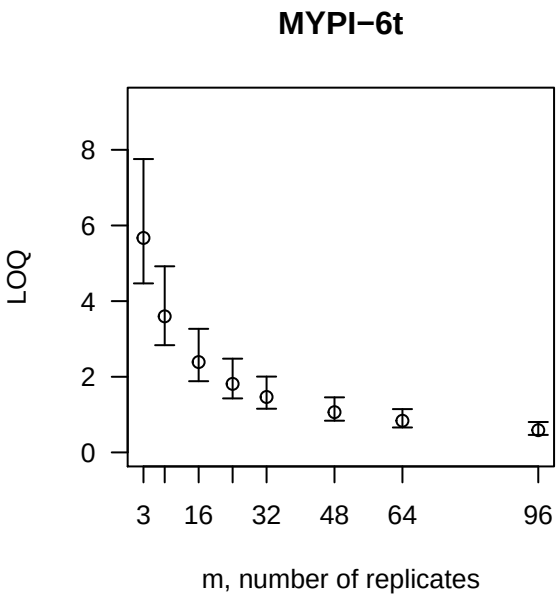
0.2
0.4
0.6
0.8
1.0
0
1
2
3
4
5
6

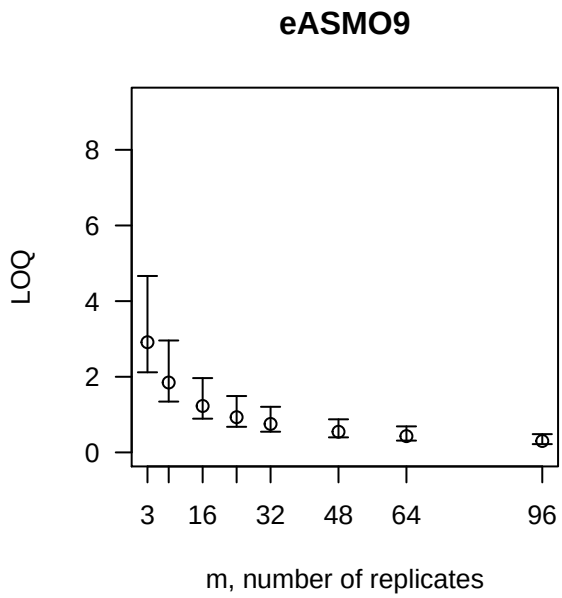

MYPI−6t
LOD
0
1
2
3
4
5
3
16
32
48
64
96
m, number of replicates

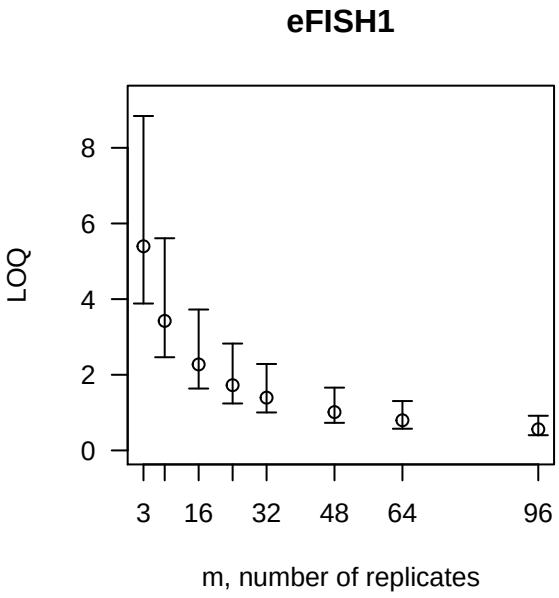

eASMO9
LOD
0
1
2
3
4
5
3
16
32
48
64
96
m, number of replicates

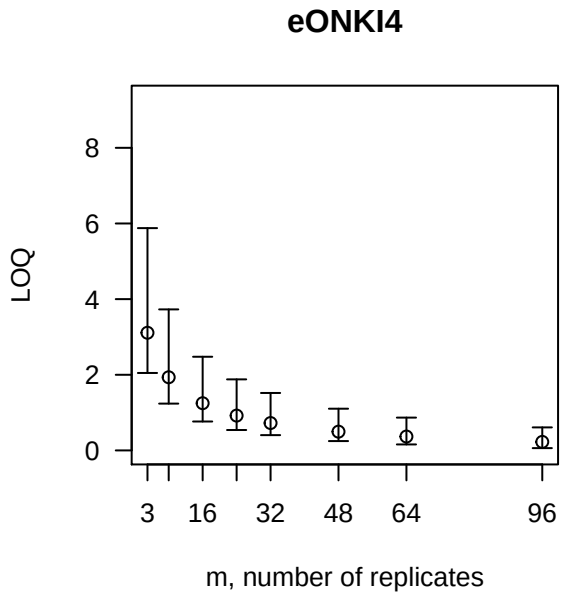

eFISH1
LOD
0
1
2
3
4
5
3
16
32
48
64
96
m, number of replicates

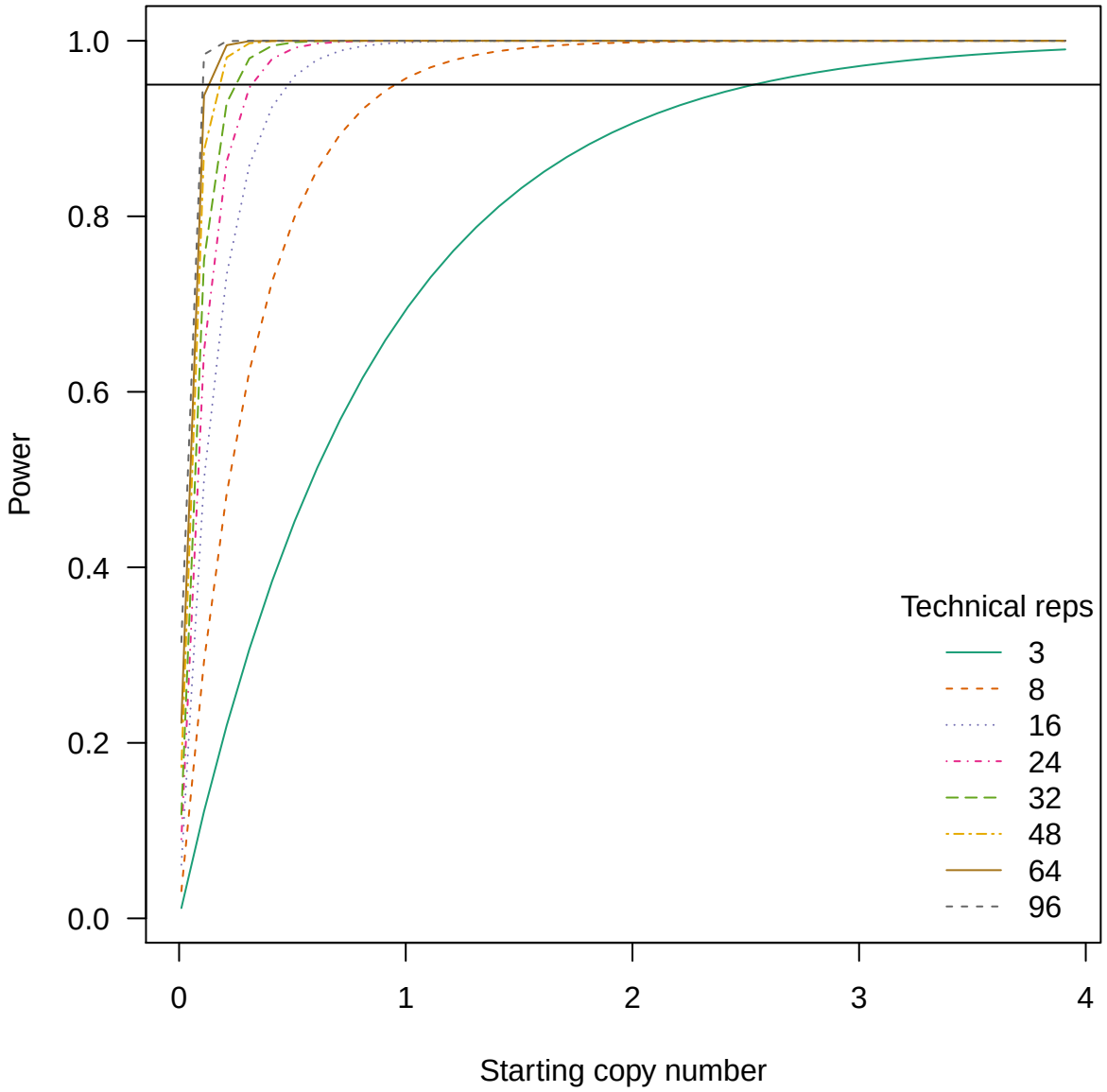

eONKI4
LOD
0
1
2
3
4
5
3
16
32
48
64
96
m, number of replicates


MYPI−6t
LOQ
0
2
4
6
8
3
16
32
48
64
96
m, number of replicates


eASMO9
LOQ
0
2
4
6
8
3
16
32
48
64
96
m, number of replicates


eFISH1
LOQ
0
2
4
6
8
3
16
32
48
64
96
m, number of replicates


eONKI4
LOQ
0
2
4
6
8
3
16
32
48
64
96
m, number of replicates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0.0
Power
0
1
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3
4
Starting copy number
Technical reps
3
8
16
24
32
48
64
96