

APPENDIX A

This appendix contains the software code written for two programs, *VictorExtract* and *GraphExtract* for use in Microsoft Excel 2004 with Visual Basic for Applications. The former was written to process the raw data output of a Victor3 luminometer (Perkin Elmer) for the neutralization assay and fit the data to a one-site dose-response model with fixed minima and maxima of 0 and 100% by minimization of the residual sum of squares. The later program processes the output from *VictorExtract* and arranges it in a format for creating figures using graphical software programs such as KaleidaGraph and Prism.

' Joshua S. Klein, California Institute of Technology, Copyright 2006

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Option Explicit
Public filePath, infoArray(10, 14), resultsArray() As Variant
Public fileList, fileName, alphaStr, rssqStr As String
Public x, y, z, a, b, c, e, zz, yy, xx, aa, bb As Integer
Public plateArray(8, 12), d, neutValue, jacobianValue, totneutValue,
avgneutValue, rssqValue, chireducedValue As Double

Sub VictorExtract()
    UserForm1.Show
End Sub

Sub extractData()
    Dim jacobianArray(1, 8)
    UserForm1.Hide
    'Read userform entries
    For x = 1 To 10
        infoArray(x, 1) = UserForm1.Controls(x - 1).Value
        infoArray(x, 2) = UserForm1.Controls(x + 16).Value
        infoArray(x, 3) = UserForm1.Controls(x + 138).Value
        infoArray(x, 4) = UserForm1.Controls(x + 28).Value
        infoArray(x, 5) = UserForm1.Controls(x + 39).Value
        infoArray(x, 6) = UserForm1.Controls(x + 50).Value
        infoArray(x, 7) = UserForm1.Controls(x + 61).Value
        infoArray(x, 8) = UserForm1.Controls(x + 72).Value
        infoArray(x, 9) = UserForm1.Controls(x + 83).Value
        infoArray(x, 10) = UserForm1.Controls(x + 94).Value
        infoArray(x, 11) = UserForm1.Controls(x + 105).Value
        infoArray(x, 12) = UserForm1.Controls(x + 116).Value
        infoArray(x, 13) = UserForm1.Controls(x + 127).Value
    Next x
    'Assign plate columns per antibody
    For x = 1 To 10
        If Trim(infoArray(x, 1)) <> "" Then
            For y = 4 To 13
                If infoArray(x, y) = "True" Then infoArray(x, 14) =
infoArray(x, 14) & Str(y - 1)
            Next y
        End If
    Next x
    'Select and open source data file
    filePath = Application.GetOpenFilename()
    MsgBox (filePath)
    Application.Workbooks.Open (filePath)
    x = 1
    y = Len(filePath)
    While x > 0
        If Mid(filePath, y, 1) <> ":" Then
            y = y - 1
        Else: x = 0
        End If
    Wend
    'Assign contents of Plate worksheet to array and close source data file
    fileName = Right(filePath, Len(filePath) - y)
    Application.Workbooks(fileName).Activate
    Application.Worksheets("Plate").Activate
    For x = 1 To 12
        For y = 1 To 8

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        plateArray(y, x) = ActiveSheet.Cells(y + 6, x).Value
    Next y
Next x
Application.Workbooks(fileName).Close
'Create new data workbook
Application.Workbooks.Add
Application.ActiveSheet.Name = fileName
Application.ActiveSheet.Cells(1, 1).Value = "Date"
Application.ActiveSheet.Cells(1, 2).Value = "Antibody"
Application.ActiveSheet.Cells(1, 3).Value = "Isolate"
Application.ActiveSheet.Cells(1, 4).Value = "IC50 (nM)"
Application.ActiveSheet.Cells(1, 5).Value = "IC50 Err (nM)"
Application.ActiveSheet.Cells(1, 6).Value = "IC50 (ug/mL)"
Application.ActiveSheet.Cells(1, 7).Value = "IC50 Err (ug/mL)"
Application.ActiveSheet.Cells(1, 8).Value = "RSSQ"
Application.ActiveSheet.Cells(1, 9).Value = "Row A (nM)"
Application.ActiveSheet.Cells(1, 10).Value = "Row B (nM)"
Application.ActiveSheet.Cells(1, 11).Value = "Row C (nM)"
Application.ActiveSheet.Cells(1, 12).Value = "Row D (nM)"
Application.ActiveSheet.Cells(1, 13).Value = "Row E (nM)"
Application.ActiveSheet.Cells(1, 14).Value = "Row F (nM)"
Application.ActiveSheet.Cells(1, 15).Value = "Row G (nM)"
Application.ActiveSheet.Cells(1, 16).Value = "Row H (nM)"
Application.ActiveSheet.Cells(1, 17).Value = "GM1"
Application.ActiveSheet.Cells(1, 18).Value = "GM2"
Application.ActiveSheet.Cells(1, 19).Value = "GM3"
Application.ActiveSheet.Cells(1, 20).Value = "GM4"
Application.ActiveSheet.Cells(1, 21).Value = "GM5"
Application.ActiveSheet.Cells(1, 22).Value = "GM6"
Application.ActiveSheet.Cells(1, 23).Value = "GM7"
Application.ActiveSheet.Cells(1, 24).Value = "GM8"
Application.ActiveSheet.Cells(1, 25).Value = "Avg GM"
Application.ActiveSheet.Cells(1, 26).Value = "VC1"
Application.ActiveSheet.Cells(1, 27).Value = "VC2"
Application.ActiveSheet.Cells(1, 28).Value = "VC3"
Application.ActiveSheet.Cells(1, 29).Value = "VC4"
Application.ActiveSheet.Cells(1, 30).Value = "VC5"
Application.ActiveSheet.Cells(1, 31).Value = "VC6"
Application.ActiveSheet.Cells(1, 32).Value = "VC7"
Application.ActiveSheet.Cells(1, 33).Value = "VC8"
Application.ActiveSheet.Cells(1, 34).Value = "Avg VC"
alphaStr = "ABCDEFGHJKLMNOPQRSTUVWXYZ"
b = 1
For y = 1 To 10
    xx = 0
    rssqStr = ""
    a = Len(infoArray(y, 14))
    c = 0
    For x = 1 To a
        If Trim(Mid(infoArray(y, 14), x, 1)) <> "" Then
            If Val(Mid(infoArray(y, 14), x, 2)) > 2 Then
                c = (Val(Mid(infoArray(y, 14), x, 2)))
                'MsgBox (c)
                Application.ActiveSheet.Cells(b + 1, 1).Value =
UserForm1.TextBox12.Value
                Application.ActiveSheet.Cells(b + 1, 2).Value =
infoArray(y, 1)

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Application.ActiveSheet.Cells(b + 1, 3).Value =
UserForm1.TextBox11.Value
If Val(infoArray(y, 2)) > 0 Then
    d = Val(infoArray(y, 2))
Else: d = 0
End If
For z = 9 To 16
    Application.ActiveSheet.Cells(b + 1, z).Value = d
    d = d / 3
Next z
'Set initial IC50 value to median concentration
Application.ActiveSheet.Cells(b + 1, 4).Value =
Application.ActiveSheet.Cells(b + 1, 12).Value
'Calculate average GM and average VC
Application.ActiveSheet.Cells(b + 1, 25).Value =
"=average(Q" & b + 1 & ":X" & b + 1 & ")"
Application.ActiveSheet.Cells(b + 1, 34).Value =
"=average(Z" & b + 1 & ":AG" & b + 1 & ")"
'Enter RL values
For z = 1 To 8
    yy = 0
    Application.ActiveSheet.Cells(b + 1, z + 16).Value
= plateArray(z, 1)
    Application.ActiveSheet.Cells(b + 1, z + 25).Value
= plateArray(z, 2)
    While yy < 1000
        If Application.ActiveSheet.Cells(b + 1, z * 4 +
31 + yy).Value = "" Then
            Application.ActiveSheet.Cells(b + 1, z * 4
+ 31 + yy).Value = plateArray(z, c)
            yy = 1000
        Else: yy = yy + 16
        End If
    Wend
Next z
'Calculate % neutralization and fitted values
For z = 1 To 8
    yy = 0
    While Application.ActiveSheet.Cells(b + 1, z * 4 +
31 + yy).Value <> ""
        If LCase(Application.ActiveSheet.Cells(b + 1, z
* 4 + 31 + yy).Value) <> "x" Then
            'Application.ActiveSheet.Cells(b + 1, z
* 4 + 31 + yy + 1).Value = (1 - ((Application.ActiveSheet.Cells(b + 1,
z * 4 + 31 + yy).Value - _
                Application.ActiveSheet.Cells(b +
1, 25).Value) / (Application.ActiveSheet.Cells(b + 1, 34).Value - _
                Application.ActiveSheet.Cells(b +
1, 25).Value))) * 100
            'Calculate obs % neutralization
            Application.ActiveSheet.Cells(b + 1, z
* 4 + 31 + yy + 1).Value = "(1 - (" & Application.ActiveSheet.Cells(b
+ 1, z * 4 + 31 + yy).Address(RowAbsolute:=False) & " - " & _
                Application.ActiveSheet.Cells(b +
1, 25).Address(RowAbsolute:=False) & ")/(" &
Application.ActiveSheet.Cells(b + 1, 34).Address(RowAbsolute:=False) &
" - " & _

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Application.ActiveSheet.Cells(b +
1, 25).Address(RowAbsolute:=False) & ") * 100"
'Calculate expected % neutralization
Application.ActiveSheet.Cells(b + 1, z
* 4 + 31 + yy + 2).Value = "=100/(1+D" & b + 1 & "/" & Mid(alphaStr, 17
- z, 1) & b + 1 & ")"
'Calculate residual
Application.ActiveSheet.Cells(b + 1, z
* 4 + 31 + yy + 3).Value = "(" & Mid(Application.ActiveSheet.Cells(b +
1, z * 4 + 31 + yy _
+ 1).Address(RowAbsolute:=False),
2) & "-" & Mid(Application.ActiveSheet.Cells(b + 1, z * 4 + 31 + yy +
2).Address(RowAbsolute:=False), 2) & ")^2"
End If
yy = yy + 16
xx = xx + 1
Wend
Next z
End If
c = 0
End If
Next x
'Calculate RSSQ = sum(y_obs - y_fit)^2, n =1 to i
If xx > 0 Then
Application.ActiveSheet.Cells(b + 1, ((yy - 16) / 32 + 1) * 32
+ 35).Value = "end"
For z = 1 To 8
rssqStr = rssqStr & "(" &
Mid(Application.ActiveSheet.Cells(b + 1, z * 4 +
33).Address(RowAbsolute:=False), 2) & " - average("
For x = 1 To xx / 16
'Ignore cells with values of "x" (assumes manual
deletion)
If LCase(Application.ActiveSheet.Cells(b + 1, z * 4 +
31 + x * 32 - 32).Value) <> "x" Then
rssqStr = rssqStr &
Mid(Application.ActiveSheet.Cells(b + 1, z * 4 + 32 + x * 32 -
32).Address(RowAbsolute:=False), 2) & ","
End If
Next x
rssqStr = Left(rssqStr, Len(rssqStr) - 1) & ")^2,"
Next z
ActiveSheet.Cells(b + 1, 8).Value = "=sum(" & Left(rssqStr,
Len(rssqStr) - 1) & ")"
End If
b = b + 1
Next y
'Minimize RSSQ using built-in solver function
For x = 1 To 10
If Application.ActiveSheet.Cells(x + 1, 8).Value > 0 Then
SolverReset
SolverOK
setCell:=Application.ActiveSheet.Cells.Range(ActiveSheet.Cells(x + 1,
8), ActiveSheet.Cells(x + 1, 8)), _
maxMinVal:=2, _
byChange:=Application.ActiveSheet.Cells.Range(ActiveSheet.Cells(x + 1,
4), ActiveSheet.Cells(x + 1, 4))

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SolverSolve UserFinish:=True
'Calculate error
xx = 1
While LCase(ActiveSheet.Cells(x + 1, xx + 34).Value) <> "end"
    xx = xx + 1
Wend
xx = (xx - 1) / 32
'Calculate asymptotic standard error
jacobianValue = 0
totneutValue = 0
avgneutValue = 0
rssqValue = 0
chireducedValue = 0
For aa = 1 To 8
    'Jacobian = d(% neut)/d(IC50)
    jacobianValue = jacobianValue + (((-100 /
ActiveSheet.Cells(x + 1, aa + 8).Value) / (1 + ActiveSheet.Cells(x + 1,
4).Value / _
        ActiveSheet.Cells(x + 1, aa + 8).Value) ^ 2)) ^ 2
Next aa
'For aa = 1 To 8
'    If xx > 0 Then
'        For bb = 1 To xx
'            'Ignore deleted values
'            If LCase(ActiveSheet.Cells(x + 1, bb * 32 + aa * 4
- 4).Value) <> "x" Then
'                totneutValue = totneutValue +
ActiveSheet.Cells(x + 1, bb * 32 + aa * 4).Value
'                End If
'            Next bb
'            avgneutValue = totneutValue / xx
'        Else:
'            If LCase(ActiveSheet.Cells(x + 1, bb * 32 + aa * 4
- 4).Value) <> "x" Then
'                avgneutValue = ActiveSheet.Cells(x + 1, bb *
32 + aa * 4).Value
'                End If
'            End If
'            If avgneutValue <> 0 Then
'                rssqValue = rssqValue + (avgneutValue -
ActiveSheet.Cells(x + 1, aa * 4 + 37 - 4).Value) ^ 2
'            End If
'            totneutValue = 0
'        Next aa
    ActiveSheet.Cells(x + 1, 5).Value = (jacobianValue ^ -0.5) *
((Application.ActiveSheet.Cells(x + 1, 8).Value / 7) ^ 0.5)
    If Application.ActiveSheet.Cells(x + 1, 4).Value > 0 Then
        'Calculate IC50 and error in ug/mL
        If Val(infoArray(x, 3)) > 0 Then
            Application.ActiveSheet.Cells(x + 1, 6).Value =
Application.ActiveSheet.Cells(x + 1, 4).Value * (10 ^ -9) *
infoArray(x, 3) * (10 ^ 3)
            Application.ActiveSheet.Cells(x + 1, 7).Value =
Application.ActiveSheet.Cells(x + 1, 5).Value * (10 ^ -9) *
infoArray(x, 3) * (10 ^ 3)
        End If
    End If
End If
End If

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Next x
'Format results
Rows("1:1").Select
Selection.Font.Bold = True
Rows("1:10").Select
Selection.Columns.AutoFit
Selection.Columns.HorizontalAlignment = xlCenter
End Sub
Sub GraphExtract()
    UserForm2.Show
End Sub
Sub getCurves()
    UserForm2.Hide
    alphaStr = "ABCDEFGHIJKLMNOPQRSTUVWXYZ"
    xx = 1
    While LCase(ActiveSheet.Cells((UserForm2.TextBox1.Value), xx +
34).Value) <> "end"
        xx = xx + 1
    Wend
    xx = (xx - 1) / 32
    ReDim resultsArray(3, 8 + xx, 8) As Variant
    resultsArray(1, 1, 1) = "Date"
    resultsArray(1, 2, 1) = "Antibody"
    resultsArray(1, 3, 1) = "Isolate"
    resultsArray(1, 4, 1) = "GM"
    resultsArray(1, 5, 1) = "VC"
    resultsArray(1, 6, 1) = "Conc (nM)"
    resultsArray(1, 7, 1) = "Avg % neut"
    resultsArray(1, 8, 1) = "Err % neut"
    resultsArray(2, 1, 1) =
Application.ActiveSheet.Cells((UserForm2.TextBox1.Value), 1).Value
    resultsArray(2, 2, 1) =
Application.ActiveSheet.Cells((UserForm2.TextBox1.Value), 2).Value
    resultsArray(2, 3, 1) =
Application.ActiveSheet.Cells((UserForm2.TextBox1.Value), 3).Value
    For x = 1 To 8
        resultsArray(2, 4, x) =
Application.ActiveSheet.Cells((UserForm2.TextBox1.Value), x + 16).Value
        resultsArray(2, 5, x) =
Application.ActiveSheet.Cells((UserForm2.TextBox1.Value), x + 25).Value
        resultsArray(2, 6, x) =
Application.ActiveSheet.Cells((UserForm2.TextBox1.Value), 17 - x).Value
    Next x
    For aa = 1 To 8
        'Get neutralization values for each replicate
        For bb = 1 To xx
            resultsArray(1, 8 + bb, 1) = "Exp " & bb
            If Val(ActiveSheet.Cells((UserForm2.TextBox1.Value), bb *
32 + aa * 4 - 1).Value) <> 0 Then
                resultsArray(2, 8 + bb, aa) =
ActiveSheet.Cells((UserForm2.TextBox1.Value), bb * 32 + aa * 4).Value
                resultsArray(2, 7, aa) = resultsArray(2, 7, aa) +
resultsArray(2, 8 + bb, aa)
            Else: resultsArray(2, 8 + bb, aa) = "x"
            End If
        Next bb
        'Calculate avg and stdev neut
        If xx > 1 Then

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        resultsArray(2, 7, aa) = "=average(I" & aa + 1 & ":" &
Mid(alphaStr, xx + 8, 1) & aa + 1 & ")"
        resultsArray(2, 8, aa) = "=stdev(I" & aa + 1 & ":" &
Mid(alphaStr, xx + 8, 1) & aa + 1 & ")"
    End If
Next aa
Application.Workbooks.Add
For a = 1 To UBound(resultsArray, 2)
    Application.ActiveSheet.Cells(1, a).Value = resultsArray(1, a,
1)
    For x = 1 To 8
        Application.ActiveSheet.Cells(x + 1, a).Value =
resultsArray(2, a, x)
    Next x
Next a
Application.ActiveSheet.Cells(10, 3).Value = "Average"
Application.ActiveSheet.Cells(11, 3).Value = "St dev"
Application.ActiveSheet.Cells(10, 4).Value = "=average(D2:D9)"
Application.ActiveSheet.Cells(10, 5).Value = "=average(E2:E9)"
Application.ActiveSheet.Cells(11, 4).Value = "=stdev(D2:D9)"
Application.ActiveSheet.Cells(11, 5).Value = "=stdev(E2:E9)"
'Format results
Rows("1:1").Select
Selection.Font.Bold = True
Rows("10:11").Select
Selection.Font.Bold = True
Rows("1:11").Select
Selection.Columns.AutoFit
Selection.Columns.HorizontalAlignment = xlCenter
End Sub

```


Victor Extract user form:

Date:	<input type="text" value="2006-00-00"/>												
HIV Isolate:	<input type="text"/>	<u>Plate column</u>											
<u>Antibody name</u>	<u>Top conc. (nM)</u>	<u>MW</u>		<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>	<u>11</u>	<u>12</u>
<input type="text"/>	<input type="text"/>	<input type="text"/>	→	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
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<input type="text"/>	<input type="text"/>	<input type="text"/>	→	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
				<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
				<input type="button" value="GO!"/> <input type="button" value="Cancel"/>									

Graph Extract user form:

Enter row number: <input type="text"/>
<input type="button" value="GO!"/> <input type="button" value="Cancel"/>