

Automating exclusion of post-peak fluorescence for nonlinear regression/curve fitting in analysis of T_m for thermal shift assay (12.1.2006; CLP)

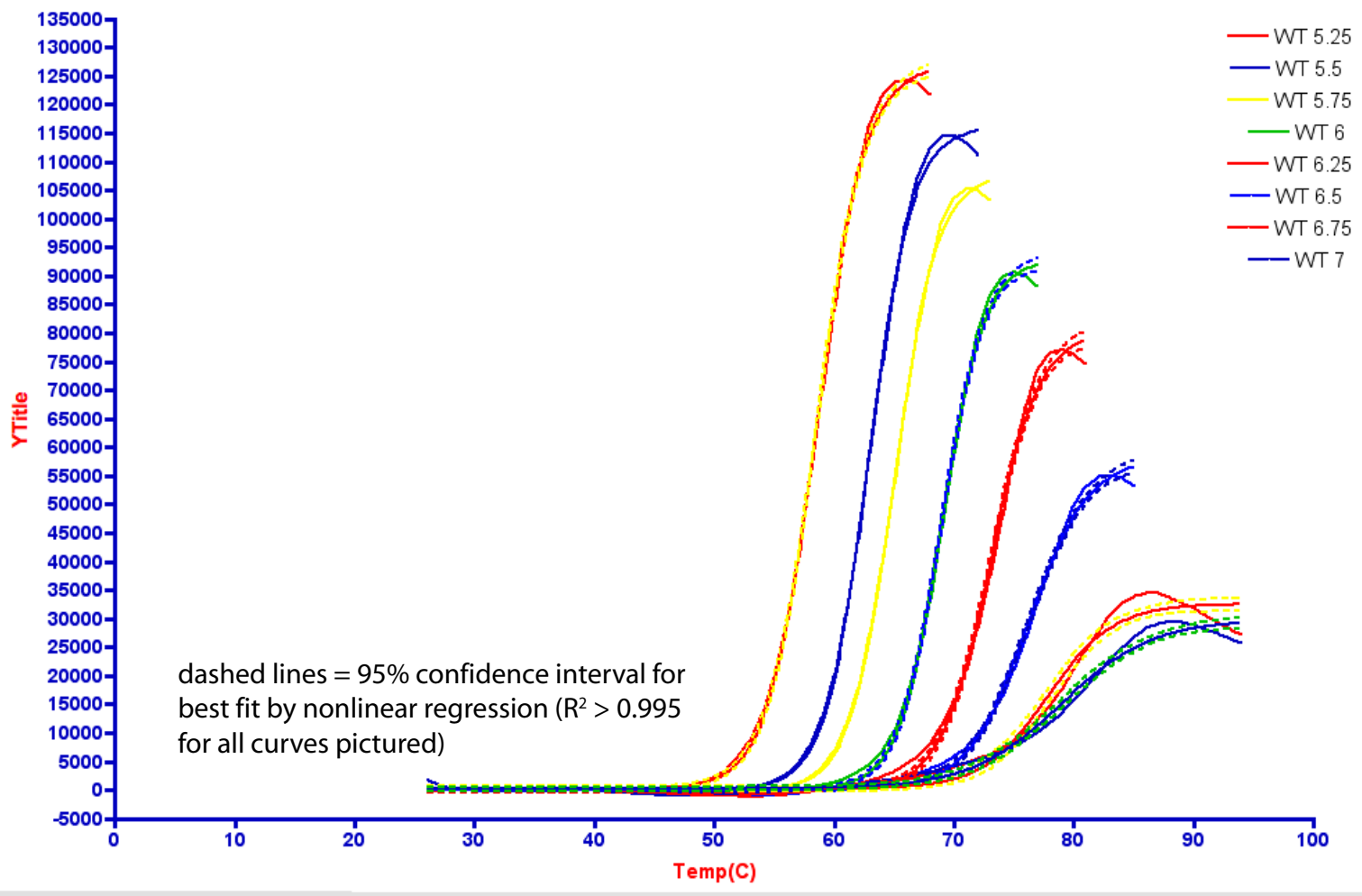
1. Import raw data from SDS file into Excel worksheet "rawdata" and transpose to columns
2. Copy data to new worksheet, labeled "peakdeleted". Run macro 'deletaftermax'.
3. Copydata into GraphPad prism and Analyze by nonlinear regression iusing "Melting Boltzmann"

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + \exp((T_m - X) / \text{Slope}))$$

4. Copy 'Table of Results' back to Excel for further analysis and plotting.

Temp(C)	WT 5.25	WT 5.5	WT 5.75	WT 6	WT 6.25	WT 6.5	WT 6.75	WT 7
47	504.768000	-889.485400	-588.804800	-446.194000	-646.823100	-413.017000	-373.425800	-317.235000
48	871.432000	-768.270300	-707.518000	-511.948000	-776.180700	-561.108400	-546.273400	-397.781700
49	1433.348000	-685.501400	-777.919000	-576.197000	-803.852300	-723.912000	-684.883700	-487.958900
50	2346.460000	-539.827000	-815.286000	-607.898000	-892.997300	-826.377000	-781.218800	-559.948100
51	3726.720000	-117.412100	-601.236400	-637.112300	-1046.707000	-880.033000	-840.945800	-607.841800
52	6772.267000	136.379800	-733.239000	-633.698000	-1069.702000	-927.464100	-883.572300	-684.958900
53	8773.027000	477.877000	-568.823700	-628.851300	-1029.877000	-946.970000	-894.948100	-697.956000
54	10270.180000	866.416000	-335.823000	-546.284000	-1023.084000	-883.860000	-744.421000	-619.420700
55	18922.890000	1766.882000	23.873500	-386.812000	-888.801000	-748.873000	-637.425200	-399.781300
56	28984.320000	3086.963000	522.882100	-133.467600	-706.666000	-678.666000	-624.864000	-354.862000
57	40824.700000	4789.317000	1288.284000	155.746600	-526.792000	-480.141000	-388.614000	-231.216000
58	54808.890000	7924.250000	2441.950000	462.848700	-314.278400	-338.310000	-229.297400	-170.419900
59	69666.080000	11884.660000	4201.342000	848.281000	-88.965400	-134.866000	-52.708000	-98.348100
60	14066.140000	20431.320000	6874.466000	1522.251000	286.501000	91.233700	167.527000	678.162300
61	58173.790000	30889.640000	11581.830000	2282.377000	681.328600	362.177000	481.252900	890.781300
62	103887.000000	44072.290000	18106.100000	3308.880000	1133.830000	640.880000	860.858400	1138.956000
63	116863.000000	56881.810000	27675.220000	4754.879000	1889.644000	991.555100	974.057000	1423.593000
64	121969.700000	74289.270000	38669.160000	6648.184000	2387.875000	1389.956000	1389.786000	1732.162000
65	124899.700000	92202.470000	52869.400000	10203.730000	3337.486000	1884.030000	1659.210000	2046.800000
66	124879.900000	95642.180000	68876.830000	15642.370000	4255.428000	2388.740000	2082.559000	2487.680000
67	123664.890000	112858.810000	107578.100000	21278.100000	6116.884000	3014.288000	2666.830000	3825.320000
68	121692.000000	114677.800000	99598.140000	40585.770000	11483.540000	4758.870000	3662.384000	3788.514000
69	114678.000000	103683.800000	56657.800000	15643.840000	6460.774000	4097.738000	4295.365000	
70	113373.000000	105938.800000	7038.900000	1124.270000	758.850000	4771.768000	4631.821000	
71	111183.800000	105023.800000	68408.800000	28462.800000	19058.570000	9521.804000	9411.800000	
72	103395.800000	89786.310000	37713.800000	1281.810000	648.224000	6088.363000		
73	90000.700000	60284.800000	1854.800000	7466.278000	894.878000			
74	80000.700000	50284.800000	1854.800000	7466.278000	894.878000			
75	60044.200000	67662.730000	2674.260000	10774.650000	1685.148000			
76	89297.800000	73561.540000	32888.800000	12427.160000	10321.280000			
77	76654.310000	80218.910000	38218.800000	14826.970000	11858.420000			
78	73312.950000	46081.510000	17587.260000	13685.710000				
79	7644.400000	4888.810000	1135.510000	16741.810000				
80	74867.910000	52326.200000	26480.800000	10008.400000				
81	5408.730000	27889.800000	20403.800000					
82	65240.340000	30636.130000	22831.800000					
83	5467.270000	32786.800000	25646.800000					
84	53584.210000	24111.180000	26953.160000					
85	34833.870000	28385.130000						
86	34485.180000	28284.230000						
87	33862.800000	26875.770000						
88	32840.000000	28288.310000						
89	31938.020000	28987.870000						
90	30866.620000	3175.750000						
91	29487.360000	27387.870000						
92	28285.800000	2658.800000						
93	27189.800000	26878.130000						

Left: data after running macro in Excel
 Right: raw data with post-peak fluorescence manually excluded (blue) in GraphPad Prism



Excel macro: 'deleteaftermax'

```
Sub deleteaftermax()
```

```
Dim i As Integer
```

```
For i = 1 To ActiveSheet.UsedRange.Columns.Count
```

```
    If WorksheetFunction.Count(Columns(i)) > 0 Then
```

```
        Range(Columns(i).Find(What:=WorksheetFunction.Max(Columns(i)),  
LookIn:=xlFormulas, LookAt:=xlWhole).Offset(3), _
```

```
        Cells(Rows.Count, i).End(xlUp)).Clear
```

```
    End If
```

```
Next
```

```
End Sub
```