

ABI Taqman primer / probe design using Primer Express

1. Open Primer Express
2. Go to File
 - ☐ New
 - ☐ TaqMan Probe and Primer Design (can also choose MGB)
3. Select Import DNA file
 - Choose the text file which has the DNA sequence of interest
 - If this is an mRNA sequence and you want the probes to end up on exon-exon boundaries, mark these boundaries using the Junction tool (palette on left)
4. Click the Parameters tab
 - ☐ Do not change the default parameters (you can see More parameters by clicking that button).
5. Click the Primers tab
 - ☐ Go to Options
 - ☐ Find Primers and Probes now (this can take a minute or two)
6. A list of primer sets will be displayed
7. Scroll through the probe list and select a probe that has more Cs than Gs
8. With this probe (and its primers) highlighted, click on the Sequence tab
9. Using the Tool palette (on left), click on the Probe tool. Highlight the probe sequence which is contained within the green box. Once you highlight the sequence, it should turn to small green letters. This defines the probe.
10. Click on the Primers tab
 - ☐ Go to Options
 - ☐ Find Primers and Probes now (this can take a minute or two)
11. A new list of primer sets (all with the same probe) will be displayed.
12. Scroll through the Forward primer list and select a primer that has at least 3 As and/or Ts out of the last 5 bases on the 3' end of the primer. Highlight this primer/probe set.
13. Return to the Sequence Tab screen.
14. Using the Forward Primer tool, highlight the forward primer sequence. Once it has been highlighted, a blue arrow should appear under the primer sequence. The forward primer is now locked on.
15. Go back to the Primers tab
16. From this list, select a reverse primer that also has at least 3 As and/or Ts out of the last 5 bases on the 3' end. With this primer/probe set highlighted, go back to the Sequence tab.
17. Using the Reverse Primer tool, highlight the reverse primer sequence. After it is highlighted, it should have a blue arrow underneath it.
18. Go back to the Primer tab one more time.
19. This final search contains your primer and probe set.
20. If you hit the Order button on the bottom of the Primer screen, it gives the sequences of primers and probes and can be printed from here.
21. If you go to the Results tab, it will display this primer and probe set. If you hit Save Result, the program will save this search in the Archives. To retrieve it in the future, you can open it under Open Results (from the File menu).

If the first probe you pick doesn't allow primers that fit the 3' rule, then you will have to select another probe.