




**Applied Biosystems ViiA™ 7 实时荧光定量 PCR 仪 V1.X 相对定量简易操作流程**

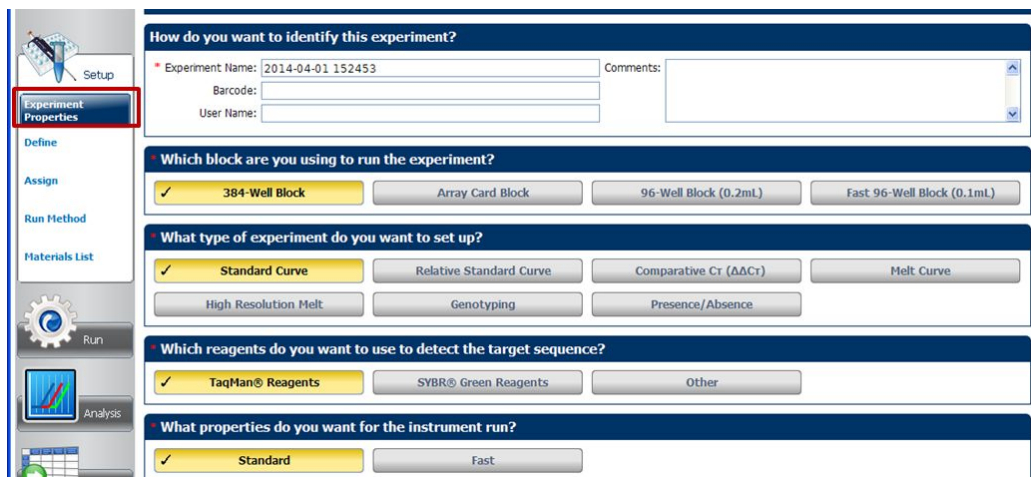


1. 双击桌面图标 ， 或从 Start > All programs > Applied

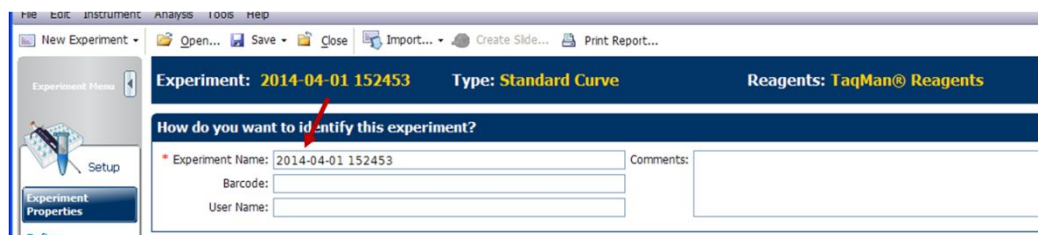
Biosystems > ViiA 7 Software > ViiA 7 Software v1.X 开启软件。进入主界面后选择“Experiment Setup”。



2. 选择“Setup”下的“Experiment Properties”界面。



2.1 输入实验名称 (Experiment Name)。



## 2.2 选择 Block 类型。



Which block are you using to run the experiment?

384-Well Block     Array Card Block     96-Well Block (0.2mL)     Fast 96-Well Block (0.1mL)

## 2.3 选择相对定量实验类型，“Comparative C<sub>T</sub>”。



What type of experiment do you want to set up?

Standard Curve     Relative Standard Curve     Comparative C<sub>T</sub> (ΔΔC<sub>T</sub>)     Melt Curve

High Resolution Melt     Genotyping     Presence/Absence

## 2.4 选择试剂种类。Taqman 探针法选择“Taqman Reagents”，SYBR 染料法选择“SYBR Green Reagents”。



Which reagents do you want to use to detect the target sequence?

TaqMan® Reagents     SYBR® Green Reagents     Other

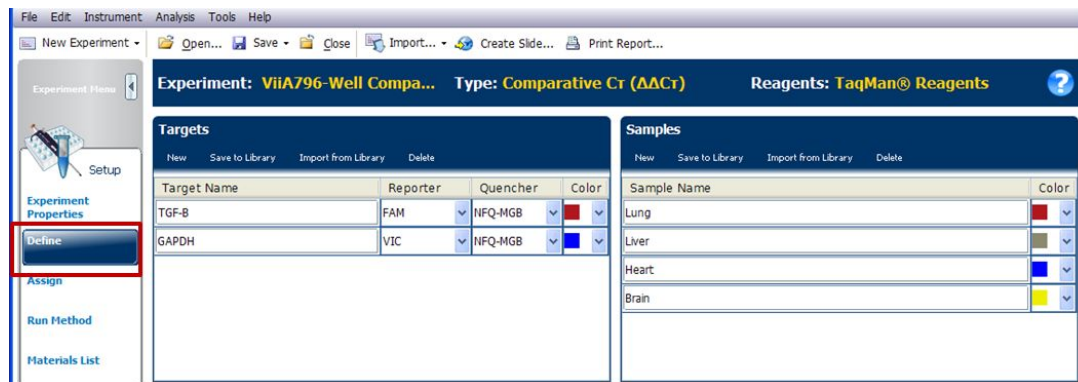
## 2.5 选择运行模式。普通试剂选择“Standard”，快速试剂选择“Fast”。



What properties do you want for the instrument run?

Standard     Fast

## 3. 选择“Setup”下的“Define”界面设置基因名称 (Target) 和样品名称 (Sample)。



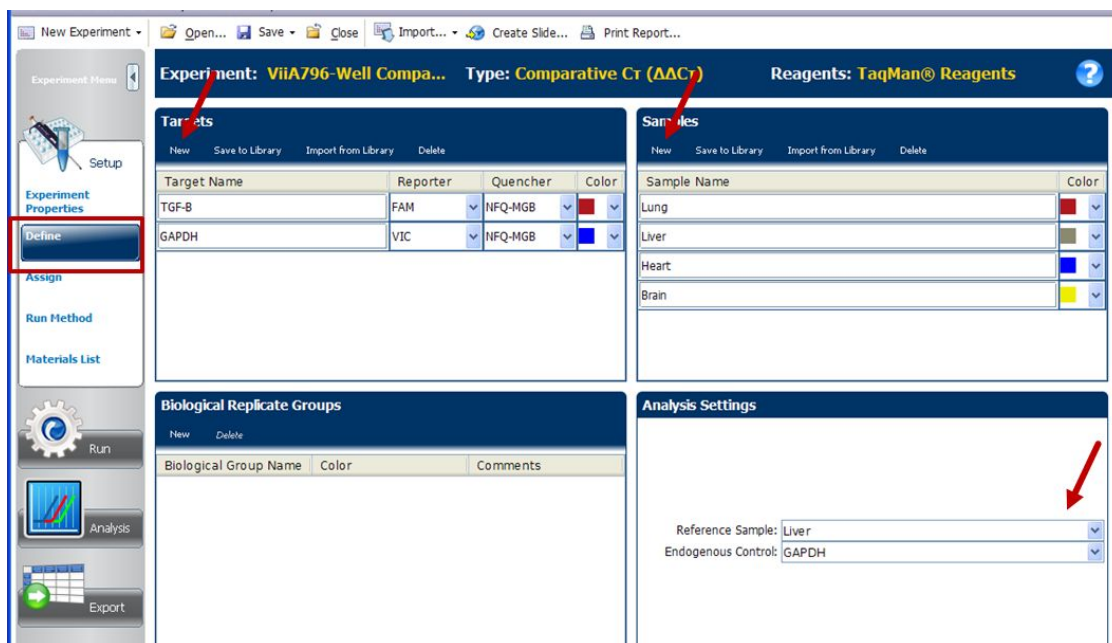
File Edit Instrument Analysis Tools Help

Experiment: ViiA796-Well Compa... Type: Comparative C<sub>T</sub> (ΔΔC<sub>T</sub>) Reagents: TaqMan® Reagents

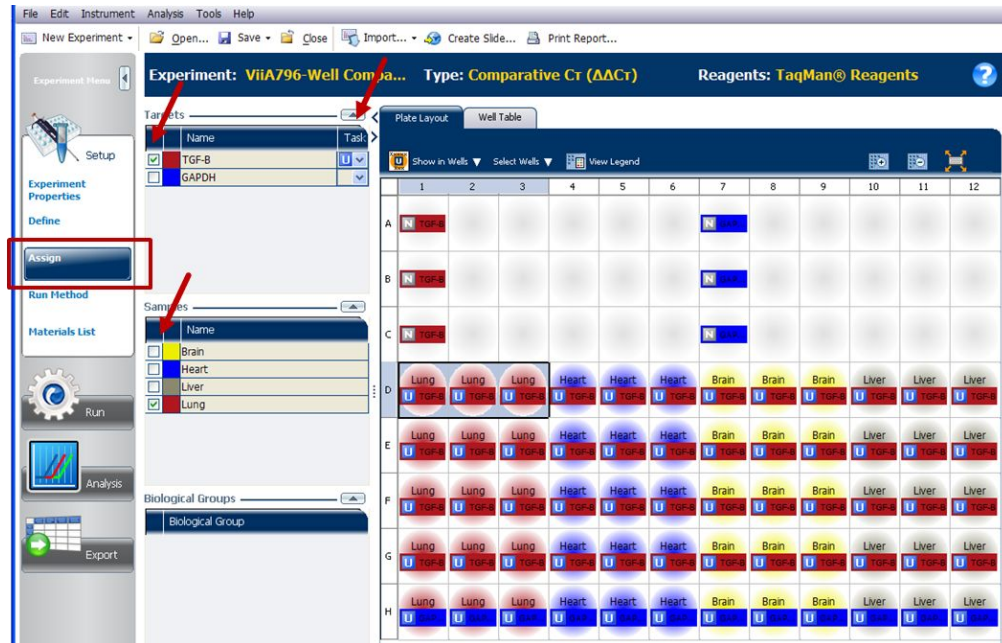
Targets			
Target Name	Reporter	Quencher	Color
TGF-β	FAM	NFQ-MGB	Red
GAPDH	VIC	NFQ-MGB	Blue

Samples	
Sample Name	Color
Lung	Red
Liver	Green
Heart	Blue
Brain	Yellow

- 3.1** 在“Targets”下点击“New”，添加待测基因。在“Target Name”中编辑基因名称，“Reporter”和“Quencher”中选择所标记的荧光基团及淬灭基团。对于“Quencher”的选择，如果是 MGB 探针，请选择 NFQ-MGB；如果是 TAMRA 探针，请选择 TAMRA；如果是其他形式的非荧光淬灭基团则选择 None。
- 3.2** 在“Samples”下点击“New”，添加待测样品。在“Sample Name”中编辑样品名称。
- 3.3** 在“Analysis Settings”下选择合适的“Reference Sample”（对照样品）和“Endogenous Control”（内参基因）。



4. 选择“Setup”下的“Assign”界面编辑样品板。利用鼠标单选或拖拽以选择反应孔，然后勾选左侧的基因及样本，同时在“Task”选项中指定该反应孔的类型 (U 代表未知样本，N 代表阴性对照)。



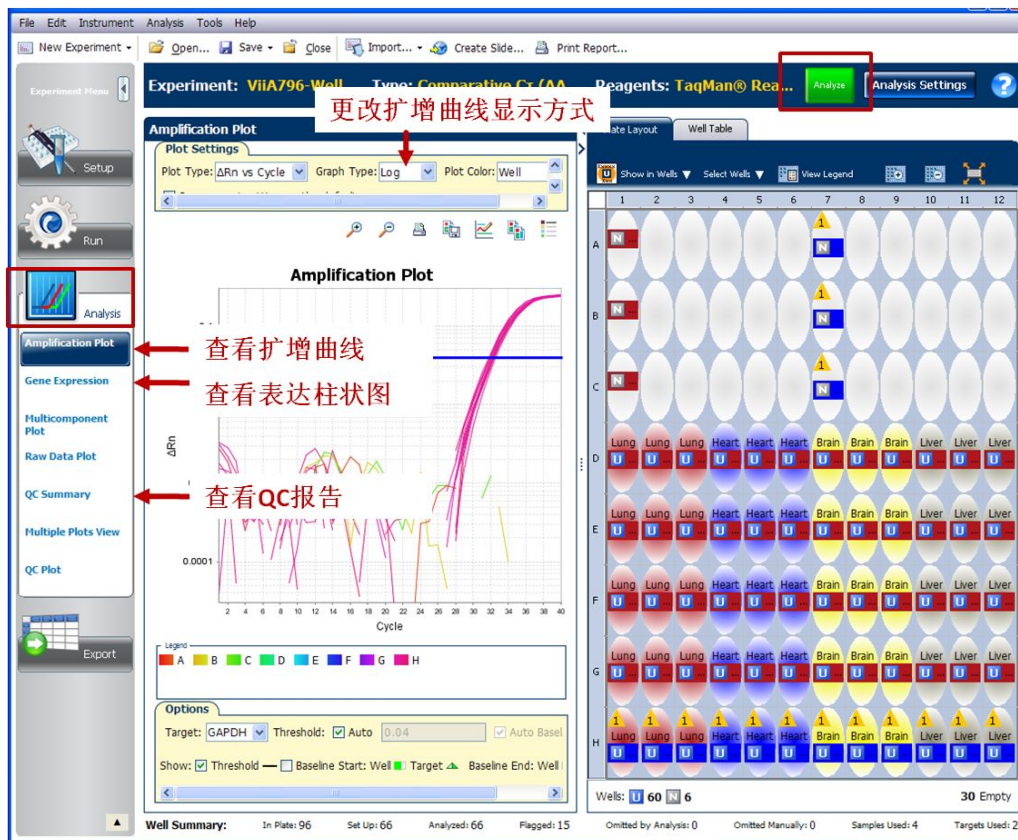
5. 选择“Setup”下的“Run Method”界面，编辑运行条件。



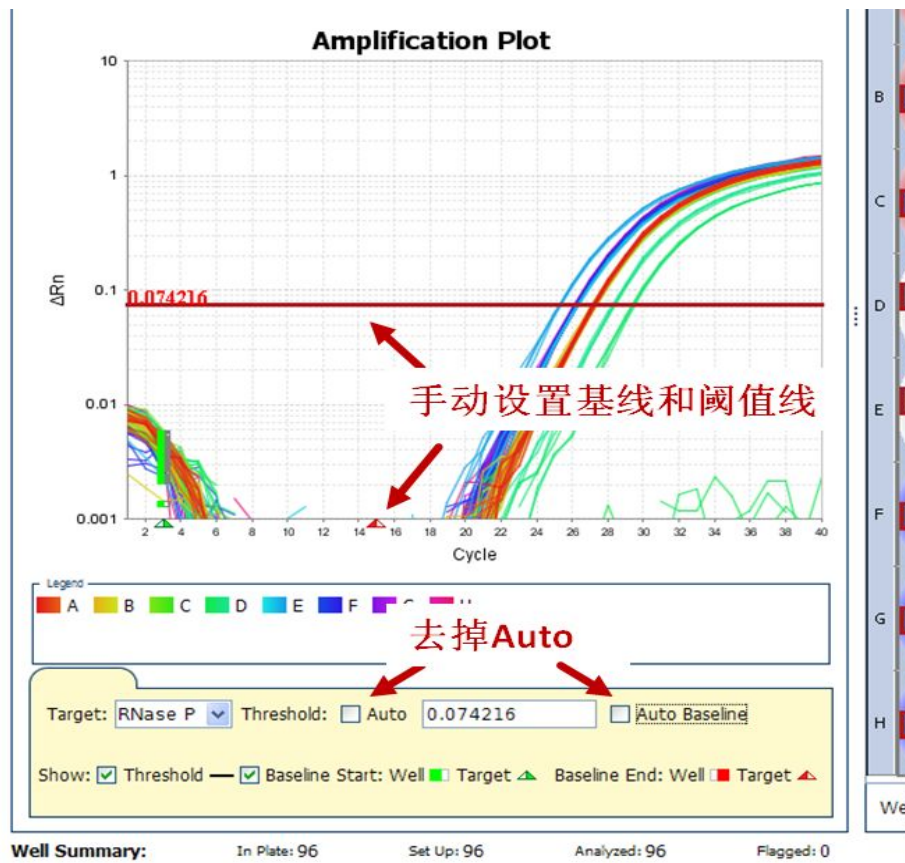
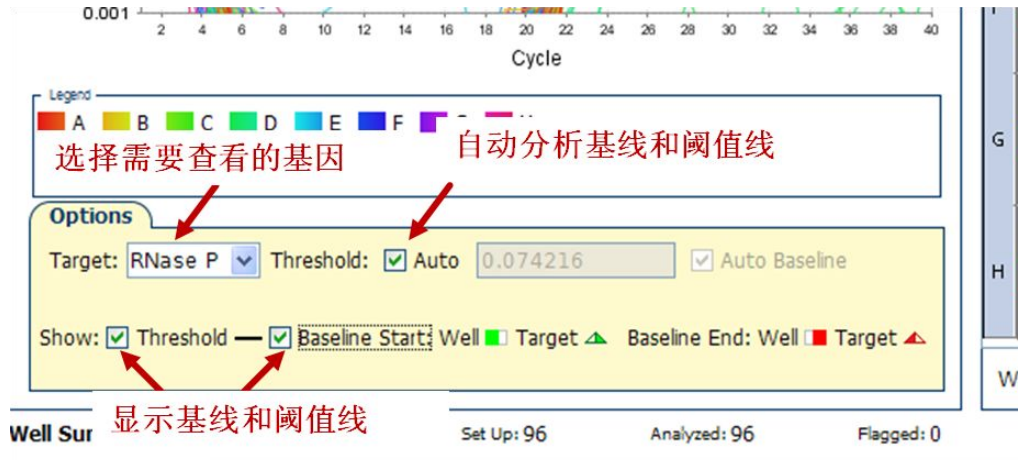
6. 选择“Run”下的“Amplification Plot”界面，点击“Save As”保存文件，点击“Start Run”开始运行。



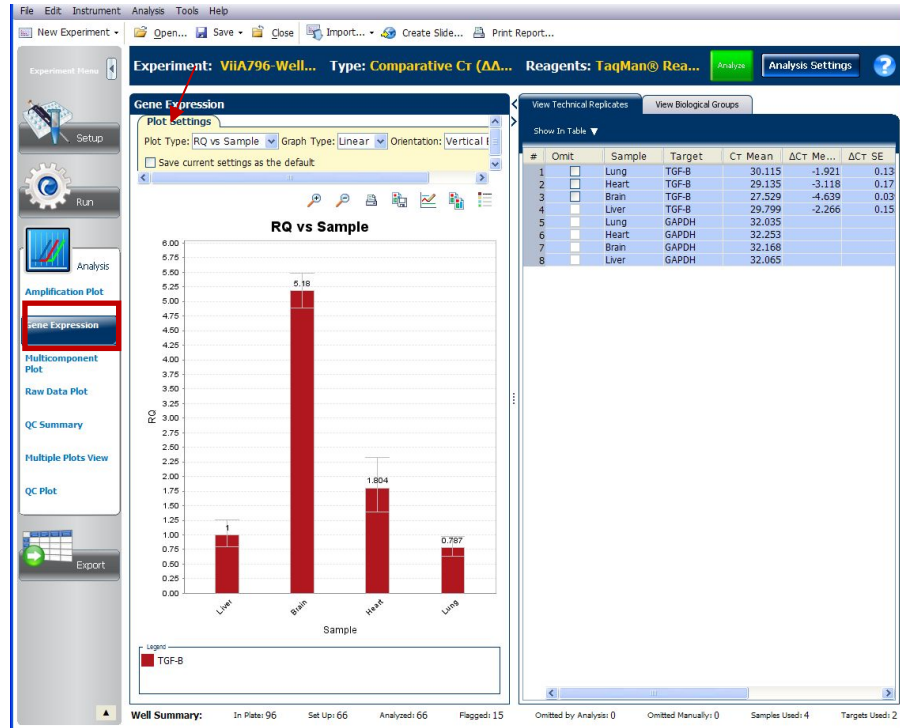
7. 实验运行结束后，进入“Analysis”界面，点击右上角的“Analyze”按钮分析数据并查看扩增结果。



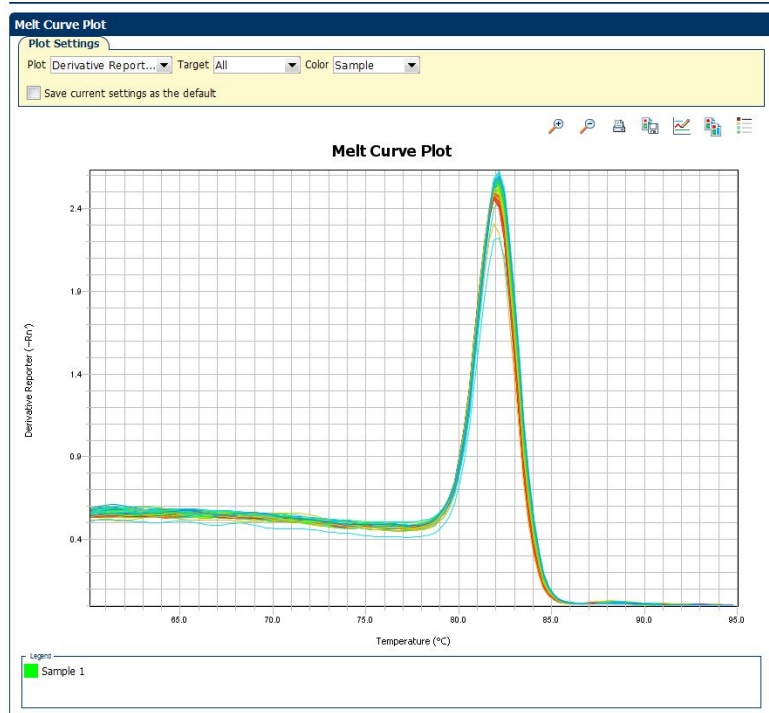
**7.1 设置基线和阈值线：** 软件默认使用“Auto”功能自动设定基线和阈值线。查看阈值线或基线：选择需要查看的基因，将 show 后的“Threshold”及“Baseline”选择打勾。扩增曲线图上会出现相应的基线范围和阈值线。



7.2 点击“Gene Expression”查看基因表达柱状图。



7.3 对于 SYBR Green 实验，在“Melt Curve Plot”界面中查看熔解曲线。





7.4 查看“QC Summary”结果：反应孔存在异常情况时，会出现黄色三角，数字 1 代表有一种情况，2 代表有两种情况，以此类推。详细信息及解决方案可以在“Flag Details”中查看。

The screenshot shows the 'QC Summary' window. On the left is a sidebar with buttons for Setup, Run, Analysis, Amplification Plot, Standard Curve, Multicomponent Plot, Raw Data Plot, **QC Summary** (highlighted), and Multiple Plots View. The main window is divided into two panes. The left pane, titled 'Flag Details', contains a table of flags:

Flag	Description	Freq...	Wells
AMPNC	Amplification in negative control	3	H3, H6, H12
BADROX	Bad passive reference signal	0	
OFFSCALE	Fluorescence is offscale	0	
HIGHSD	High standard deviation in replicat...	12	A1, A2, A3, A...
NOAMP	No amplification	0	
NOISE	Noise higher than others in plate	1	H12
SPIKE	Noise spikes	1	H12
NOSIGN...	No signal in well	0	
OUTLIE...	Outlier in replicate group	0	
EXPFAIL	Exponential algorithm failed	0	
BLFAIL	Baseline algorithm failed	0	
THOLD...	Thresholding algorithm failed	48	A7, A8, A9, A...
CTFAIL	Cr algorithm failed	1	H9

Below the table, a 'Flag Details' section for 'NOISE' is shown, with a red arrow pointing to a link: 'View NOISE Troubleshooting Information'. A red text annotation '点击查看解决方案' (Click to view solution) is placed over this link. At the bottom of the window, a 'Well Summary' shows: In Plate: 96, Set Up: 96, Analyzed: 96, Flagged: 56. The right pane shows a 'Well Table' with a grid of wells (A1-H12) and yellow triangles indicating flagged wells. A status bar at the bottom right shows 'Wells: 84 0 12'.

8. 数据导出：在“Export”界面下导出需要的数据。

The screenshot shows the 'Export' window. The left sidebar has buttons for Setup, Run, Analysis, **Export** (highlighted), and Export. The main window has options for 'Auto Export', 'Format: ViiA™ 7', 'Export Data To: One File', and 'Open file(s) when export is complete'. The 'Export File Location' is 'D:\Applied Biosystems\ViiA7 Softw...' and the 'Export File Name' is 'ViiA7 96-Well St...'. A red text annotation '选择需要导出的数据' (Select data to export) points to the 'Results' checkbox in the 'Select Content' section. The 'Select Content' section has a list of checkboxes: All Fields, Well, Well Position, Sample Name, Sample Color, Biogroup Name, Biogroup Color, Target Name, Target Color, and Task. Below this is a table of well data:

Well	Well Po...	Sample ...	Sample ...	Biogrou...	Biogrou...	Target ...
1 A1	5K	RGB(176,2...				RNase P
2 A2	5K	RGB(176,2...				RNase P
3 A3	5K	RGB(176,2...				RNase P
4 A4	5K	RGB(176,2...				RNase P
5 A5	5K	RGB(176,2...				RNase P
6 A6	5K	RGB(176,2...				RNase P
7 A7	5K	RGB(176,2...				RNase P
8 A8	5K	RGB(176,2...				RNase P
9 A9	5K	RGB(176,2...				RNase P
10 A10	5K	RGB(176,2...				RNase P
11 A11	5K	RGB(176,2...				RNase P
12 A12	5K	RGB(176,2...				RNase P
13 B1	5K	RGB(176,2...				RNase P
14 B2	5K	RGB(176,2...				RNase P
15 B3	5K	RGB(176,2...				RNase P
16 B4	5K	RGR/176,2...				RNase P

At the bottom are buttons for 'Start Export', 'Save Export Set As', and 'Load Export Set'.

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