Takara Bio USA, Inc.

SmartChip® MyDesign Kit User Manual

Cat. Nos. 640032 & 640036
(102124)

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I. Introduction

A. Thank You for Your Order!

Congratulations on the purchase of the **SmartChip MyDesign Kit, 150 nl** (Cat. Nos. 640032 & 640036). These chips are designed to run up to 5,184 real-time PCR reactions at once on the SmartChip ND™ Real-Time PCR System (Takara Bio, Cat. No. 640290) or the SmartChip Real-Time PCR System (Cat. No. 640022). See Figure 1 (next page) for the protocol overview.

NOTE: The SmartChip system is intended for Research Use Only and is not approved for use as a diagnostic tool for the treatment of patients.

B. About this Manual

This manual provides instructions for preparing samples and PCR assays for use with the SmartChip system. Please follow these directions, paying special attention to information designated as follows:

NOTE	Helpful ancillary information
IMPORTANT	Information on proper system information
WARNING	Instructions for safe operation of Takara Bio instruments

C. Technical Support

Review the information in this manual thoroughly before starting your reactions. Also review documentation supplied with the accessory equipment you are using. If you require technical support, you can contact your authorized Takara Bio service technician or send email to field_support@takarabio.com.

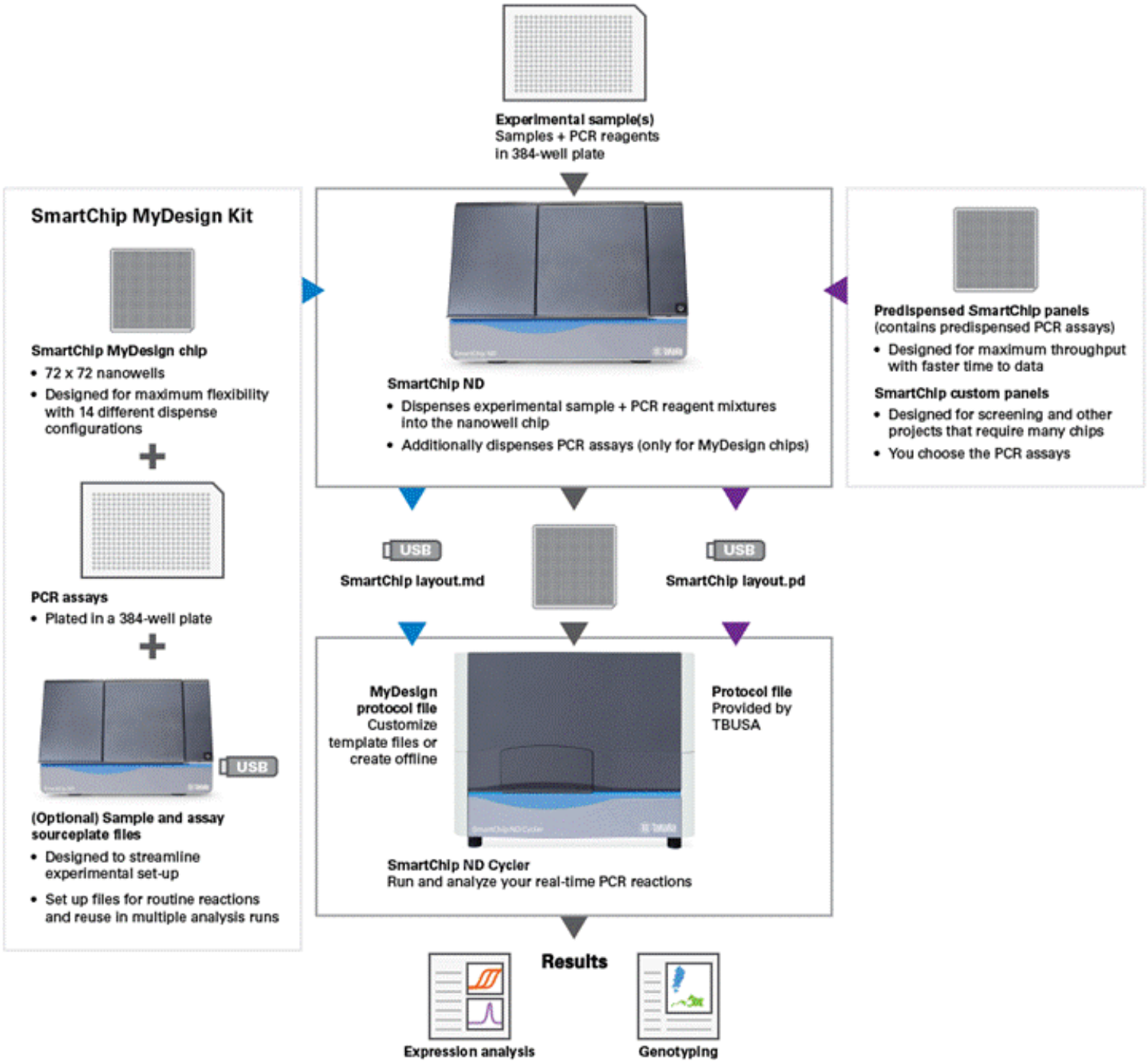

Figure 1. Overview of the SmartChip ND system protocol. (Cat. No. 640290) Although not depicted, the workflow is identical on the SmartChip Real-Time PCR System (Cat. No. 640022).

II. List of Components

SmartChip nanowell chips are thin metal alloy chips with 5,184 precision-manufactured nanowells (72 x 72) designed for real-time applications. SmartChip MyDesign chips are supplied empty. You will need to use the SmartChip ND (SmartChip ND dispenser or dispenser), part of the SmartChip ND Real-Time PCR System (Cat. No. 640290), or the SmartChip MultiSample Nanodispenser (SmartChip MSND), part of the SmartChip Real-Time PCR System (Cat. No. 640022), to fill the chip first with sample mixtures, and then with PCR assay mixtures appropriate for your application (i.e., PCR primer pairs for intercalating dye-based real-time PCR or primer/probe sets for probe-based real-time PCR).

Table 1. SmartChip MyDesign Kit, 150 nl components.

SmartChip MyDesign Kit, 150 nl (Store at room temperature)	640032 (1 Chip)	640036 (20 Chips)
SmartChip MyDesign Chip	1 each	20 each
Blotting Paper	2 each	4 each
Nanodispenser Chip Intermediate Film	1 each	2 each
Cycler Sealing and Pressure Film	1 each	2 each

III. Additional Materials Required (Not Provided)

- Nanodispenser 384-Well Source Plate and Seal (Takara Bio, Cat. No. 640018 [20/Pack] or 640037 [120/Pack])

  IMPORTANT: The Nanodispenser 384-Well Source Plate is used as a reservoir for the SmartChip ND dispenser and SmartChip MSND. This specific brand and model is required.

- Ice bucket and/or cold rack
- Calibrated pipette and nuclease-free aerosol-resistant tips
- Vortex
- Centrifuge capable of spinning tubes, 96-well plates, and 384-well plates at 2,750*g*
- Nuclease-free 1.5 ml tubes (from any supplier)
- 0.2 ml nuclease-free PCR tubes and 96-well PCR plates and sealing film (from any supplier)

For mRNA Expression Analysis via Intercalating Dye-Based Real-Time PCR

- Standard thermal cycler that can accommodate your RT reaction tubes or plates
- 1X TE, pH 8.0 (from any supplier)
- PrimeScript 1st strand cDNA Synthesis Kit (Cat. No. 6110A or 6110B)
- Nuclease-free PCR-grade water (from any supplier)
- PCR assays: PCR primer sets for intercalating dye-based real-time PCR that your lab has used successfully in routine real-time PCR (from any supplier)
- SmartChip TB Green Gene Expression Master Mix (Takara Bio, Cat. No. 640211)

  NOTE: Precipitate may be observed in the SmartChip TB Green Gene Expression Master Mix. This precipitate does not affect the performance of the kit. The precipitate can be dissolved easily by warming to room temperature and mixing for a few minutes. Ensure that the precipitate is fully dissolved before use.

For mRNA Expression Analysis via Probe-Based Real-Time PCR

- Standard thermal cycler that can accommodate your RT reaction tubes or plates
- ROX Reference Dye (50X; Thermo Fisher Scientific, Cat. No. 12223-012)
- 1X TE, pH 8.0, nuclease-free
- PrimeScript 1st strand cDNA Synthesis Kit (Cat. No. 6110A or 6110B)
- SmartChip Probe qPCR Master Mix (Takara Bio, Cat No 640209)
- Nuclease-free PCR-grade water (from any supplier)
- PCR assays: PCR primer/FAM-labeled probe sets for probe-based (5' nuclease or hydrolysis probe-based) real-time PCR that your lab has successfully used in routine qPCR. We have tested PrimeTime qPCR Assays (Integrated DNA Technologies, Inc.) and TaqMan Gene Expression Assays (Thermo Fisher Scientific). In principle, the SmartChip system can be used with other fluorescent dyes; please contact Takara Bio Technical Support for current information.

For SNP Genotyping Analysis

- 1X TE, pH 8.0 (from any supplier)
- SmartChip Probe qPCR Master Mix (Takara Bio, Cat. No. 640209)
- Nuclease-free PCR-grade water (from any supplier)

IV. General Considerations

A. Sample Requirements

RNA for mRNA Expression Analysis

High-quality RNA at 70–100 ng/µl in RT- and PCR-compatible buffer (e.g., water or 1X TE)

- **Purity:** RNA can be purified using any method, but should be free of contaminants, including RT and PCR inhibitors and genomic DNA (gDNA)—you may want to treat your RNA with DNase to remove gDNA
- **Integrity:** We recommend using highly intact RNA (free of degradation) with an RNA Integrity Number (RIN) ≥8, if possible (as measured on an Agilent Bioanalyzer)

DNA for SNP Genotyping Analysis

High-quality gDNA in PCR-compatible buffer (e.g., water or 1X TE)

B. How to use the SmartChip MyDesign Kit

SmartChip MyDesign Chips are designed for use with the SmartChip system. First, fill the chips using the SmartChip ND dispenser with mixtures containing your experimental cDNA or genomic DNA samples plus PCR reagents. Seal and spin the chip, then dispense mixtures containing your PCR assays (primer sets) and PCR reagents into the same MyDesign Chip. Finally, place your filled chip on the SmartChip Cycler, program the instrument to run your real-time PCR reactions, capture data, and analyze your results. We currently support the use of SmartChip MyDesign Chips for mRNA expression analysis and SNP genotyping.

1. mRNA Expression Analysis

For mRNA expression analysis, the SmartChip Real-Time PCR System has been tested with cDNA synthesized from total RNA using the PrimeScript™ 1st strand cDNA Synthesis Kit

(Cat. No. 6110A or 6110B) and SmartChip TB Green® Gene Expression Master Mix (Takara Bio, Cat. No. 640210). The SmartChip system can be used with other fluorescent dyes; contact Takara Bio technical support for current information. The SmartChip system also supports green intercalating dye-based real-time PCR for the analysis of microRNA and long noncoding RNA.

NOTE: Precipitate may be observed in the SmartChip TB Green Gene Expression Master Mix. This precipitate does not affect the performance of the kit. The precipitate can be dissolved easily by warming to room temperature and mixing for a few minutes. Ensure that the precipitate is fully dissolved before use.

2. SNP Genotyping

For SNP Genotyping, the SmartChip Real-Time PCR System has been tested with SmartChip Probe qPCR Master Mix (Takara Bio, Cat. No. 640208).

C. Precautions for Avoiding RT-PCR and PCR Contamination

1. Avoiding RNases When Working With RNA

Reverse transcription (RT)-PCR is susceptible to contamination with RNases from equipment, consumables, and reagents that can lead to false-negative results. Here are some tips for avoiding RNase contamination:

- Wear powder-free laboratory gloves and use dedicated pipettes with nuclease-free, aerosol-resistant tips
- Use nuclease-free, disposable plastic ware and keep plates, tubes, and tip dispensers closed when possible
- Store RNA at –70°C and avoid multiple freeze/thaw cycles
- Store nucleases away from reagents used for cDNA production and reactions containing RNA
- Use proper microbiological aseptic technique when working with RNA, as dust particles are a common source of ribonuclease contamination

2. Avoiding Contamination with PCR Product from Previous Reactions

PCR assays are subject to false-positive results from the carryover of DNA from previous amplifications. To prevent this, we recommend that you take the following precautions:

- **Never bring amplified PCR products into the PCR setup area.** Maintain separate work areas for sample preparation/PCR setup and PCR amplification. Use equipment, consumables, and laboratory coats that are dedicated to pre- or post-PCR handling.
- Wipe down lab benches daily with a 10% hypochlorite solution or other PCR decontamination product after use. If possible, further decontaminate the work area using ultra-violet light radiation.
- Dispense PCR reagents into small-volume aliquots to limit handling and freeze/thaw cycles.
- Pulse-spin reagent tubes before opening. Uncap and close tubes carefully to prevent aerosols.

V. Protocol: mRNA Expression Analysis Using Intercalating Dye-Based PCR

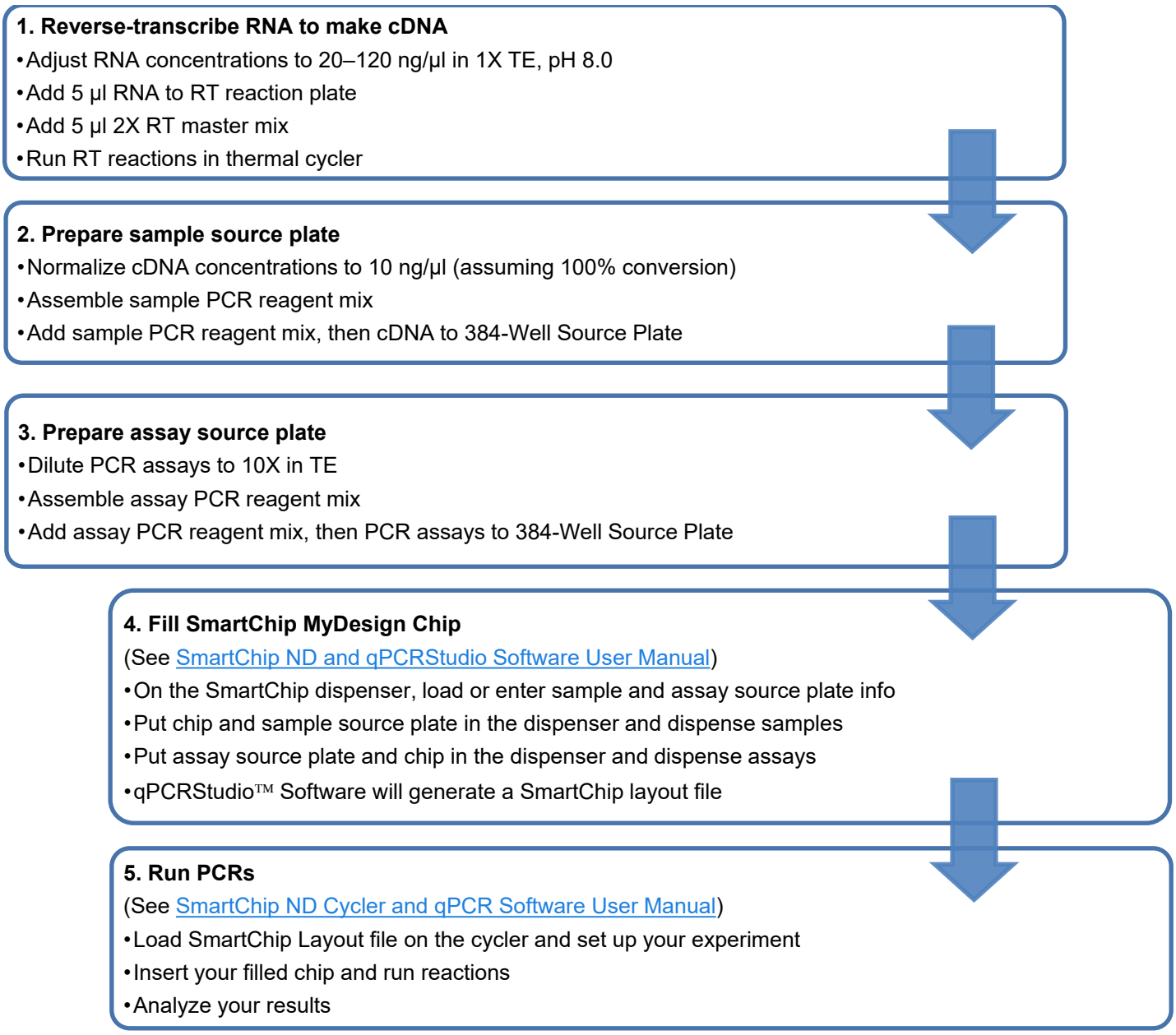

Figure 2. Procedure overview for dye-based mRNA expression analysis. Steps 1–3 are done on the bench; Steps 4 & 5 are performed in the SmartChip system.

A. Reverse Transcription of RNA Sample(s) to Generate cDNA

When working with RNA, it is critical that large amounts of high-quality cDNA is generated. In principle, any method compatible with RT-qPCR can be used with the SmartChip system. However, we recommend using our PrimeScript 1st strand cDNA Synthesis Kit (Cat. Nos. 6110A or 6110B). This kit is powered by PrimeScript Reverse Transcriptase, which has exceptionally strong strand-displacement and extension capabilities that can synthesize up to 12 kb cDNA, high specificity and efficiency, works with challenging GC-rich of secondary structure templates, and exhibits outstanding accuracy.

For details on how to use this kit, please refer to the PrimeScript 1st Strand cDNA Synthesis Kit User Manual.

B. Preparation of Sample Source Plate

This section describes how to mix your cDNA samples with real-time PCR master mix and distribute the resulting Sample/PCR reagent mix to the wells of a 384-Well Source Plate (sample source plate).

Plan to plate your samples into specific wells in the sample source plate and record their locations in one of the following ways:

- **Follow a sample source plate layout guide:** Follow the sample source plate layout guides (maps) in the SmartChip Dispenser Software. Alternatively, place the SmartChip plate layout guide corresponding to your SmartChip layout in the plate lid, under your sample source plate, and use it as a pipetting guide.
  These maps indicate samples with numbers; they include a single replicate of each reaction. To run multiple replicates, use the Sample/PCR reagent mixture for more than one sample shown in the source plate map. Record this in the sample source plate file or in a spreadsheet for transfer to the Dispenser Software.
- **Reuse a sample source plate layout from a previous experiment:** If you are analyzing a sample set more than once, you can enter your sample information and locations in the source plate into the SmartChip Dispenser Software and save the resulting sample source plate file for use in subsequent experiments.
- **Prepare sample source plate files with your own software:** If you are processing many samples or are using an automated sample preparation system, you may want to prepare sample source plate files in a text editor, then load them into the SmartChip Dispenser Software. See the SmartChip ND dispenser manual for instructions.

1. Thaw cDNA sample(s) on ice or a cold rack. Thaw nuclease-free PCR-grade water at room temperature, and then place on ice or a cold rack.
2. If necessary, add 1X TE, pH 8.0 to the RT reactions to normalize the cDNA concentrations to 10 ng/µl. Assume 100% conversion of RNA to cDNA in the RT reaction. Table 2 below indicates the volume of 1X TE, pH 8.0 to add to 10-µl RT reactions to bring the final concentration to 10 ng/µl.

Table 2. Normalizing cDNA concentrations, intercalating dye-based PCR mRNA expression analysis.

Chip format					
Assays	Samples	Replicates	RNA input to RT rxn (ng)	RNA concentration in RT rxn (ng/µl)	Volume of 1X TE to add (µl)
12–144	384–36	1	100	10	–
		4	200	20	10
216	24	1	100	10	–
		4	400	40	30
248	20	1	100	10	–
		4	400	40	30
296	16	1	200	20	10
		4	600	60	50
384	12	1	200	20	10
		4	600	60	50

3. Prepare a sample PCR reagent mix in a nuclease-free tube on ice or a cold rack. See Table 3 for volumes. Close the tube and vortex gently to mix well. Place on ice or a cold rack. Minimize light exposure to the SmartChip TB Green Gene Expression Mix.

Table 3. Sample PCR reagent mix preparation, intercalating dye-based PCR mRNA expression analysis.

Chip format		SmartChip TB Green Gene Expression Mix (2X) (µl)	Nuclease-free PCR-grade water (µl)	Total volume (µl)
Assays	Samples			
12	384	2,350	1,410	3,760
24	216	1,430	858	2,288
36	144	1,039	623	1,662
48	108	850	510	1,360
54	96	784	470	1,254
72	72	652	391	1,043
80	64	610	366	976
96	54	556	333	890
120	42	493	296	788
144	36	458	275	733
216	24	523	313	836
248	20	481	289	770
296	16	610	366	976
384	12	523	313	836

4. On ice, add the sample PCR reagent mix and then cDNA samples to a 384-Well Source Plate (this will be your sample source plate) as outlined below (see Table 4, next page).
   a. Dispense sample PCR reagent mix into wells of the 384-Well Source Plate using the volume appropriate for your SmartChip format, following the sample source plate map.
   b. Add the indicated volume of cDNA to each well containing PCR reagent mix, following the sample source plate map.

IMPORTANT: It is very important that you plate your samples into specific wells in the sample source plate. You will need multiple wells of each cDNA sample for some SmartChip formats.

- You can place the SmartChip source plate layout guide for your chip format in the plate lid, under your source plate to serve as a pipetting guide.
- Alternatively, you can find sample source plate layout guides (maps) in the SmartChip Dispenser Software.
- Finally, you can load the sample source plate file for the sample set from previous runs into the SmartChip Dispenser Software and use it as a pipetting guide (map).

   c. Seal the plate and vortex vigorously to mix well. Centrifuge for 5 min at 2,750*g*.

Table 4. Dispense volumes for sample source plate, intercalating dye-based PCR mRNA expression analysis.

Chip format		4a. Sample PCR reagent mix per well (µl)	4b. cDNA at 10 ng/µl per well (µl)
Assays	Samples		
12	384	9.4	2.3
24	216	9.9	2.5
36	144	10.5	2.6
48	108	11.2	2.8
54	96	11.5	2.9
72	72	12.4	3.1
80	64	12.9	3.2
96	54	13.7	3.4
120	42	15.2	3.8
144	36	16.2	4.1
216	24	14.3	3.6
248	20	15.5	3.9
296	16	12.9	3.2
384	12	14.3	3.6

C. Preparing the Assay Source Plate

This section describes how to mix your PCR assays with real-time PCR master mix and distribute the resulting Assay/PCR reagent mix to wells of the assay source plate.

Set up your assay source plate following the same general recommendations described above for the sample source plate. You will need to enter PCR assay information into the SmartChip Dispenser Software that runs the SmartChip ND dispenser; PCR assay information is stored in assay source plate files.

IMPORTANT: Immediately after completing this procedure, you will need to dispense the mixtures into your SmartChip MyDesign Chip and start your reactions.

– Plan for adequate time to dispense your reagents and run your real-time PCR

– Power on the SmartChip ND dispenser and run the daily warmup procedure before starting this part of the protocol

1. Thaw PCR Assays and dilute with 1X TE to 10X on ice, as described in Steps 1a–1b below. Use nuclease-free 1X TE, pH 8.0 and plate 10X PCR assays in a nuclease-free 96-well plate on ice. We recommend that you plate the assays as shown in Appendix B.

   a. Prepare the volume of 10X PCR assay shown for your SmartChip format (Table 5, next page). We recommend that you prepare enough diluted PCR assay for 10 SmartChip MyDesign Chips, but the table also shows the amount needed for a single chip in brackets and gray text.

   b. Plate the diluted PCR assays into a nuclease-free 96-well PCR plate in the configuration shown for your SmartChip format in Appendix B.

Table 5. PCR assay volumes by chip format, intercalating dye-based PCR mRNA expression analysis.

Chip format		[Volume for 1 chip (µl)]	Volume for 10 chips (µl)
Assays	Samples		
12	384	[18]	158
24	216	[11]	79
36	144	[8]	45
48	108	[7]	39
54	96	[7]	38
72	72	[7]	34
80	64	[7]	33
96	54	[6]	32
120	42	[6]	30
144	36	[6]	29
216	24	[6]	27
248	20	[6]	27
296	16	[6]	26
384	12	[14.3]	26

2. Prepare the assay PCR reagent mix in a nuclease-free tube on ice or a cold rack, following Table 6. Close the tube and vortex gently to mix well. Place on ice or a cold rack. Minimize light exposure to the SmartChip TB Green Gene Expression Mix.

Table 6. Assay PCR reagent mix preparation, intercalating dye-based PCR mRNA expression analysis.

Chip format		SmartChip TB Green Gene Expression Mix (2X) (µl)	Nuclease-free PCR-grade water (µl)	Total volume (µl)
Assays	Samples			
12	384	523	314	836
24	216	523	314	836
36	144	458	275	733
48	108	523	314	836
54	96	556	334	890
72	72	652	391	1,043
80	64	699	419	1,118
96	54	784	470	1,254
120	42	911	547	1,458
144	36	1,039	623	1,662
216	24	1,430	858	2,288
248	20	1,613	968	2,580
296	16	1,870	1,122	2,992
384	12	2,350	1,410	3,760

3. On ice, add the assay PCR reagent mix and then 10X PCR Assays to a 384-Well Source Plate (this will be your assay source plate) as outlined below (see Table 7, next page).

   a. Dispense assay PCR reagent mix into wells of the 384-Well Source Plate using the volume appropriate for your SmartChip format, following the assay source plate map.

b. Add the indicated volume of 10X PCR Assay to each well containing PCR reagent mix, following the assay source plate map.

NOTES:
- It is very important that you plate your assays into specific wells in the assay source plate. You can find the assay source plate layout guides (maps) in the SmartChip Dispenser Software.
- You will need to put reagents into multiple wells for some SmartChip formats.

c. Seal the plate and vortex vigorously to mix well. Centrifuge for 5 min at 2,750*g*.

Table 7. Dispense volumes for assay source plate, intercalating dye-based PCR mRNA expression analysis.

Chip format		3a. Assay PCR reagent mix per well (µl)	3b. 10X PCR Assay per well (µl)
Assays	Samples		
12	384	14.3	3.6
24	216	14.3	3.6
36	144	16.2	4.1
48	108	14.3	3.6
54	96	13.7	3.4
72	72	12.4	3.1
80	64	12.1	3.0
96	54	11.5	2.9
120	42	10.9	2.7
144	36	10.5	2.6
216	24	9.9	2.5
248	20	9.8	2.4
296	16	9.6	2.4
384	12	9.4	2.3

4. Dispense reagents into your SmartChip MyDesign Chip with the SmartChip ND dispenser. See the instructions in the SmartChip ND and qPCRStudio Software User Manual.
   a. Program the instrument for your experiment: specify the chip format, the type of analysis, and chip identification number.
   b. Load the Sample and assay source plate files or enter new Sample and Assay information to create new source plate files.
   c. Place your empty SmartChip MyDesign Chip and sample source plate on the dispenser and dispense Sample/PCR reagent mixes into your chip.
   d. Seal the chip and spin.
   e. Load the assay source plate on the dispenser and dispense Assay/PCR reagent mixes into the chip. The SmartChip ND dispenser will create a SmartChip Layout file.
5. Run the real-time PCR and analyze data using the SmartChip Cycler. See the instructions in the SmartChip ND Real-Time PCR Cycler and Software User Manual. To run your PCR reactions, you'll load your SmartChip MyDesign Chip into the SmartChip Cycler and program the instrument with information about your experiment, including the SmartChip Layout file from Step 4e. The SmartChip Cycler is designed to run the reactions, capture the data, and help you analyze your results.

VI. Protocol: mRNA Expression Analysis using Probe-Based PCR

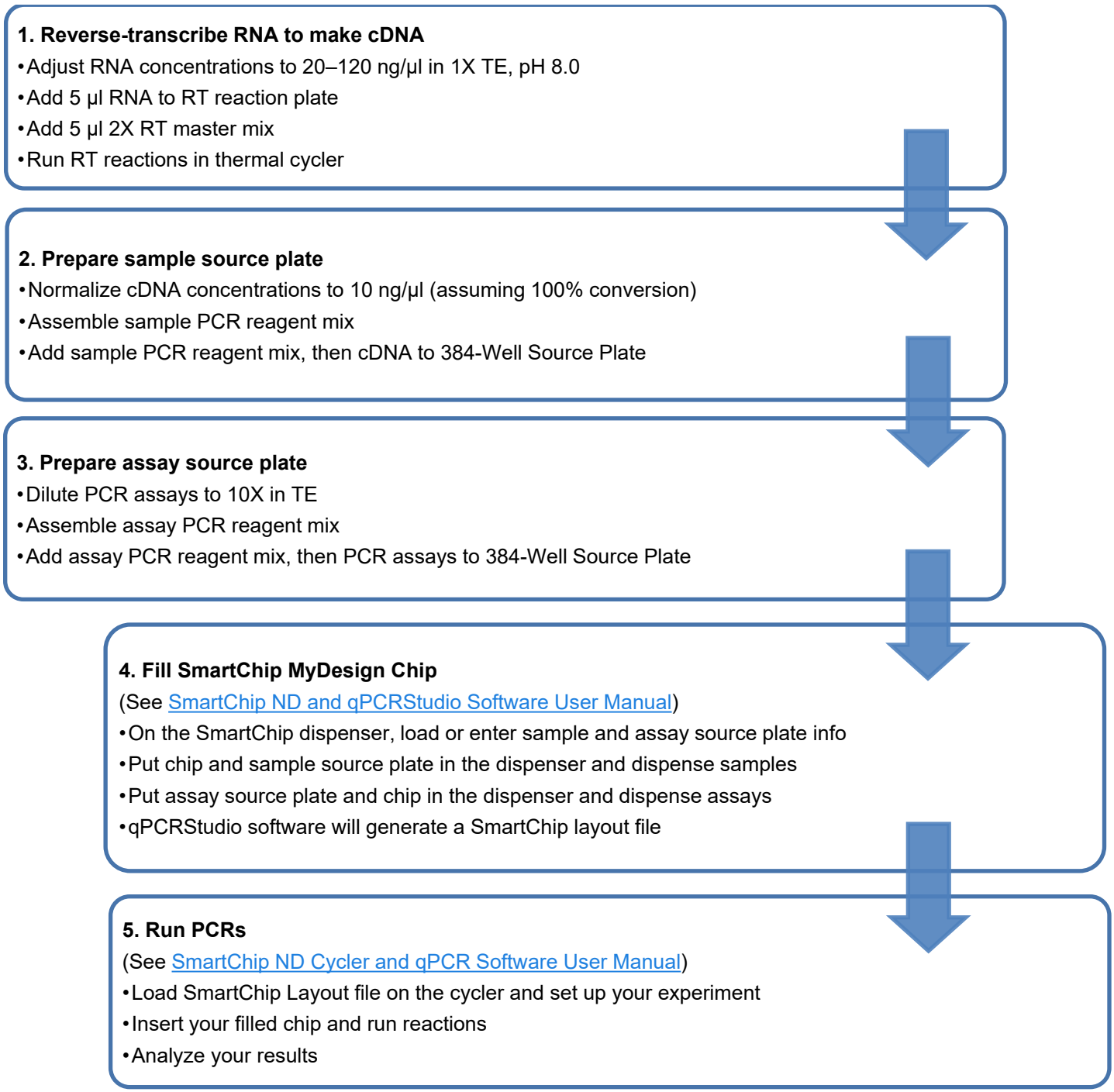

Figure 3. Procedure overview for probe-based mRNA expression analysis. Steps 1–3 are done on the bench; Steps 4 & 5 are performed in the SmartChip system.

A. Reverse Transcription of RNA Sample(s) to Generate cDNA

Please refer to the PrimeScript 1st Strand cDNA Synthesis Kit User Manual (also see Section VI.A).

B. Preparation of Sample Source Plate

This section describes how to mix your cDNA samples with real-time PCR master mix and distribute the resulting Sample/PCR reagent mix to the wells of the 384-Well Source Plate (sample source plate).

Plan to plate your samples into specific wells in the sample source plate and record their locations in one of the following ways:

- **Follow a sample source plate layout guide:** Follow the sample source plate layout guides (maps) in the SmartChip Dispenser Software. Alternatively, place the SmartChip Plate layout guide corresponding to your SmartChip layout in the plate lid, under your sample source plate, and use it as a pipetting guide.

  These maps indicate samples with numbers; they include a single replicate of each reaction. To run multiple replicates, use the Sample/PCR reagent mixture for more than one sample shown in the source plate map. Record this in the sample source plate file or in a spreadsheet for transfer to the Dispenser Software.
- **Reuse a sample source plate layout from a previous experiment:** If you are analyzing a sample set more than once, you can enter your sample information and locations in the source plate into the SmartChip Dispenser Software and save the resulting sample source plate file for use in subsequent experiments.
- **Prepare sample source plate files with your own software:** If you are processing many samples or are using an automated sample preparation system, you may want to prepare sample source plate files in a text editor, then load them into the SmartChip Dispenser Software. See the SmartChip ND dispenser manual for instructions.

1. Thaw cDNA sample(s) on ice or a cold rack. Thaw nuclease-free PCR-grade water at room temperature, and then place on ice or a cold rack.
2. If necessary, add 1X TE, pH 8.0 to the RT reactions to normalize the cDNA concentrations to 10 ng/µl. Assume 100% conversion of RNA to cDNA in the RT reaction. Table 8 indicates the volume of 1X TE, pH 8.0 to add to 10-µl RT reactions to bring the final concentration to 10 ng/µl.

Table 8. Normalizing cDNA concentrations, probe-based PCR mRNA expression analysis.

Chip format			RNA input to RT rxn (ng)	RNA concentration in RT rxn (ng/µl)	Volume of 1X TE to add (µl)
Assays	Samples	Replicates			
12–144	384–36	1	100	10	–
		4	200	20	10
216	24	1	100	10	–
		4	400	40	30
248	20	1	100	10	–
		4	400	40	30
296	16	1	200	20	10
		4	600	60	50
384	12	1	200	20	10
		4	600	60	50

3. Prepare a sample PCR reagent mix in a nuclease-free tube on ice or a cold rack. See Table 9 for volumes.

NOTE: Minimize light exposure to the SmartChip Probe qPCR Master Mix.

a. Swirl the bottle of SmartChip Probe qPCR Master Mix gently to mix well before use.

b. After assembling the reagent mix, close the tube and vortex gently to mix well. Place on ice or a cold rack.

Table 9. Sample PCR reagent mix preparation, probe-based PCR mRNA expression analysis.

Chip format		SmartChip Probe qPCR Master Mix (2X) (µl)	Nuclease-free PCR-grade water (µl)	Total volume (µl)
Assays	Samples			
12	384	2,350	1,391	3,760
24	216	1,430	847	2,288
36	144	1,039	615	1,662
48	108	850	503	1,360
54	96	784	464	1,254
72	72	652	386	1,043
80	64	610	361	976
96	54	556	329	890
120	42	493	292	788
144	36	458	271	733
216	24	523	309	836
248	20	481	285	770
296	16	610	361	976
384	12	523	309	836

4. On ice, add the sample PCR reagent mix and then cDNA samples to a 384-Well Source Plate (this will be your sample source plate) as outlined below (see Table 10, next page).

a. Dispense sample PCR reagent mix into wells of the 384-Well Source Plate using the volume appropriate for your SmartChip format, following the sample source plate map.

b. Add the indicated volume of cDNA to each well containing PCR reagent mix, following the sample source plate map.

IMPORTANT: It is very important that you plate your samples into specific wells in the sample source plate. You will need multiple wells of each cDNA sample for some SmartChip formats.

- You can place the SmartChip source plate layout guide for your chip format in the plate lid, under your source plate to serve as a pipetting guide.
- Alternatively, you can find sample source plate layout guides (maps) in the SmartChip Dispenser Software.
- Finally, you can load the sample source plate file for the sample set from previous runs into the SmartChip Dispenser Software and use it as a pipetting guide (map).

c. Seal the plate and vortex vigorously to mix well. Centrifuge for 5 min at 2,750*g*.

NOTE: To run replicates, you can use the same Sample/PCR reagent mix for more than one sample shown in the sample source plate layout guide (map).

Table 10. Dispense volumes for sample source plate, probe-based PCR mRNA expression analysis.

Chip format		4a. Sample PCR reagent mix per well (µl)	4b. cDNA at 10 ng/µl per well (µl)
Assays	Samples		
12	384	9.4	2.3
24	216	9.9	2.5
36	144	10.5	2.6
48	108	11.2	2.8
54	96	11.5	2.9
72	72	12.4	3.1
80	64	12.9	3.2
96	54	13.7	3.4
120	42	15.2	3.8
144	36	16.2	4.1
216	24	14.3	3.6
248	20	15.5	3.9
296	16	12.9	3.2
384	12	14.3	3.6

C. Preparing the Assay Source Plate

This section describes how to mix your PCR assays with real-time PCR master mix and distribute the resulting Assay/PCR reagent mix to wells of the assay source plate.

Set up your assay source plate following the same general recommendations described above for the sample source plate. You will need to enter PCR assay information into the SmartChip Dispenser Software that runs the SmartChip ND dispenser; PCR assay information is stored in assay source plate files. Minimize light exposure to your PCR assays.

IMPORTANT: Immediately after completing this procedure, you will need to dispense the mixtures into your SmartChip MyDesign Chip and start your reactions.

- Plan for adequate time to dispense your reagents and run your real-time PCR
- Power on the SmartChip ND dispenser and run the daily warmup procedure before starting this part of the protocol

1. Thaw PCR Assays and dilute with 1X TE to 10X on ice, as described in Steps 1a–1b below. Use nuclease-free 1X TE, pH 8.0 and plate 10X PCR assays in a nuclease-free 96-well plate on ice. We recommend that you plate the assays as shown in Appendix B.

   a. Prepare the volume of 10X PCR assay shown for your SmartChip format in Table 11 (next page). We recommend that you prepare enough diluted PCR assay for 10 SmartChip MyDesign Chips, but the table also shows the amount needed for a single chip in brackets and gray text.

   b. Plate the diluted PCR assays into a nuclease-free 96-well PCR plate in the configuration shown for your SmartChip format in Appendix B.

Table 11. PCR assay volumes by chip format, probe-based PCR mRNA expression analysis.

Chip format		[Volume for 1 chip (µl)]	Volume for 10 chips (µl)
Assays	Samples		
12	384	[18]	158
24	216	[11]	79
36	144	[8]	45
48	108	[7]	39
54	96	[7]	38
72	72	[7]	34
80	64	[7]	33
96	54	[6]	32
120	42	[6]	30
144	36	[6]	29
216	24	[6]	27
248	20	[6]	27
296	16	[6]	26
384	12	[14.3]	26

2. Prepare the assay PCR reagent mix in a nuclease-free tube on ice or a cold rack, following Table 12. Minimize light exposure to the SmartChip Probe qPCR Master Mix. Swirl the bottle of SmartChip Probe qPCR Master Mix gently to mix well before use. After assembling the mixture, close the tube and vortex gently to mix well. Place on ice or a cold rack.

Table 12. Assay PCR reagent mix preparation, probe-based PCR mRNA expression analysis.

Chip format		SmartChip Probe qPCR Master Mix (2X) (µl)	ROX Reference Dye (50X) (µl)	Nuclease-free PCR-grade water (µl)	Total volume (µl)
Assays	Samples				
12	384	523	20.9	293	836
24	216	523	20.9	293	836
36	144	458	18.3	257	733
48	108	523	20.9	293	836
54	96	556	22.2	311	890
72	72	652	26.1	365	1,043
80	64	699	28.0	391	1,118
96	54	784	31.4	439	1,254
120	42	911	36.5	510	1,458
144	36	1,039	41.6	582	1,662
216	24	1,430	57.2	801	2,288
248	20	1,613	64.5	903	2,580
296	16	1,870	74.8	1,047	2,992
384	12	2,350	94.0	1,316	3,760

3. On ice, add the assay PCR reagent mix and then 10X PCR Assays to a 384-Well Source Plate (this will be your assay source plate) as outlined below (see Table 13, next page).

   a. Dispense assay PCR reagent mix into wells of the 384-Well Source Plate using the volume appropriate for your SmartChip format, following the assay source plate map.

b. Add the indicated volume of 10X PCR Assay to each well containing PCR reagent mix, following the assay source plate map.

NOTES:

– It is very important that you plate your assays into specific wells in the assay source plate. You can find the assay source plate layout guides (maps) in the SmartChip Dispenser Software.

– You will need to put reagents into multiple wells for some SmartChip formats.

c. Seal the plate and vortex vigorously to mix well. Centrifuge for 5 min at 2,750*g*.

Table 13. Dispense volumes for assay source plate, probe-based PCR mRNA expression analysis.

Chip format		3a. Assay PCR reagent mix per well (µl)	3b. 10X PCR Assay per well (µl)
Assays	Samples		
12	384	14.3	3.6
24	216	14.3	3.6
36	144	16.2	4.1
48	108	14.3	3.6
54	96	13.7	3.4
72	72	12.4	3.1
80	64	12.1	3.0
96	54	11.5	2.9
120	42	10.9	2.7
144	36	10.5	2.6
216	24	9.9	2.5
248	20	9.8	2.4
296	16	9.6	2.4
384	12	9.4	2.3

4. Dispense reagents into your SmartChip MyDesign Chip with the SmartChip ND dispenser. See the instructions in the SmartChip ND and qPCRStudio Software User Manual.
   a. Program the instrument for your experiment: specify the chip format, the type of analysis, and chip identification number.
   b. Load the Sample and assay source plate files or enter new Sample and Assay information to create new source plate files.
   c. Place your empty SmartChip MyDesign Chip and sample source plate on the dispenser and dispense Sample/PCR reagent mixes into your chip.
   d. Seal the chip and spin.
   e. Load the assay source plate on the dispenser and dispense Assay/PCR reagent mixes into the chip. The SmartChip ND dispenser will create a SmartChip Layout file.
5. Run the real-time PCR and analyze data using the SmartChip Cycler. See the instructions in the SmartChip ND Real-Time PCR Cycler and Software User Manual. To run your PCR reactions, you'll load your SmartChip MyDesign Chip into the SmartChip Cycler and program the instrument with information about your experiment, including the SmartChip Layout file from Step 4e. The SmartChip Cycler is designed to run the reactions, capture the data, and help you analyze your results.

VII. Protocol: SNP Genotyping

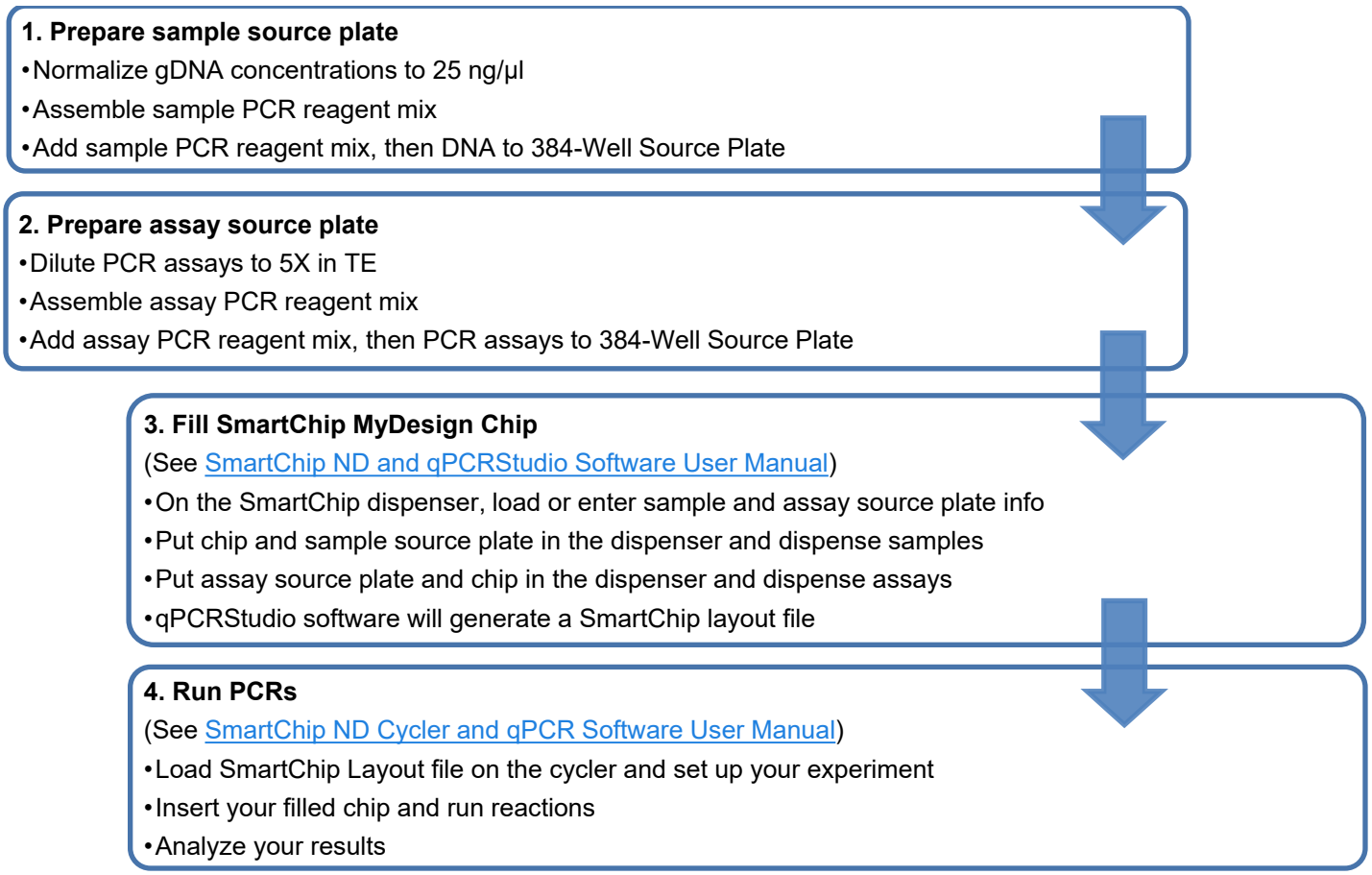

Figure 4. Procedure overview for SNP genotyping. Steps 1 & 2 are done on the bench; Steps 3 & 4 are performed in the SmartChip system.

A. Preparation of Sample Source Plate

This section describes how to mix your DNA samples with real-time PCR master mix and distribute the resulting Sample/PCR reagent mix to the wells of the 384-Well Source Plate (sample source plate).

IMPORTANT: In the steps below, follow the instructions that correspond to the format (i.e., the number of samples and PCR assays) of your SmartChip MyDesign Chip(s).

Plan to plate your samples into specific wells in the sample source plate and record their locations in one of the following ways:

- **Follow a sample source plate layout guide:** Follow the sample source plate layout guides (maps) in the SmartChip Dispenser Software. Alternatively, place the SmartChip Plate layout guide corresponding to your SmartChip layout in the plate lid, under your sample source plate, and use it as a pipetting guide.
- **Reuse a sample source plate layout from a previous experiment:** If you are analyzing a sample set more than once, you can enter your sample information and locations in the source plate into the SmartChip Dispenser Software and save the resulting sample source plate file for use in subsequent experiments.
- **Prepare sample source plate files with your own software:** If you are processing many samples or are using an automated sample preparation system, you may want to prepare sample source plate files

in a text editor, then load them into the SmartChip Dispenser Software. See the SmartChip ND dispenser manual for instructions.

1. Thaw DNA sample(s) on ice or a cold rack. Thaw nuclease-free PCR-grade water at room temperature, and then place on ice or a cold rack.
2. If necessary, normalize the gDNA concentrations to 25 ng/µl with 1X TE, pH 8.0.
3. Prepare a sample PCR reagent mix in a nuclease-free tube on ice or a cold rack. See Table 14 for volumes. Minimize light exposure to the SmartChip Probe qPCR Master Mix. Swirl the bottle of SmartChip Probe qPCR Master Mix gently to mix well before use. After assembling the reagent mix, close the tube and vortex gently to mix well. Place on ice or a cold rack.

Table 14. Sample PCR reagent mix preparation, SNP genotyping.

Chip format		SmartChip Probe qPCR Master Mix (µl)	Nuclease-free PCR-grade water (µl)	Total volume (µl)
Assays	Samples			
12	384	3,140	620	3,760
24	216	1,910	378	2,288
36	144	1,388	274	1,662
48	108	1,136	224	1,360
54	96	1,047	207	1,254
72	72	871	172	1,043
80	64	815	161	976
96	54	743	147	890
120	42	658	130	788
144	36	612	121	733
216	24	698	138	836
248	20	643	127	770
296	16	815	161	976
384	12	698	138	836

4. On ice, add the sample PCR reagent mix and then DNA samples to a 384-Well Source Plate (this will be your sample source plate) as outlined below (see Table 15, next page).
   a. Dispense sample PCR reagent mix into wells of the 384-Well Source Plate using the volume appropriate for your SmartChip format, following the sample source plate map.
   b. Add the indicated volume of DNA to each well containing PCR reagent mix, following the sample source plate map.

      IMPORTANT: It is very important that you plate your samples into specific wells in the sample source plate. You will need multiple wells of each cDNA sample for some SmartChip formats.
      - You can place the SmartChip source plate layout guide for your chip format in the plate lid, under your source plate to serve as a pipetting guide.
      - Alternatively, you can find sample source plate layout guides (maps) in the SmartChip Dispenser Software.
      - Finally, you can load the sample source plate file for the sample set from previous runs into the SmartChip Dispenser Software and use it as a pipetting guide (map).

   c. Seal the plate and vortex vigorously to mix well. Centrifuge for 5 min at 2,750*g*.

Table 15. Dispense volumes for sample source plate, SNP genotyping.

SmartChip format Assays	Samples	4a. Sample PCR reagent mix per well (µl)	4b. gDNA at 25 ng/µl per well (µl)
12	384	7.0	4.7
24	216	7.4	2.5
36	144	7.8	2.6
48	108	8.4	2.8
54	96	8.6	2.9
72	72	9.3	3.1
80	64	9.6	3.2
96	54	10.2	3.4
120	42	11.4	3.8
144	36	12.2	4.1
216	24	10.7	3.6
248	20	11.6	3.9
296	16	9.6	3.2
384	12	10.7	3.6

B. Preparing the Assay Source Plate

This section describes how to mix your PCR assays with real-time PCR master mix and distribute the resulting Assay/PCR reagent mix to wells of the assay source plate.

Set up your assay source plate following the same general recommendations described above for the sample source plate. You will need to enter PCR assay information into the SmartChip Dispenser Software that runs the SmartChip ND dispenser; PCR assay information is stored in assay source plate files.

IMPORTANT: Immediately after completing this procedure, you will need to dispense the mixtures into your SmartChip MyDesign Chip and start your reactions.

- Plan for adequate time to dispense your reagents and run your real-time PCR
- Power on the SmartChip ND dispenser and run the daily warmup procedure before starting this part of the protocol

1. Thaw PCR Assays and dilute with 1X TE to 10X on ice, as described in Steps 1a–1b below. Use nuclease-free 1X TE, pH 8.0 and plate 5X PCR assays in a nuclease-free 96-well plate on ice or a cold rack. We recommend that you plate the assays as shown in Appendix B.
   a. Prepare the volume of 5X PCR assay shown for your SmartChip format in Table 16 (next page). We recommend that you prepare enough diluted PCR assay for 10 SmartChip MyDesign Chips, but the table also shows the amount needed for a single chip in brackets and gray text.

b. Plate the diluted PCR assays into a nuclease-free 96-well PCR plate in the configuration shown for your SmartChip format in Appendix B.

Table 16. PCR assay volumes by chip format, SNP genotyping.

Chip format		[Volume for	Volume for
Assays	Samples	1 chip + 20% (µl)]	10 chips + 20% (µl)
12	384	[34.4]	344
24	216	[17.2]	172
36	144	[9.7]	97
48	108	[8.6]	86
54	96	[8.2]	82
72	72	[7.4]	74
80	64	[7.2]	72
96	54	[[6.9]	69
120	42	[6.5]	65
144	36	[6.3]	63
216	24	[6.0]	60
248	20	[5.9]	59
296	16	[5.8]	58
384	12	[5.6]	56

2. Prepare the assay PCR reagent mix in a nuclease-free tube on ice or a cold rack, following Table 17. Minimize light exposure to the SmartChip Probe qPCR Master Mix. Swirl the bottle of SmartChip Probe qPCR Master Mix gently to mix well before use. After assembling the mixture, close the tube and vortex gently to mix well. Place on ice or a cold rack.

Table 17. Assay PCR reagent mix preparation, SNP genotyping.

Chip format		SmartChip Probe qPCR Master Mix (2X) (µl)	ROX Reference Dye (50X) (µl)	Nuclease-free PCR-grade water (µl)	Total volume (µl)
Assays	Samples				
12	384	698	16.7	121.2	836
24	216	698	16.7	121.2	836
36	144	612	14.7	106.3	733
48	108	698	16.7	121.2	836
54	96	743	17.8	129.1	890
72	72	871	20.9	151.2	1,043
80	64	934	22.4	162.1	1,118
96	54	1,047	25.1	181.8	1,254
120	42	1,217	29.2	211.4	1,458
144	36	1,388	33.2	241.0	1,662
216	24	1,910	45.8	331.8	2,288
248	20	2,154	51.6	374.1	2,580
296	16	2,498	59.8	433.8	2,992
384	12	3,140	75.2	545.2	3,760

3. On ice, add the assay PCR reagent mix and then 5X PCR Assays to a 384-Well Source Plate (this will be your assay source plate) as outlined below (see Table 18).
   a. Dispense assay PCR reagent mix into wells of the 384-Well Source Plate using the volume appropriate for your SmartChip format, following the assay source plate map.
   b. Add the indicated volume of 5X PCR Assay to each well containing PCR reagent mix, following the assay source plate map.
   c. Seal the plate and vortex vigorously to mix well. Centrifuge for 5 min at 2,750*g*.

Table 18. Dispense volumes for assay source plate, SNP genotyping.

Chip format		3a. Assay PCR reagent mix per well (µl)	3b. 5X PCR Assay per well (µl)
Assays	Samples		
12	384	10.7	7.2
24	216	10.7	7.2
36	144	12.2	8.1
48	108	10.7	7.2
54	96	10.2	6.8
72	72	9.3	6.2
80	64	9.0	6.0
96	54	8.6	5.8
120	42	8.1	5.4
144	36	7.8	5.2
216	24	7.4	5.0
248	20	7.3	4.9
296	16	7.2	4.8
384	12	7.0	4.7

It is very important that you plate your assays into specific wells in the assay source plate. You will need to put reagents into multiple wells for some SmartChip formats.

- You can place the SmartChip source plate layout guide for your chip format in the plate lid, under your source plate to serve as a pipetting guide.
- You can find the assay source plate layout guides (maps) in the SmartChip Dispenser Software.

4. Dispense reagents into your SmartChip MyDesign Chip with the SmartChip ND dispenser. See the instructions in the SmartChip ND and qPCRStudio Software User Manual.
   a. Program the instrument for your experiment: specify the chip format, the type of analysis, and chip identification number.
   b. Load the Sample and assay source plate files or enter new Sample and Assay information to create new source plate files.
   c. Place your empty SmartChip MyDesign Chip and sample source plate on the dispenser and dispense Sample/PCR reagent mixes into your chip.
   d. Seal the chip and spin.
   e. Load the assay source plate on the dispenser and dispense Assay/PCR reagent mixes into the chip. The SmartChip ND dispenser will create a SmartChip Layout file.
5. Run the real-time PCR and analyze data using the SmartChip Cycler. See the instructions in the SmartChip ND Real-Time PCR Cycler and Software User Manual. To run your PCR reactions, you'll load your SmartChip MyDesign Chip into the SmartChip Cycler and program the

instrument with information about your experiment, including the SmartChip Layout file from Step 4e. The SmartChip Cycler is designed to run the reactions, capture the data, and help you analyze your results.

Appendix A. Suggested RT Reaction Plate Layouts

We recommend that you follow these suggested layouts to assemble the reactions/mixtures that will later be loaded into your SmartChip MyDesign Chips. They are designed to make it easy to transfer sample and assay mixtures from tubes or 96-well setup plates to the 384-Well Source Plates that you will load onto the SmartChip ND dispenser.

12 Assay X 384 Sample Format

RT Reaction Plates: 1 sample replicate

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	73	81	89
B	2	10	18	26	34	42	50	58	66	74	82	90
C	3	11	19	27	35	43	51	59	67	75	83	91
D	4	12	20	28	36	44	52	60	68	76	84	92
E	5	13	21	29	37	45	53	61	69	77	85	93
F	6	14	22	30	38	46	54	62	70	78	86	94
G	7	15	23	31	39	47	55	63	71	79	87	95
H	8	16	24	32	40	48	56	64	72	80	88	96

	1	2	3	4	5	6	7	8	9	10	11	12
A	97	105	113	121	129	137	145	153	161	169	177	185
B	98	106	114	122	130	138	146	154	162	170	178	186
C	99	107	115	123	131	139	147	155	163	171	179	187
D	100	108	116	124	132	140	148	156	164	172	180	188
E	101	109	117	125	133	141	149	157	165	173	181	189
F	102	110	118	126	134	142	150	158	166	174	182	190
G	103	111	119	127	135	143	151	159	167	175	183	191
H	104	112	120	128	136	144	152	160	168	176	184	192

	1	2	3	4	5	6	7	8	9	10	11	12
A	193	201	209	217	225	233	241	249	257	265	273	281
B	194	202	210	218	226	234	242	250	258	266	274	282
C	195	203	211	219	227	235	243	251	259	267	275	283
D	196	204	212	220	228	236	244	252	260	268	276	284
E	197	205	213	221	229	237	245	253	261	269	277	285
F	198	206	214	222	230	238	246	254	262	270	278	286
G	199	207	215	223	231	239	247	255	263	271	279	287
H	200	208	216	224	232	240	248	256	264	272	280	288

	1	2	3	4	5	6	7	8	9	10	11	12
A	289	297	305	313	321	329	337	345	353	361	369	377
B	290	298	306	314	322	330	338	346	354	362	370	378
C	291	299	307	315	323	331	339	347	355	363	371	379
D	292	300	308	316	324	332	340	348	356	364	372	380
E	293	301	309	317	325	333	341	349	357	365	373	381
F	294	302	310	318	326	334	342	350	358	366	374	382
G	295	303	311	319	327	335	343	351	359	367	375	383
H	296	304	312	320	328	336	344	352	360	368	376	384

RT Reaction Plate: 4 sample replicates

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	73	81	89
B	2	10	18	26	34	42	50	58	66	74	82	90
C	3	11	19	27	35	43	51	59	67	75	83	91
D	4	12	20	28	36	44	52	60	68	76	84	92
E	5	13	21	29	37	45	53	61	69	77	85	93
F	6	14	22	30	38	46	54	62	70	78	86	94
G	7	15	23	31	39	47	55	63	71	79	87	95
H	8	16	24	32	40	48	56	64	72	80	88	96

Figure 5. 12 assay x 384 sample format.

24 Assay X 216 Sample Format

RT Reaction Plates: 1 sample replicate

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	73	81	89
B	2	10	18	26	34	42	50	58	66	74	82	90
C	3	11	19	27	35	43	51	59	67	75	83	91
D	4	12	20	28	36	44	52	60	68	76	84	92
E	5	13	21	29	37	45	53	61	69	77	85	93
F	6	14	22	30	38	46	54	62	70	78	86	94
G	7	15	23	31	39	47	55	63	71	79	87	95
H	8	16	24	32	40	48	56	64	72	80	88	96

	1	2	3	4	5	6	7	8	9	10	11	12
A	97	105	113	121	129	137	145	153	161	169	177	185
B	98	106	114	122	130	138	146	154	162	170	178	186
C	99	107	115	123	131	139	147	155	163	171	179	187
D	100	108	116	124	132	140	148	156	164	172	180	188
E	101	109	117	125	133	141	149	157	165	173	181	189
F	102	110	118	126	134	142	150	158	166	174	182	190
G	103	111	119	127	135	143	151	159	167	175	183	191
H	104	112	120	128	136	144	152	160	168	176	184	192

	1	2	3	4	5	6	7	8	9	10	11	12
A	193	199	205	211								
B	194	200	206	212								
C	195	201	207	213								
D	196	202	208	214								
E	197	203	209	215								
F	198	204	210	216								
G												
H												

RT Reaction Plate: 4 sample replicates

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49					
B	2	10	18	26	34	42	50					
C	3	11	19	27	35	43	51					
D	4	12	20	28	36	44	52					
E	5	13	21	29	37	45	53					
F	6	14	22	30	38	46	54					
G	7	15	23	31	39	47						
H	8	16	24	32	40	48						

Figure 6. 24 assay x 216 sample format.

36 Assay X 144 Sample Format

RT Reaction Plates: 1 sample replicate

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	73	81	89
B	2	10	18	26	34	42	50	58	66	74	82	90
C	3	11	19	27	35	43	51	59	67	75	83	91
D	4	12	20	28	36	44	52	60	68	76	84	92
E	5	13	21	29	37	45	53	61	69	77	85	93
F	6	14	22	30	38	46	54	62	70	78	86	94
G	7	15	23	31	39	47	55	63	71	79	87	95
H	8	16	24	32	40	48	56	64	72	80	88	96

	1	2	3	4	5	6	7	8	9	10	11	12
A	97	105	113	121	129	133	137	141				
B	98	106	114	122	130	134	138	142				
C	99	107	115	123	131	135	139	143				
D	100	108	116	124	132	136	140	144				
E	101	109	117	125								
F	102	110	118	126								
G	103	111	119	127								
H	104	112	120	128								

RT Reaction Plate: 4 sample replicates

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33							
B	2	10	18	26	34							
C	3	11	19	27	35							
D	4	12	20	28	36							
E	5	13	21	29								
F	6	14	22	30								
G	7	15	23	31								
H	8	16	24	32								

Figure 7. 36 assay x 144 sample format.

48 Assay X 108 Sample Format

RT Reaction Plates: 1 sample replicate

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	73	81	89
B	2	10	18	26	34	42	50	58	66	74	82	90
C	3	11	19	27	35	43	51	59	67	75	83	91
D	4	12	20	28	36	44	52	60	68	76	84	92
E	5	13	21	29	37	45	53	61	69	77	85	93
F	6	14	22	30	38	46	54	62	70	78	86	94
G	7	15	23	31	39	47	55	63	71	79	87	95
H	8	16	24	32	40	48	56	64	72	80	88	96

	1	2	3	4	5	6	7	8	9	10	11	12
A	97	105	113	121	129	133	137	141				
B	98	106	114	122	130	134	138	142				
C	99	107	115	123	131	135	139	143				
D	100	108	116	124	132	136	140	144				
E	101	109	117	125								
F	102	110	118	126								
G	103	111	119	127								
H	104	112	120	128								

RT Reaction Plate: 4 sample replicates

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33							
B	2	10	18	26	34							
C	3	11	19	27	35							
D	4	12	20	28	36							
E	5	13	21	29								
F	6	14	22	30								
G	7	15	23	31								
H	8	16	24	32								

Figure 8. 48 assay x 108 sample format.

54 Assay X 96 Sample Format

RT Reaction Plates: 1 sample replicate

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	73	81	89
B	2	10	18	26	34	42	50	58	66	74	82	90
C	3	11	19	27	35	43	51	59	67	75	83	91
D	4	12	20	28	36	44	52	60	68	76	84	92
E	5	13	21	29	37	45	53	61	69	77	85	93
F	6	14	22	30	38	46	54	62	70	78	86	94
G	7	15	23	31	39	47	55	63	71	79	87	95
H	8	16	24	32	40	48	56	64	72	80	88	96

	1	2	3	4	5	6	7	8	9	10	11	12
A	97	100	103	106								
B	98	101	104	107								
C	99	102	105	108								
D												
E												
F												
G												
H												

RT Reaction Plate: 4 sample replicates

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25								
B	2	10	18	26								
C	3	11	19	27								
D	4	12	20									
E	5	13	21									
F	6	14	22									
G	7	15	23									
H	8	16	24									

Figure 9. 54 assay x 96 sample format.

72 Assay X 72 Sample Format

RT Reaction Plate: 1 sample replicate

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	67	69	71
B	2	10	18	26	34	42	50	58	66	68	70	72
C	3	11	19	27	35	43	51	59				
D	4	12	20	28	36	44	52	60				
E	5	13	21	29	37	45	53	61				
F	6	14	22	30	38	46	54	62				
G	7	15	23	31	39	47	55	63				
H	8	16	24	32	40	48	56	64				

RT Reaction Plate: 4 sample replicates

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17									
B	2	10	18									
C	3	11										
D	4	12										
E	5	13										
F	6	14										
G	7	15										
H	8	16										

Figure 10. 72 assay x 72 sample format.

80 Assay X 64 Sample Format

RT Reaction Plate: 1 sample replicate

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57				
B	2	10	18	26	34	42	50	58				
C	3	11	19	27	35	43	51	59				
D	4	12	20	28	36	44	52	60				
E	5	13	21	29	37	45	53	61				
F	6	14	22	30	38	46	54	62				
G	7	15	23	31	39	47	55	63				
H	8	16	24	32	40	48	56	64				

RT Reaction Plate: 4 sample replicates

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9										
B	2	10										
C	3	11										
D	4	12										
E	5	13										
F	6	14										
G	7	15										
H	8	16										

Figure 11. 80 assay x 64 sample format.

96 Assay X 54 Sample Format

RT Reaction Plate: 1 sample replicate

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	39	44	50				
B	2	10	18	26	34	40	45	51				
C	3	11	19	27	35	41	46	52				
D	4	12	20	28	36	42	47	53				
E	5	13	21	29	37	43	48	54				
F	6	14	22	30	38		49					
G	7	15	23	31								
H	8	16	24	32								

RT Reaction Plate: 4 sample replicates

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9										
B	2	10										
C	3	11										
D	4	12										
E	5	13										
F	6											
G	7											
H	8											

Figure 12. 96 assay x 54 sample format.

120 Assay X 42 Sample Format

RT Reaction Plate: 1 sample replicate

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	36	38	41				
B	2	10	18	26	34	37	39	42				
C	3	11	19	27	35		40					
D	4	12	20	28								
E	5	13	21	29								
F	6	14	22	30								
G	7	15	23	31								
H	8	16	24	32								

RT Reaction Plate: 4 sample replicates

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9										
B	2	10										
C	3											
D	4											
E	5											
F	6											
G	7											
H	8											

Figure 13. 120 assay x 42 sample format.

144 Assay X 36 Sample Format

RT Reaction Plate: 1 sample replicate

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33		35					
B	2	10	18	26	34		36					
C	3	11	19	27								
D	4	12	20	28								
E	5	13	21	29								
F	6	14	22	30								
G	7	15	23	31								
H	8	16	24	32								

RT Reaction Plate: 4 sample replicates

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9										
B	2											
C	3											
D	4											
E	5											
F	6											
G	7											
H	8											

Figure 14. 144 assay x 36 sample format.

216 Assay X 24 Sample Format

RT Reaction Plate: 1 sample replicate

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	21								
B	2	10	18	22								
C	3	11	19	23								
D	4	12	20	24								
E	5	13										
F	6	14										
G	7	15										
H	8	16										

RT Reaction Plate: 4 sample replicates

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	5										
B	2	6										
C	3	5										
D	4	6										
E	1											
F	2											
G	3											
H	4											

Figure 15. 216 assay x 24 sample format.

248 Assay X 20 Sample Format

RT Reaction Plate: 1 sample replicate

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	19								
B	2	10	18	20								
C	3	11										
D	4	12										
E	5	13										
F	6	14										
G	7	15										
H	8	16										

RT Reaction Plate: 4 sample replicates

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	5										
B	2	5										
C	3											
D	4											
E	1											
F	2											
G	3											
H	4											

Figure 16. 248 assay x 20 sample format.

296 Assay X 16 Sample Format

RT Reaction Plate: 1 sample replicate

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9										
B	2	10										
C	3	11										
D	4	12										
E	5	13										
F	6	14										
G	7	15										
H	8	16										

RT Reaction Plate: 4 sample replicates

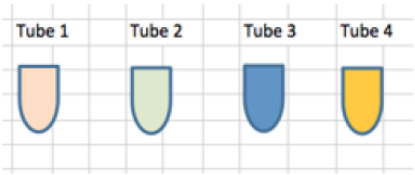

Figure 17. 296 assay x 16 sample format.

Appendix B. Suggested 5X PCR Assay Plate Layouts

We recommend that you follow these suggested layouts to assemble the reactions/mixtures that will later be loaded into your SmartChip MyDesign Chips. They are designed to make it easy to transfer sample and assay mixtures from tubes or 96-well setup plates to the 384-Well Source Plates that you will load onto the SmartChip ND dispenser.

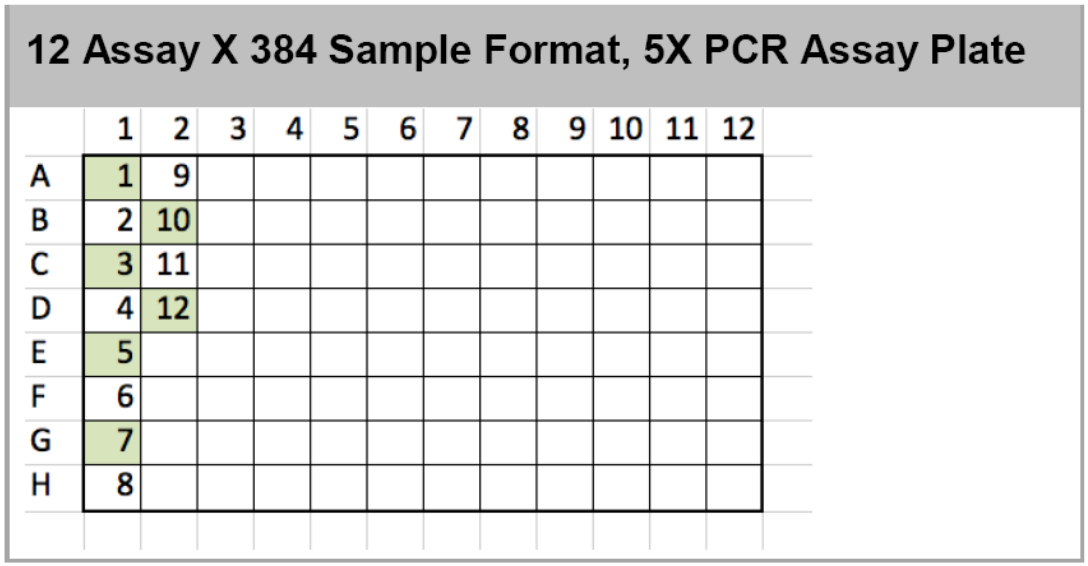

Figure 18. 12 assay x 384 sample format, 5X PCR assay plate.

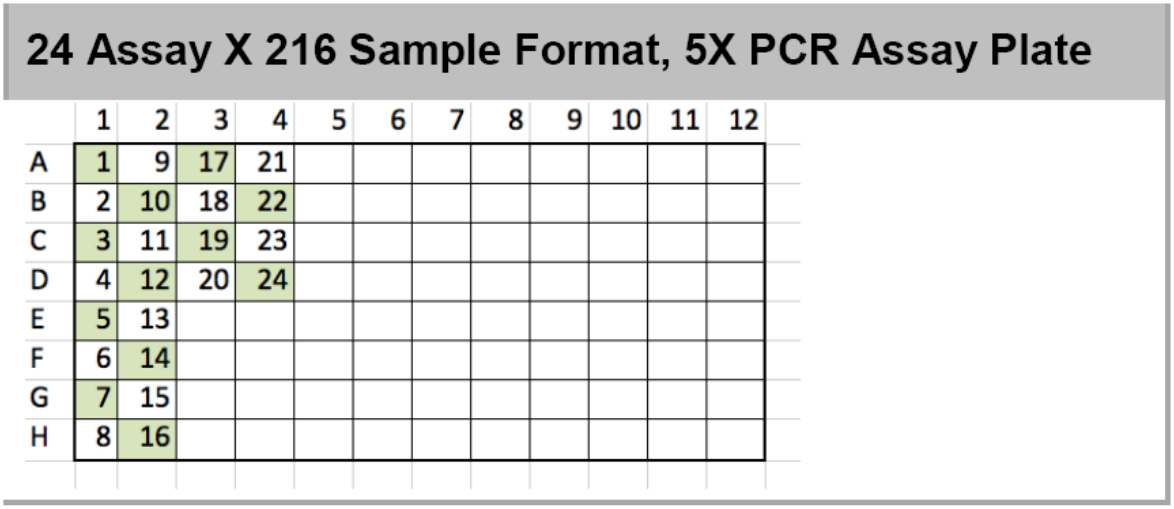

Figure 19. 24 assay x 216 sample format, 5X PCR assay plate.

36 Assay X 144 Sample Format, 5X PCR Assay Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	35						
B	2	10	18	26	34	36						
C	3	11	19	27								
D	4	12	20	28								
E	5	13	21	29								
F	6	14	22	30								
G	7	15	23	31								
H	8	16	24	32								

Figure 20. 36 assay x 144 sample format, 5X PCR assay plate.

48 Assay X 108 Sample Format, 5X PCR Assay Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41						
B	2	10	18	26	34	42						
C	3	11	19	27	35	43						
D	4	12	20	28	36	44						
E	5	13	21	29	37	45						
F	6	14	22	30	38	46						
G	7	15	23	31	39	47						
H	8	16	24	32	40	48						

Figure 21. 48 assay x 108 sample format, 5X PCR assay plate.

54 Assay X 96 Sample Format, 5X PCR Assay Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	37	41	45				
B	2	10	18	26	34	38	42	46				
C	3	11	19	27	35	39	43	47				
D	4	12	20	28	36	40	44	48				
E	5	13	21	29								
F	6	14	22	30								
G	7	15	23	31								
H	8	16	24	32								

Figure 22. 54 assay x 96 sample format, 5X PCR assay plate.

72 Assay X 72 Sample Format, 5X PCR Assay Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	67	69	71
B	2	10	18	26	34	42	50	58	66	68	70	72
C	3	11	19	27	35	43	51	59				
D	4	12	20	28	36	44	52	60				
E	5	13	21	29	37	45	53	61				
F	6	14	22	30	38	46	54	62				
G	7	15	23	31	39	47	55	63				
H	8	16	24	32	40	48	56	64				

Figure 23. 72 assay x 72 sample format, 5X PCR assay plate.

80 Assay X 64 Sample Format, 5X PCR Assay Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	69	73	77
B	2	10	18	26	34	42	50	58	66	70	74	78
C	3	11	19	27	35	43	51	59	67	71	75	79
D	4	12	20	28	36	44	52	60	68	72	76	80
E	5	13	21	29	37	45	53	61				
F	6	14	22	30	38	46	54	62				
G	7	15	23	31	39	47	55	63				
H	8	16	24	32	40	48	56	64				

Figure 24. 80 assay x 64 sample format, 5X PCR assay plate.

96 Assay X 54 Sample Format, 5X PCR Assay Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	73	81	89
B	2	10	18	26	34	42	50	58	66	74	82	90
C	3	11	19	27	35	43	51	59	67	75	83	91
D	4	12	20	28	36	44	52	60	68	76	84	92
E	5	13	21	29	37	45	53	61	69	77	85	93
F	6	14	22	30	38	46	54	62	70	78	86	94
G	7	15	23	31	39	47	55	63	71	79	87	95
H	8	16	24	32	40	48	56	64	72	80	88	96

Figure 25. 96 assay x 54 sample format, 5X PCR assay plate.

120 Assay X 42 Sample Format, 5X PCR Assay Plate

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	1	16	31	46	61	76	91	106																
B	2	17	32	47	62	77	92	107																
C	3	18	33	48	63	78	93	108																
D	4	19	34	49	64	79	94	109																
E	5	20	35	50	65	80	95	110																
F	6	21	36	51	66	81	96	111																
G	7	22	37	52	67	82	97	112																
H	8	23	38	53	68	83	98	113																
I	9	24	39	54	69	84	99	114																
J	10	25	40	55	70	85	100	115																
K	11	26	41	56	71	86	101	116																
L	12	27	42	57	72	87	102	117																
M	13	28	43	58	73	88	103	118																
N	14	29	44	59	74	89	104	119																
O	15	30	45	60	75	90	105	120																
P																								

Figure 26. 120 assay x 42 sample format, 5X PCR assay plate.

144 Assay X 36 Sample Format, 5X PCR Assay Plate

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	1	17	33	49	65	81	97	113	129	137														
B	2	18	34	50	66	82	98	114	130	138														
C	3	19	35	51	67	83	99	115	131	139														
D	4	20	36	52	68	84	100	116	132	140														
E	5	21	37	53	69	85	101	117	133	141														
F	6	22	38	54	70	86	102	118	134	142														
G	7	23	39	55	71	87	103	119	135	143														
H	8	24	40	56	72	88	104	120	136	144														
I	9	25	41	57	73	89	105	121																
J	10	26	42	58	74	90	106	122																
K	11	27	43	59	75	91	107	123																
L	12	28	44	60	76	92	108	124																
M	13	29	45	61	77	93	109	125																
N	14	30	46	62	78	94	110	126																
O	15	31	47	63	79	95	111	127																
P	16	32	48	64	80	96	112	128																

Figure 27. 144 assay x 36 sample format, 5X PCR assay plate.

216 Assay X 24 Sample Format, 5X PCR Assay Plate

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	1	17	33	49	65	81	97	113	129	145	161	177	193	205										
B	2	18	34	50	66	82	98	114	130	146	162	178	194	206										
C	3	19	35	51	67	83	99	115	131	147	163	179	195	207										
D	4	20	36	52	68	84	100	116	132	148	164	180	196	208										
E	5	21	37	53	69	85	101	117	133	149	165	181	197	209										
F	6	22	38	54	70	86	102	118	134	150	166	182	198	210										
G	7	23	39	55	71	87	103	119	135	151	167	183	199	211										
H	8	24	40	56	72	88	104	120	136	152	168	184	200	212										
I	9	25	41	57	73	89	105	121	137	153	169	185	201	213										
J	10	26	42	58	74	90	106	122	138	154	170	186	202	214										
K	11	27	43	59	75	91	107	123	139	155	171	187	203	215										
L	12	28	44	60	76	92	108	124	140	156	172	188	204	216										
M	13	29	45	61	77	93	109	125	141	157	173	189												
N	14	30	46	62	78	94	110	126	142	158	174	190												
O	15	31	47	63	79	95	111	127	143	159	175	191												
P	16	32	48	64	80	96	112	128	144	160	176	192												

Figure 28. 216 assay x 24 sample format, 5X PCR assay plate.

248 Assay X 20 Sample Format, 5X PCR Assay Plate

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	1	17	33	49	65	81	97	113	129	145	161	177	193	209	225	241	245							
B	2	18	34	50	66	82	98	114	130	146	162	178	194	210	226	242	246							
C	3	19	35	51	67	83	99	115	131	147	163	179	195	211	227	243	247							
D	4	20	36	52	68	84	100	116	132	148	164	180	196	212	228	244	248							
E	5	21	37	53	69	85	101	117	133	149	165	181	197	213	229									
F	6	22	38	54	70	86	102	118	134	150	166	182	198	214	230									
G	7	23	39	55	71	87	103	119	135	151	167	183	199	215	231									
H	8	24	40	56	72	88	104	120	136	152	168	184	200	216	232									
I	9	25	41	57	73	89	105	121	137	153	169	185	201	217	233									
J	10	26	42	58	74	90	106	122	138	154	170	186	202	218	234									
K	11	27	43	59	75	91	107	123	139	155	171	187	203	219	235									
L	12	28	44	60	76	92	108	124	140	156	172	188	204	220	236									
M	13	29	45	61	77	93	109	125	141	157	173	189	205	221	237									
N	14	30	46	62	78	94	110	126	142	158	174	190	206	222	238									
O	15	31	47	63	79	95	111	127	143	159	175	191	207	223	239									
P	16	32	48	64	80	96	112	128	144	160	176	192	208	224	240									

Figure 29. 248 assay x 20 sample format, 5X PCR assay plate.

296 Assay X 16 Sample Format, 5X PCR Assay Plate

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	1	17	33	49	65	81	97	113	129	145	161	177	193	209	225	241	257	273	289	293				
B	2	18	34	50	66	82	98	114	130	146	162	178	194	210	226	242	258	274	290	294				
C	3	19	35	51	67	83	99	115	131	147	163	179	195	211	227	243	259	275	291	295				
D	4	20	36	52	68	84	100	116	132	148	164	180	196	212	228	244	260	276	292	296				
E	5	21	37	53	69	85	101	117	133	149	165	181	197	213	229	245	261	277						
F	6	22	38	54	70	86	102	118	134	150	166	182	198	214	230	246	262	278						
G	7	23	39	55	71	87	103	119	135	151	167	183	199	215	231	247	263	279						
H	8	24	40	56	72	88	104	120	136	152	168	184	200	216	232	248	264	280						
I	9	25	41	57	73	89	105	121	137	153	169	185	201	217	233	249	265	281						
J	10	26	42	58	74	90	106	122	138	154	170	186	202	218	234	250	266	282						
K	11	27	43	59	75	91	107	123	139	155	171	187	203	219	235	251	267	283						
L	12	28	44	60	76	92	108	124	140	156	172	188	204	220	236	252	268	284						
M	13	29	45	61	77	93	109	125	141	157	173	189	205	221	237	253	269	285						
N	14	30	46	62	78	94	110	126	142	158	174	190	206	222	238	254	270	286						
O	15	31	47	63	79	95	111	127	143	159	175	191	207	223	239	255	271	287						
P	16	32	48	64	80	96	112	128	144	160	176	192	208	224	240	256	272	288						

Figure 30. 296 assay x 16 sample format, 5X PCR assay plate.

384 Assay X 12 Sample Format, 5X PCR Assay Plate

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	1	17	33	49	65	81	97	113	129	145	161	177	193	209	225	241	257	273	289	305	321	337	353	369
B	2	18	34	50	66	82	98	114	130	146	162	178	194	210	226	242	258	274	290	306	322	338	354	370
C	3	19	35	51	67	83	99	115	131	147	163	179	195	211	227	243	259	275	291	307	323	339	355	371
D	4	20	36	52	68	84	100	116	132	148	164	180	196	212	228	244	260	276	292	308	324	340	356	372
E	5	21	37	53	69	85	101	117	133	149	165	181	197	213	229	245	261	277	293	309	325	341	357	373
F	6	22	38	54	70	86	102	118	134	150	166	182	198	214	230	246	262	278	294	310	326	342	358	374
G	7	23	39	55	71	87	103	119	135	151	167	183	199	215	231	247	263	279	295	311	327	343	359	375
H	8	24	40	56	72	88	104	120	136	152	168	184	200	216	232	248	264	280	296	312	328	344	360	376
I	9	25	41	57	73	89	105	121	137	153	169	185	201	217	233	249	265	281	297	313	329	345	361	377
J	10	26	42	58	74	90	106	122	138	154	170	186	202	218	234	250	266	282	298	314	330	346	362	378
K	11	27	43	59	75	91	107	123	139	155	171	187	203	219	235	251	267	283	299	315	331	347	363	379
L	12	28	44	60	76	92	108	124	140	156	172	188	204	220	236	252	268	284	300	316	332	348	364	380
M	13	29	45	61	77	93	109	125	141	157	173	189	205	221	237	253	269	285	301	317	333	349	365	381
N	14	30	46	62	78	94	110	126	142	158	174	190	206	222	238	254	270	286	302	318	334	350	366	382
O	15	31	47	63	79	95	111	127	143	159	175	191	207	223	239	255	271	287	303	319	335	351	367	383
P	16	32	48	64	80	96	112	128	144	160	176	192	208	224	240	256	272	288	304	320	336	352	368	384

Figure 31. 384 assay x 12 sample format, 5X PCR assay plate.

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