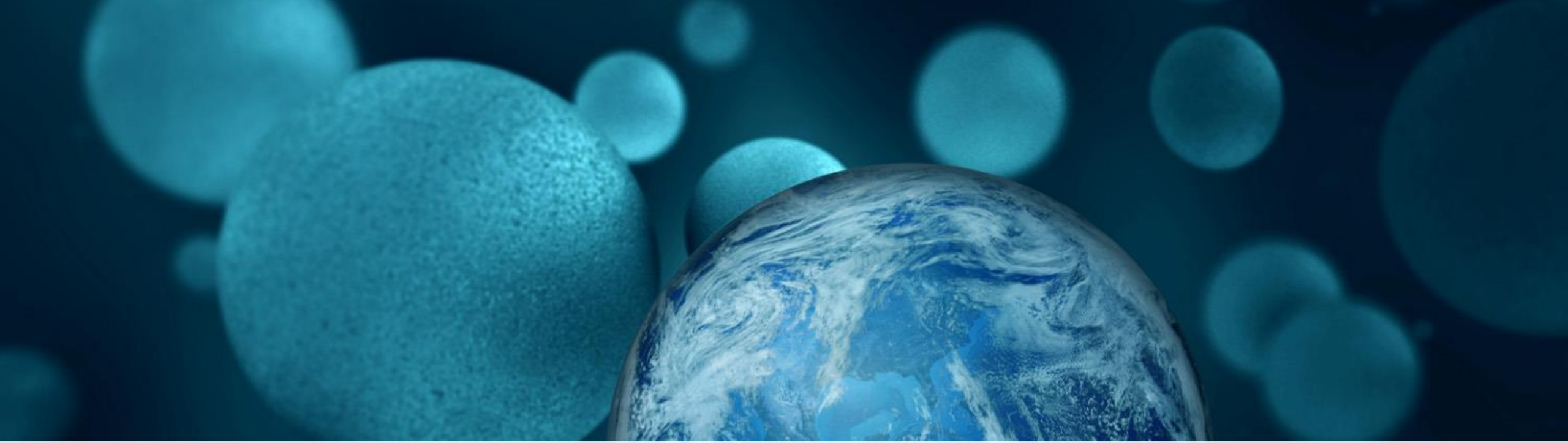


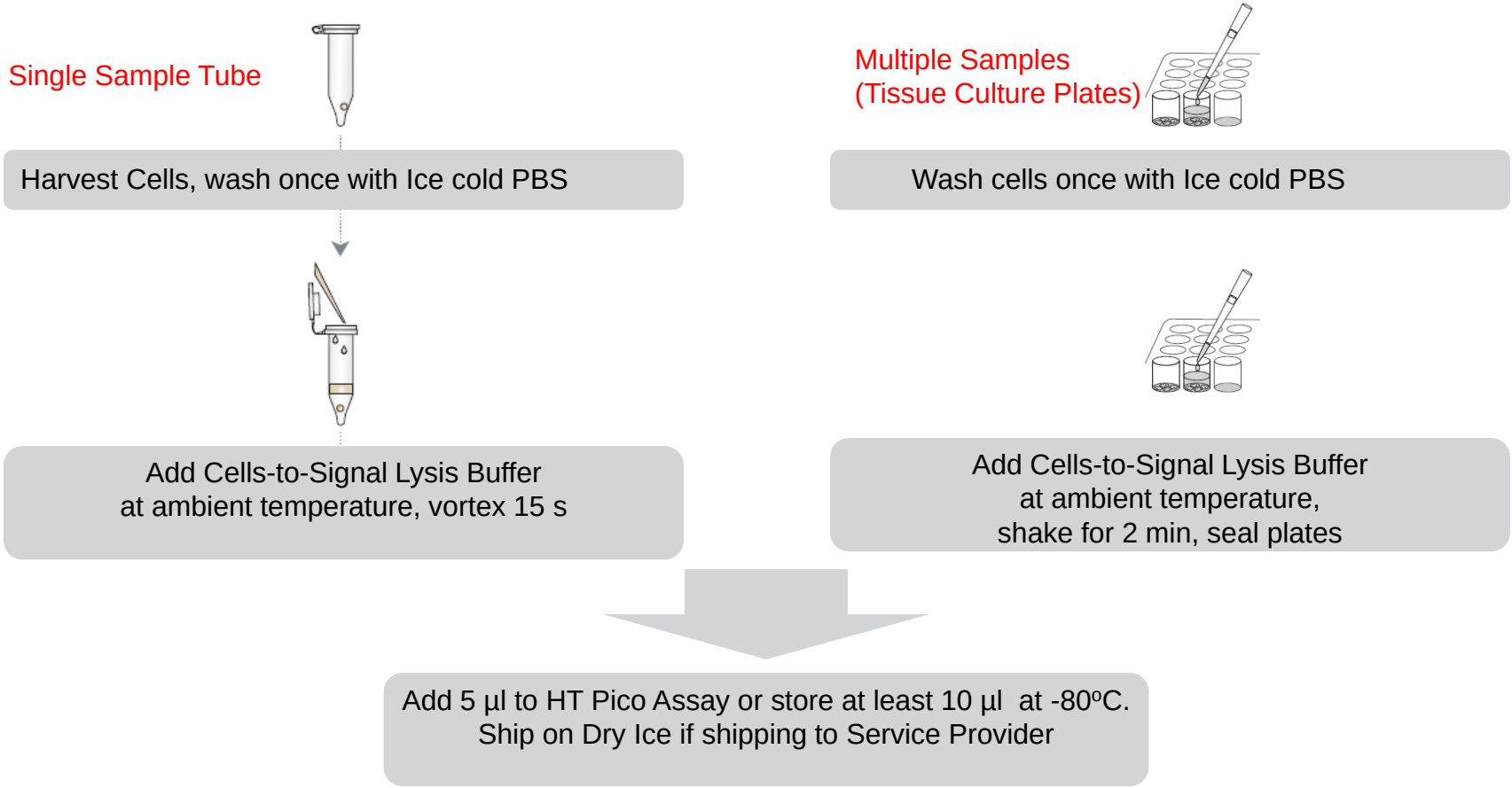
ThermoFisher SCIENTIFIC

Cells-to-Signal User Guidance for Clariom™ GO Screen sample preparation

Expression R&D, Microarray

[For training purposes only. Not for resale. Not for use in diagnostic procedures.]

Cells-to-Signal (AM8728) is a direct lysis reagent which can be used in conjunction with HT Pico sample preparation for Clariom GO Screen



- Cells-to-Signal (https://www.thermofisher.com/order/catalog/product/AM8728) is a direct lysis reagent developed for use with RT-qPCR reagents, and has a simple one-step protocol that is amenable as a direct input into HT Pico sample preparation
- Samples can be used directly, or if initial lysis cellular input exceeds 200 cells/µl (1000 Cells/Assay in 5 µl) can be further diluted in Cells-to-Signal reagent to 100-1000 cells (recommended dilution is 100 cells/µl, 500 Cells/Assay in 5µl)

Protocols for Direct Cell Lysis of 96- and 384-Well Tissue Culture plates

- The Cells-to-Signal technology uses a single buffer for cell lysis and RNase inactivation and lysates can be prepared for downstream HT Pico sample preparation in less than 10 minutes.
- After lysis steps, tissue culture plates can be sealed, and shipped to service providers on dry ice.
- If multiple experiments are to be run with the same lysate samples, we recommend partitioning of samples prior to freezing and storage as multiple freeze-thaw cycles can effect performance.
- Lysates frozen at -80^{o}C can be stored for 2.5 months without any performance decrease.
  - We recommend partitioning into hard shell RNase/DNase-Free PCR Plates (Bio-Rad, #hsp9631).
  - If using a service provider, please ship at least 10 µl of direct lysate per experimental condition on dry ice.
  - Please provide a plate map, in a fashion to be advised by your Service Provider, and confirm that designated control wells are either empty, or can be replaced with RNA controls (see below)

- ***Note: For each 384 Array plate, 8 control RNA samples will be run on each plate. This should be noted in the study design such that either 2 wells of a 96-well tissue culture plate, or 8 wells of a 384–well tissue culture plate should either be left empty, or explicitly noted that these samples will not be processed in the GO Screen Assay***

Protocol for use with 96 well Tissue Culture plates

1. Prior to beginning, bring Cells-to-Signal reagent to room temperature.
2. Remove cell culture media from 96-well tissue culture plate.
3. Add 50 µl of ice cold PBS (P/N 14190144) to each well of 96-well plate.
4. Remove PBS from plate.
5. Add 100 µl of Cells-to-Signal to each well.
6. Shake 96-well plate at 300 RPM for 2 minutes to ensure lysis.
7. Seal plates with foil plate seal and plate can be centrifuged at 500 RPM for 1 minute using standard plate centrifuge.
8. Either:

   A. Process cells immediately using 5 µl as an input into HT Pico assay. If processing with no freezing step, keep lysates at 4°C while setting up HT Pico Assay.

   B. Alternatively, store at -80°C for future processing.

      A. If freezing Lysates, Prior to HT Pico Assay, thaw lysates at 4°C for ~ 1 hour at 4°C

Protocol for use with 384 well Tissue Culture plates

1. Prior to beginning, bring Cells-to-Signal reagent to room temperature.
2. Remove cell culture media from 384-well tissue culture plate.
3. Add 40 µl of ice cold PBS (P/N 14190144) to each well of 96-well plate.
4. Remove PBS from plate.
5. Add 40 µl of Cells-to-Signal to each well.
6. Shake 384-well plate at 300 RPM for 2 minutes to ensure lysis.
7. Seal plates with foil plate seal and plate can be centrifuged at 500 RPM for 1 minute using standard plate centrifuge.
8. Either:

   A. Process cells immediately using 5 µl as an input into HT Pico assay. If processing with no freezing step, keep lysates at 4^{o}C while setting up HT Pico Assay.

   B. Alternatively, store at -80^{o}C for future processing.

      A. If freezing Lysates, Prior to HT Pico Assay, thaw lysates at 4^{o}C for ~ 1 hour at 4^{o}C

Dealing with High Cellular inputs

Cells/Well	Intial Lysis Buffer Added/well (µl)	Initial Cells/µl	Cells/5 µl	Intial Lysed Material (µl)	Volume of fresh Cells-to-Signal for Dilution (µl)	Final Cells/µl	Final Cells/5 µl
100,000	100	1000	5000	5	45	100	500
80,000	100	800	4000	5	35	100	500
50,000	100	500	2500	5	20	100	500
20,000	100	200	1000	No dilution Necessary			
10,000	100	100	500				

- For experimental systems where the final cellular density is > 1000 Cells/5 µl in the final lysis reaction, we recommend a dilution step, with Cells-to-Signal, of the primary lysate.
- This step is recommended as using cellular inputs higher than 1000 Cells may have negative effects on performance
- When diluting the final target should be 500 cells/5 µl to target the mid-range of recommended input and not fall outside the recommended input range
- The Chart above illustrates some recommended dilutions

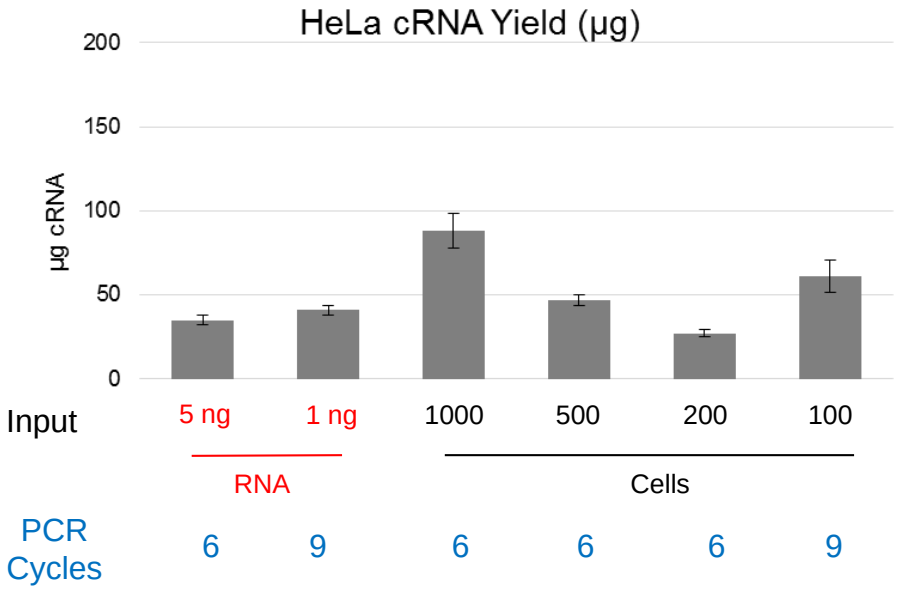
HT Pico PCR Cycling Guidelines

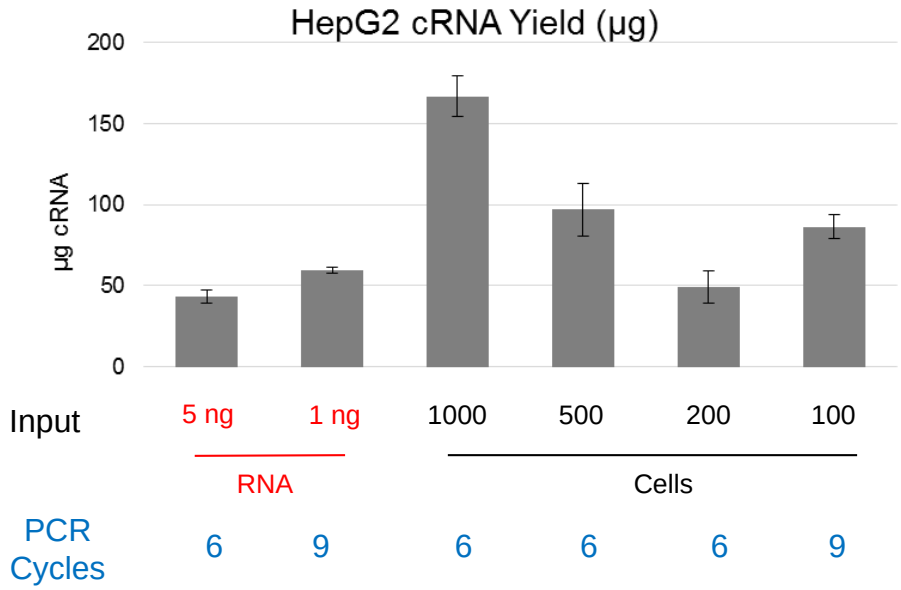
Number of Cells per 5 µl input	PCR Cycles for Fresh-Frozen Cells or Tissues
100 to < 200	9
200 to 1000	6

Total RNA Input	PCR Cycles for Fresh-Frozen Cells or Tissues
100 pg to < 500 pg	12
500 pg to < 2ng	9
2 ng to 10 ng	6

- Cellular lysates can be processed using the HT Pico assay as detailed in the Clariom GO Screen 384 Sample preparation guide.
- When performing the pre-IVT PCR cycling, the guidance for both RNA samples (controls) and lysates are shown above
  - Please note – for RNA controls on plates – 2 ng can be used for either 9 or 6 cycles. If 12 cycles are to be used (see below) please use 500 pg input.
- Our lowest recommended input is 100 cells, however Cell-to-Signal lysates down to 10 cells for HeLa and HepG2 cells have been tested.
- For Lysates containing 10 to less than 50 Cells, 12 cycles can be used, for Lysates containing 50-100 Cells, 9 cycles can be used as a starting point.

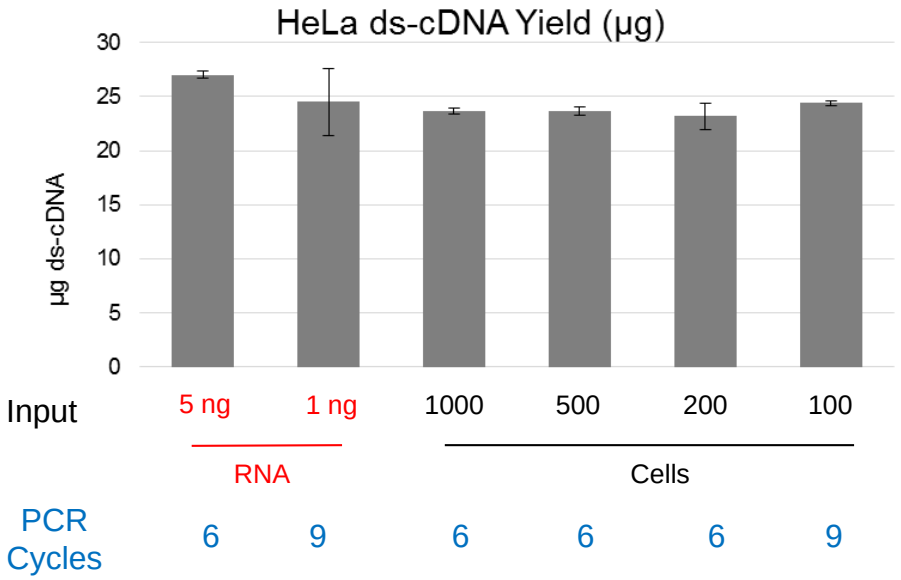
Representative cRNA yields from HeLa and HepG2 Lysates

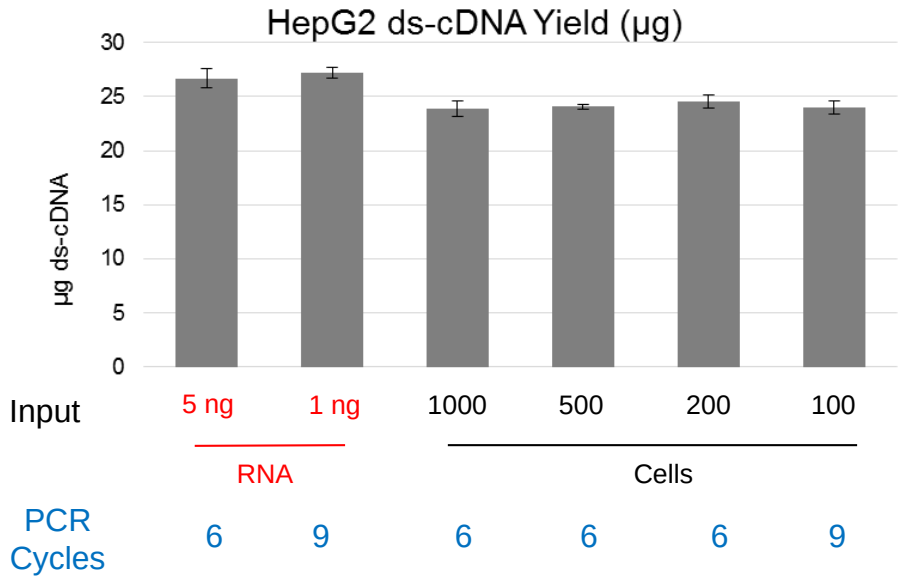




- Representative yields for cRNA for HeLa and HepG2 Lysates, all which meet the 25 µg cutoff to proceed to ds-cDNA generation
- After cRNA generation, samples are purified and normalized for input into the 2nd cycle ds-cDNA generation steps

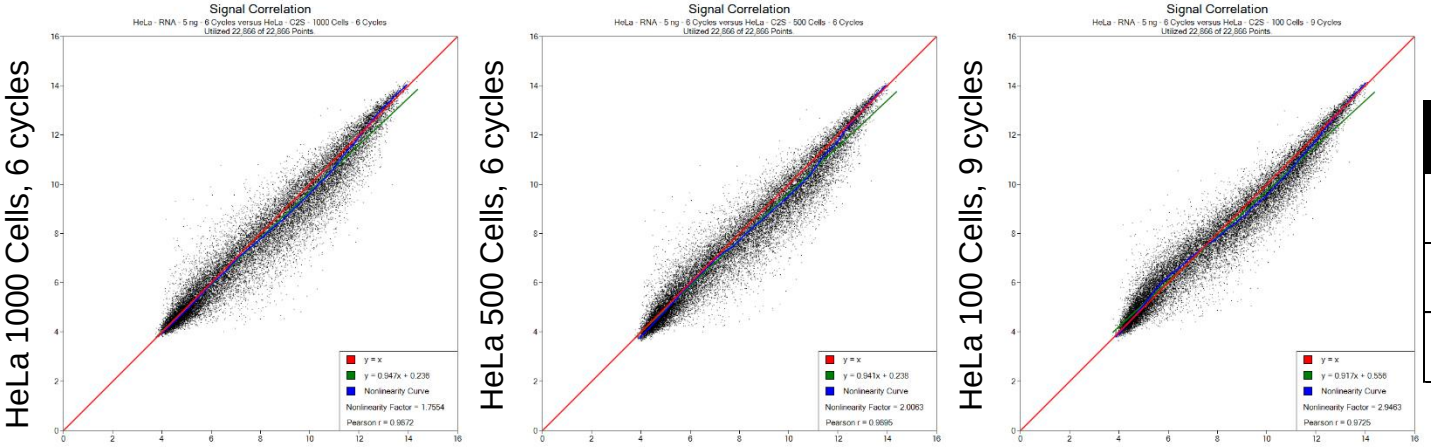
Representative ds-cDNA yields from HeLa and HepG2 Lysates



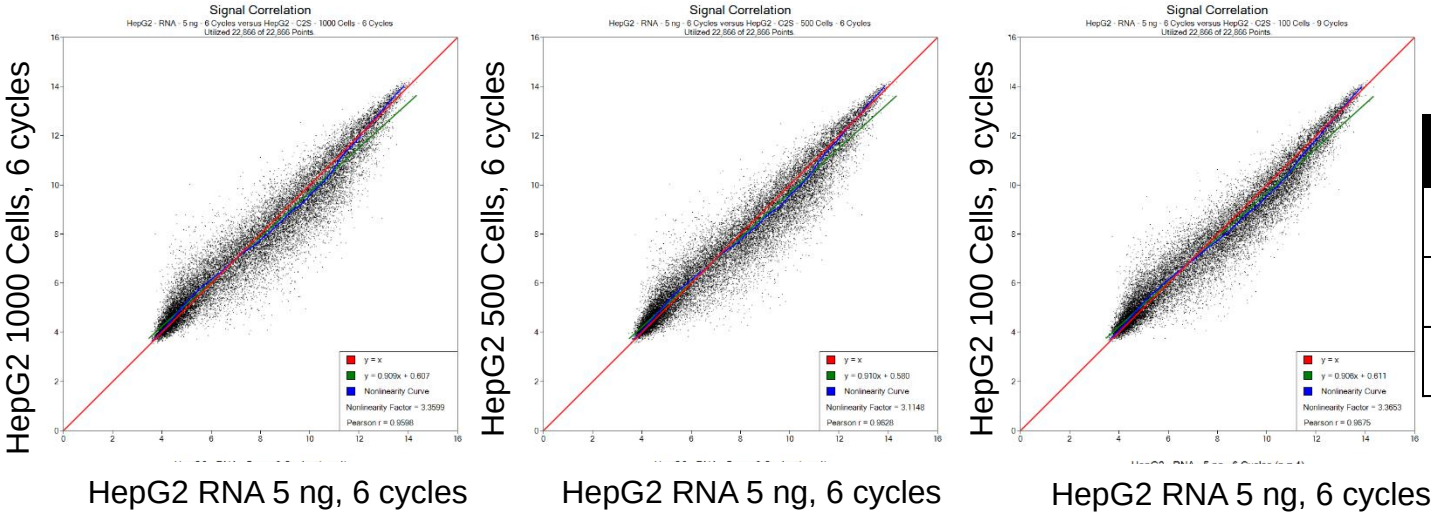


- Representative yields for ds-cDNA for HeLa and HepG2 Lysates, all meeting the 10 µg cutoff to proceed with target preparation
- The above demonstrate similar yields for lysates and RNA input controls

HeLa and HepG2 Cells-to-Signal Lysates correlate well to RNA controls across the range of cellular inputs



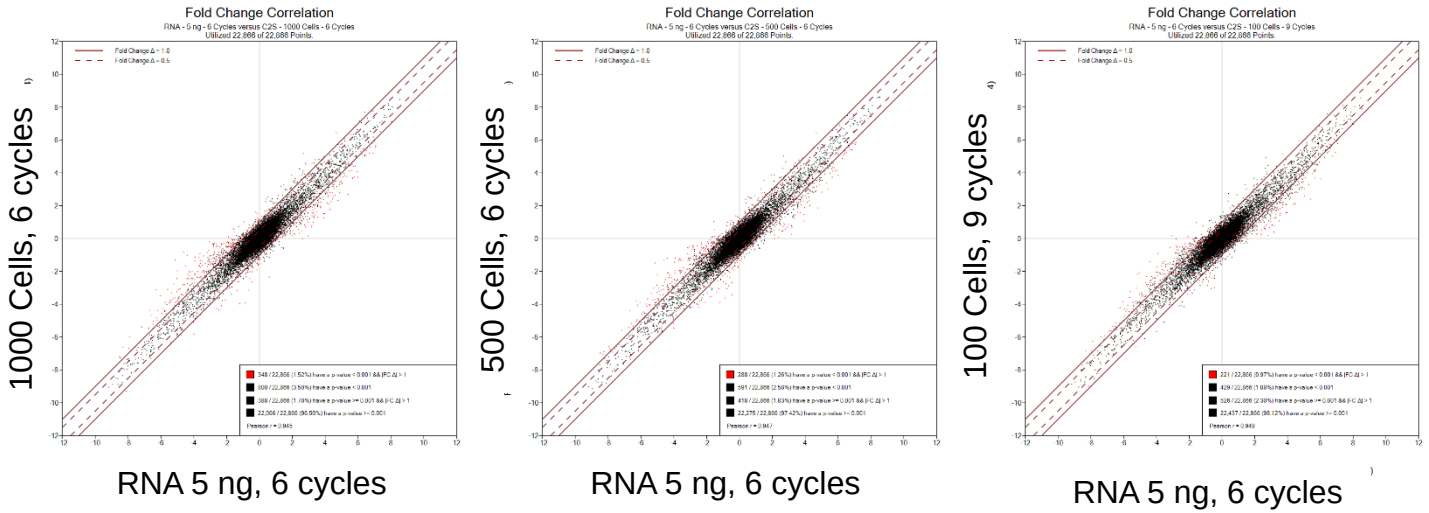
HeLa Pearson Correlation vs. 5 ng, 6 cycles	Pearson r
C2S - 1000 Cells - 6 Cycles	0.967
C2S - 500 Cells - 6 Cycles	0.970
C2S - 100 Cells - 9 Cycles	0.972



HepG2 Pearson Correlation vs. 5 ng, 6 cycles	Pearson r
C2S - 1000 Cells - 6 Cycles	0.960
C2S - 500 Cells - 6 Cycles	0.963
C2S - 100 Cells - 9 Cycles	0.968

- Cells-to-Signal lysates compare well to RNA samples across a range of cellular inputs via assessment by Pearson Correlation

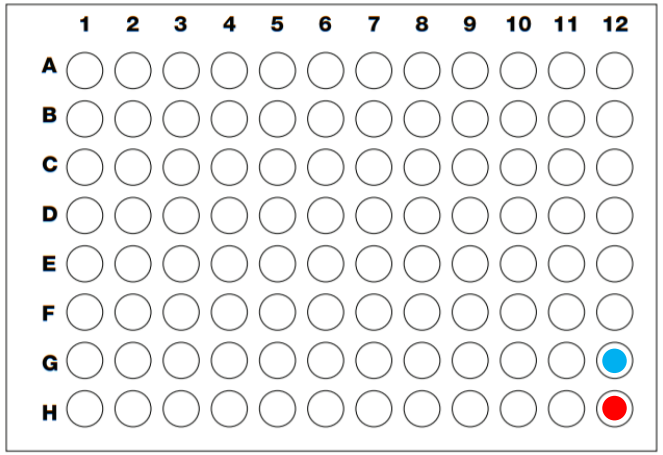
Fold Change Correlation of Cells-to-Signal lysates to RNA controls across the range of cellular inputs



Fold Change Correlation		
vs. 5 ng, 6 cycles	**Pearson r**	**FC Δ >1, p-value > 0.001**
C2S - 1000 Cells - 6 Cycles	0.945	348
C2S - 500 Cells - 6 Cycles	0.947	288
C2S - 100 Cells - 9 Cycles	0.949	221

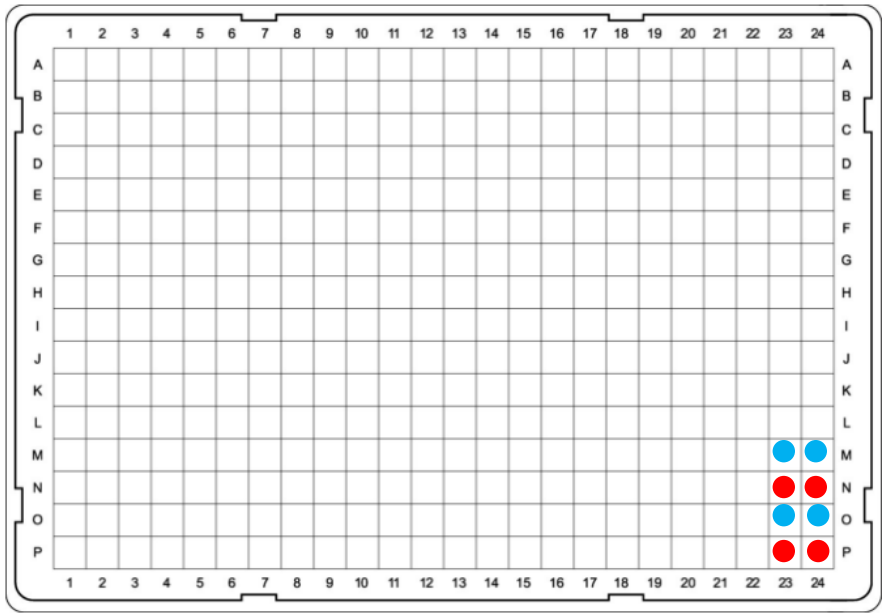
- Fold Change Correlations show comparable performance to RNA controls across the range of cellular inputs

8 Replicate controls Layout on 96- and 384-well plates



HeLa RNA

Liver RNA



HeLa RNA

Liver RNA

- For sample processing 4 x Each of the following will be run in the following positions, such that these will be reserved for controls, and no samples to be processed should be placed in these spots.
  - HeLa RNA - TFS P/N AM7852
  - Liver RNA – TFS P/N AM7960
  - For each
    - For 6 or 9 Pre-IVT cycles use 2 ng input per well
    - For 12 Pre-IVT cycles use 500 pg per well

Summary

- The Cells-to-Signal technology uses a single buffer for cell lysis and RNase inactivation and lysates can be prepared for downstream HT Pico sample preparation in less than 10 minutes.
- For best performance, we recommend that cells be treated with an ice-cold PBS wash step prior to cellular lysis
- It is important to not use lysates above the recommended input range (1000 cells). More sample does not gain better performance. As lysates are not purified and contain cellular debris, increasing the input can have negative effects on assay performance.
- For samples above 1000 Cells, we suggest a further dilution step.
- Cells-to-Signal has been tested with a number of cell lines, and for GO Screen applications we have tested HepG2 and HeLa lysates extensively. For other cell types, a small pilot experiment can be performed
- For a Pilot experiment, lysates can be run with either the 3'IVT Pico Assay or HT Pico assay with the input guidelines described here. One can assay on a Clariom S Peg array (e.g. 24 Array Plate). This performance will be indicative of how lysates perform.
- For Pilot Experiments, we recommend triplicate samples and comparison to an RNA extraction control for your cell line of interest as well as running a HeLa RNA control to assure assay performance
  - One may also wish to use a test compound or perturbation to assess performance (e.g. fold changes of known transcripts which respond to variables).

Revision history

Revision	Date	Description
0	27 June 2019	