

# Monitoring Differential Expression in Stem Cells

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## Abstract

Stem cell differentiation is routinely monitored by measuring gene expression using real-time reverse transcription PCR. The Plexor® Differential Expression System is a novel real-time PCR system for the detection and relative quantitation of RNA expression levels, and can be used to monitor stem cell differentiation using multiplexed amplification.

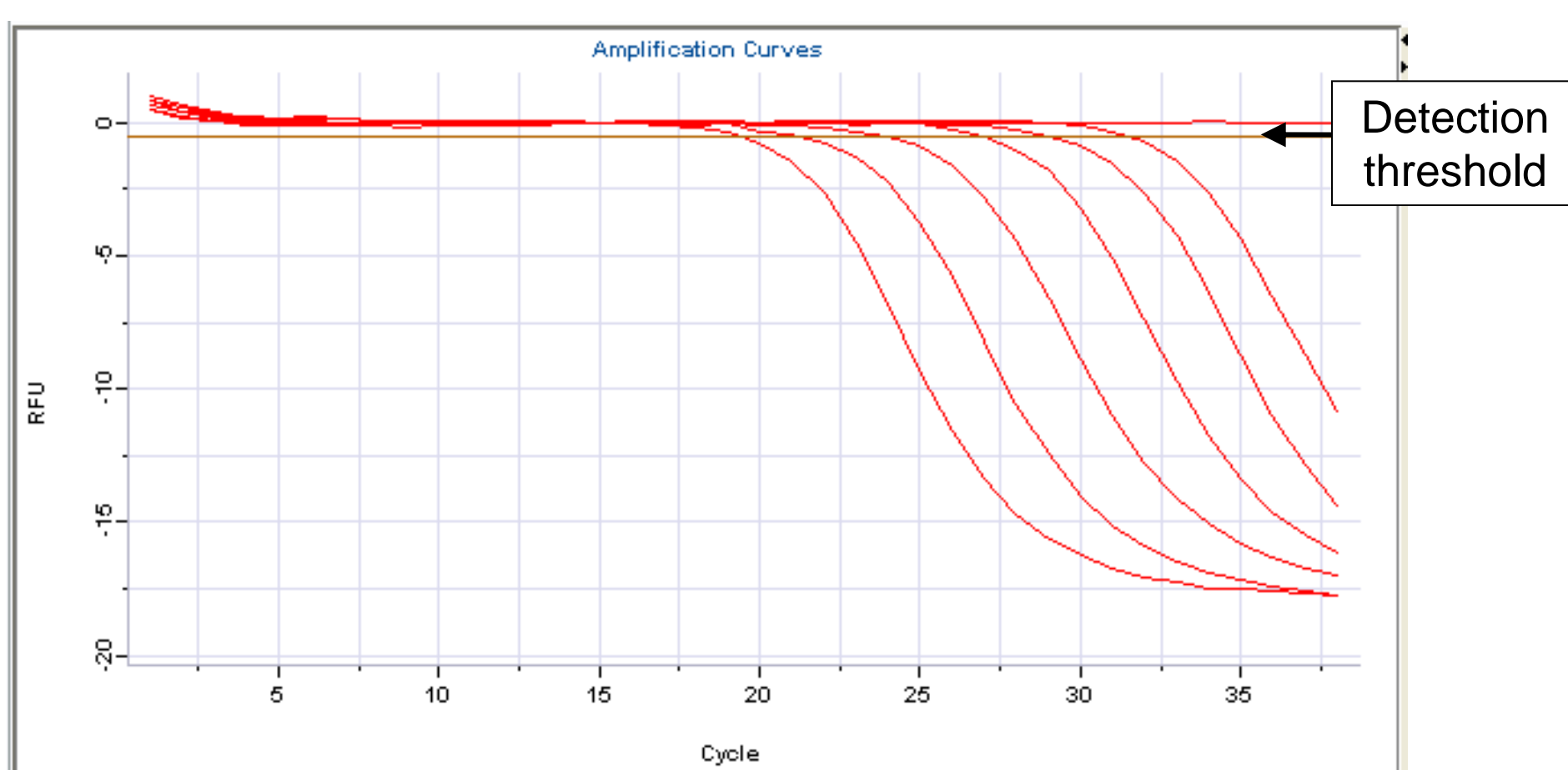
Optimized primer pairs will allow users to quantitatively amplify specific transcripts in a two-color duplex reaction. This duplex amplification strategy enables the user to amplify a transcript of interest as well as a reference transcript (GAPDH) in a single reaction.

The first set of optimized primer pairs to be made available will focus on transcripts associated with stem cell pluripotency: NANOG, SOX2, OCT3/4, LIN28, KLF4, and cMYC, each in a duplex with GAPDH. These primers target genes that are expressed at high levels in pluripotent cells (i.e., stem cells and induced pluripotent stem cells [iPS]). In contrast, these genes are expressed at low levels in differentiated cells.

## Plexor® Technology for Real-Time PCR

### Plexor® qPCR – Amplification Curves

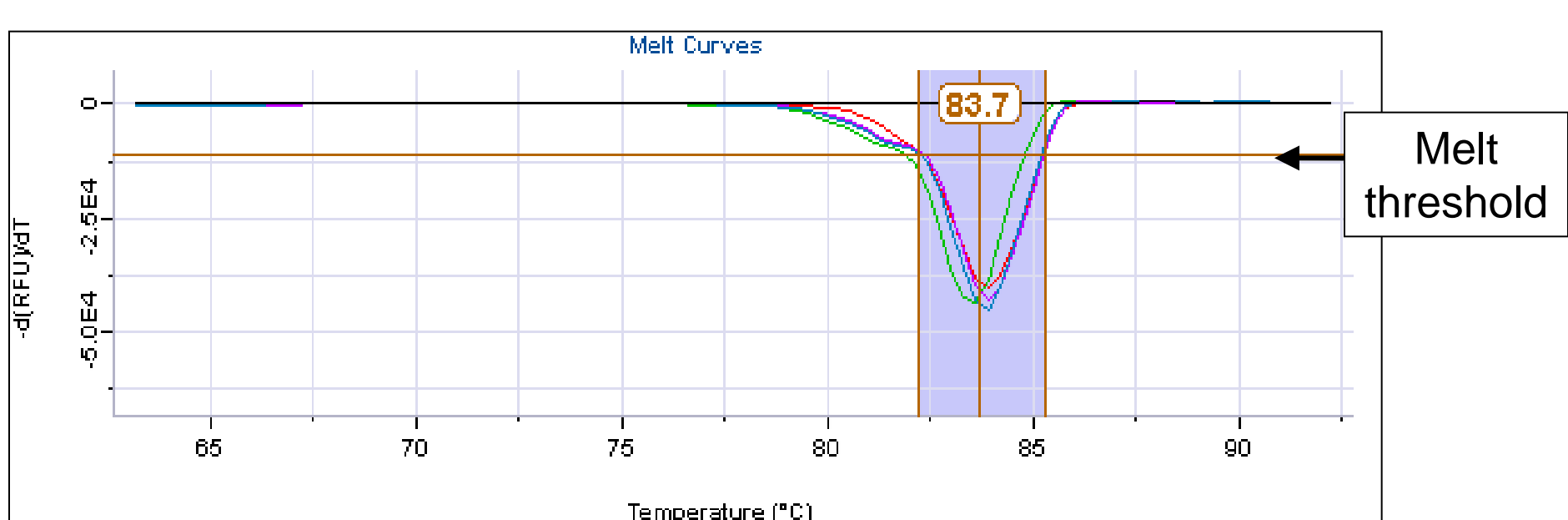
- As amplification proceeds, fluorescent dyes are quenched.
- Results in a DECREASE in fluorescence during amplification.
- The point where the decrease in fluorescence crosses the detection threshold is the C<sub>T</sub>; samples are compared using this number.



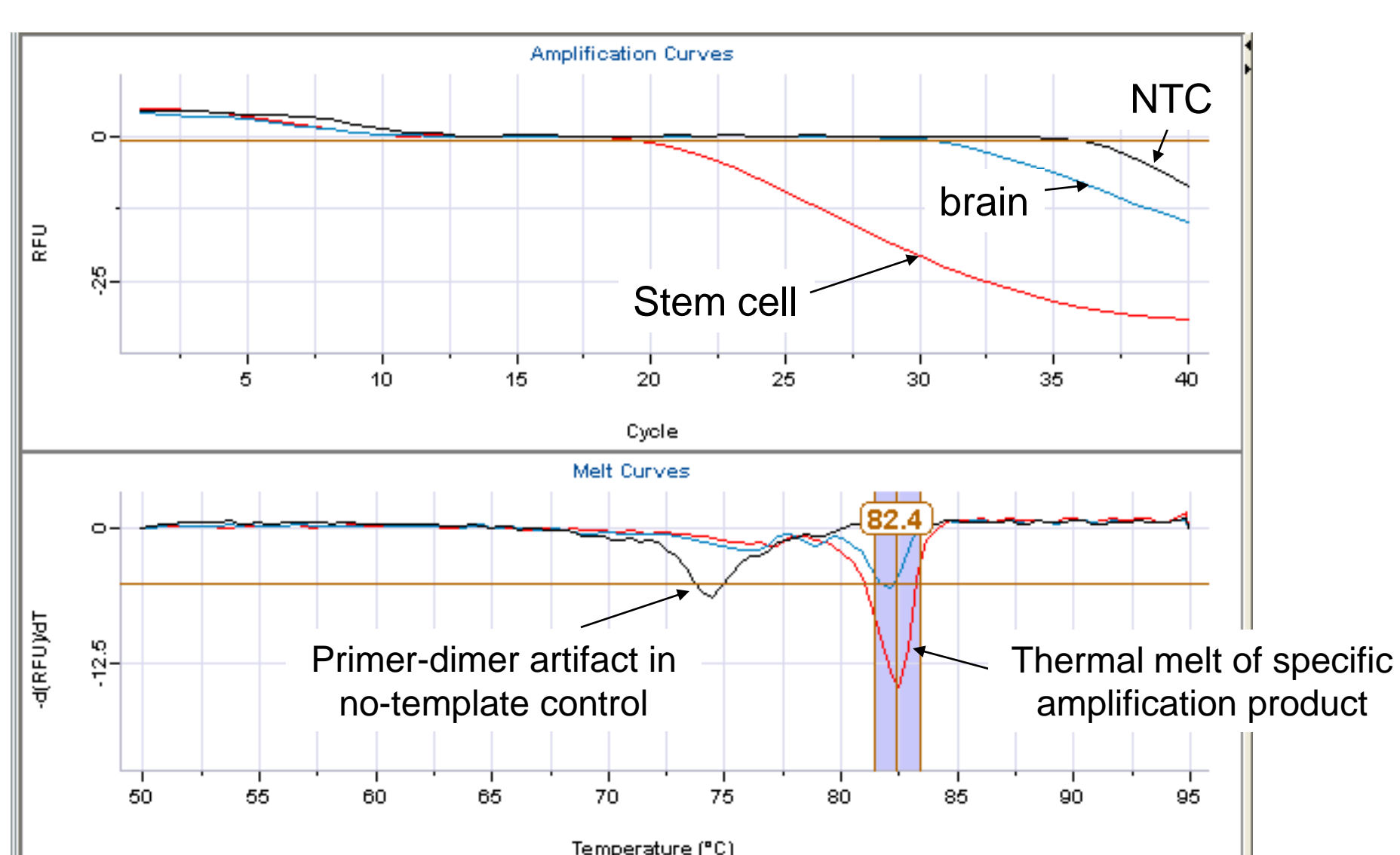
smaller C<sub>T</sub> value = higher level of gene expression

### Plexor® qPCR – Thermal Melt Analysis

- After amplification is complete, the amplified product is denatured during a "melt" step.



- Primer-dimer artifacts in the no-template control (NTC) are easily distinguished from the thermal melt of the specific amplification product.

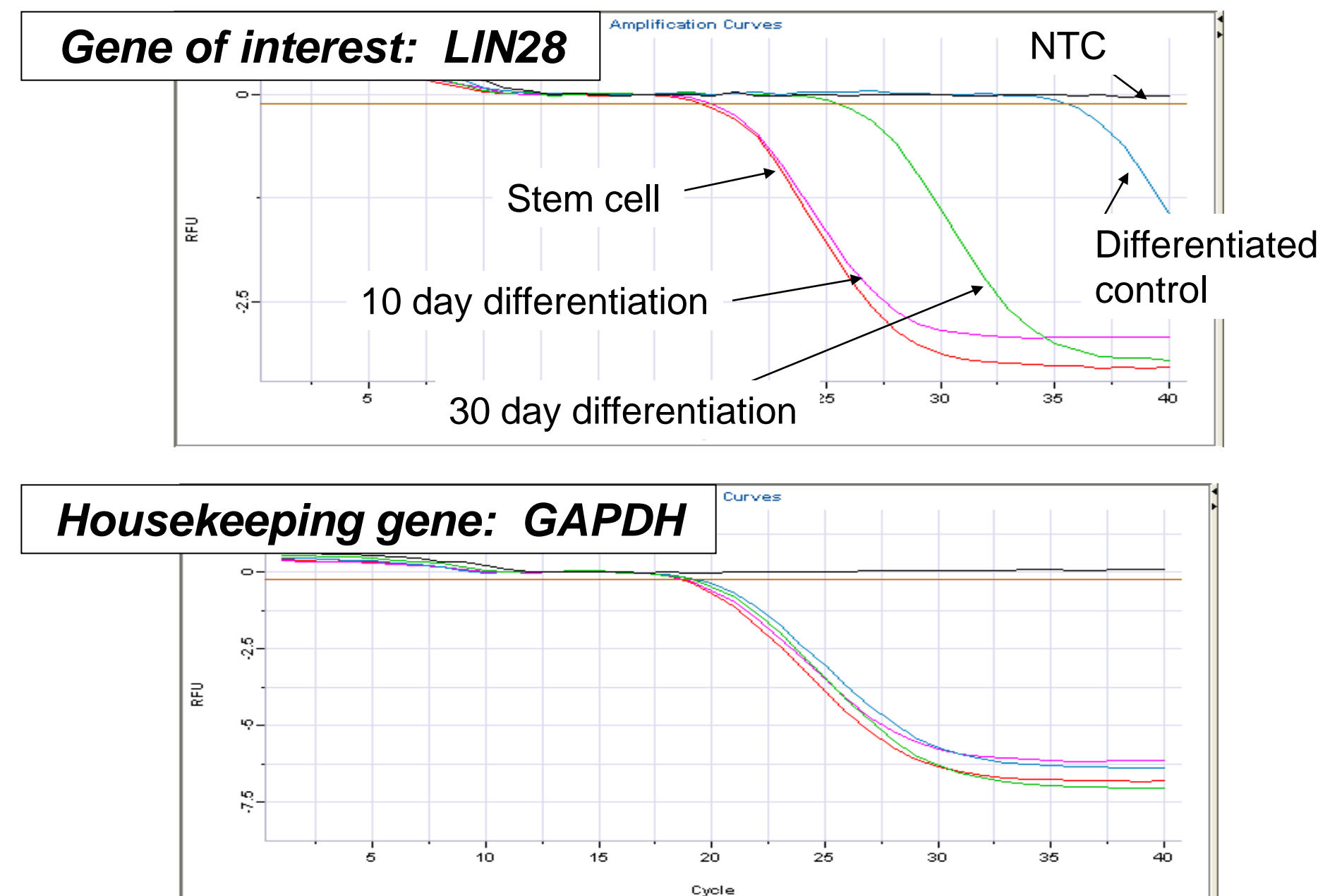


## Relative Quantitation: $\Delta C_T$

Expression levels of a particular gene of interest can be more easily compared across different RNA samples after normalization to a reference transcript (typically, a housekeeping gene). To achieve this normalization, a  $\Delta C_T$  is calculated for each sample:

$$\Delta C_T = (C_T \text{ gene of interest}) - (C_T \text{ GAPDH})$$

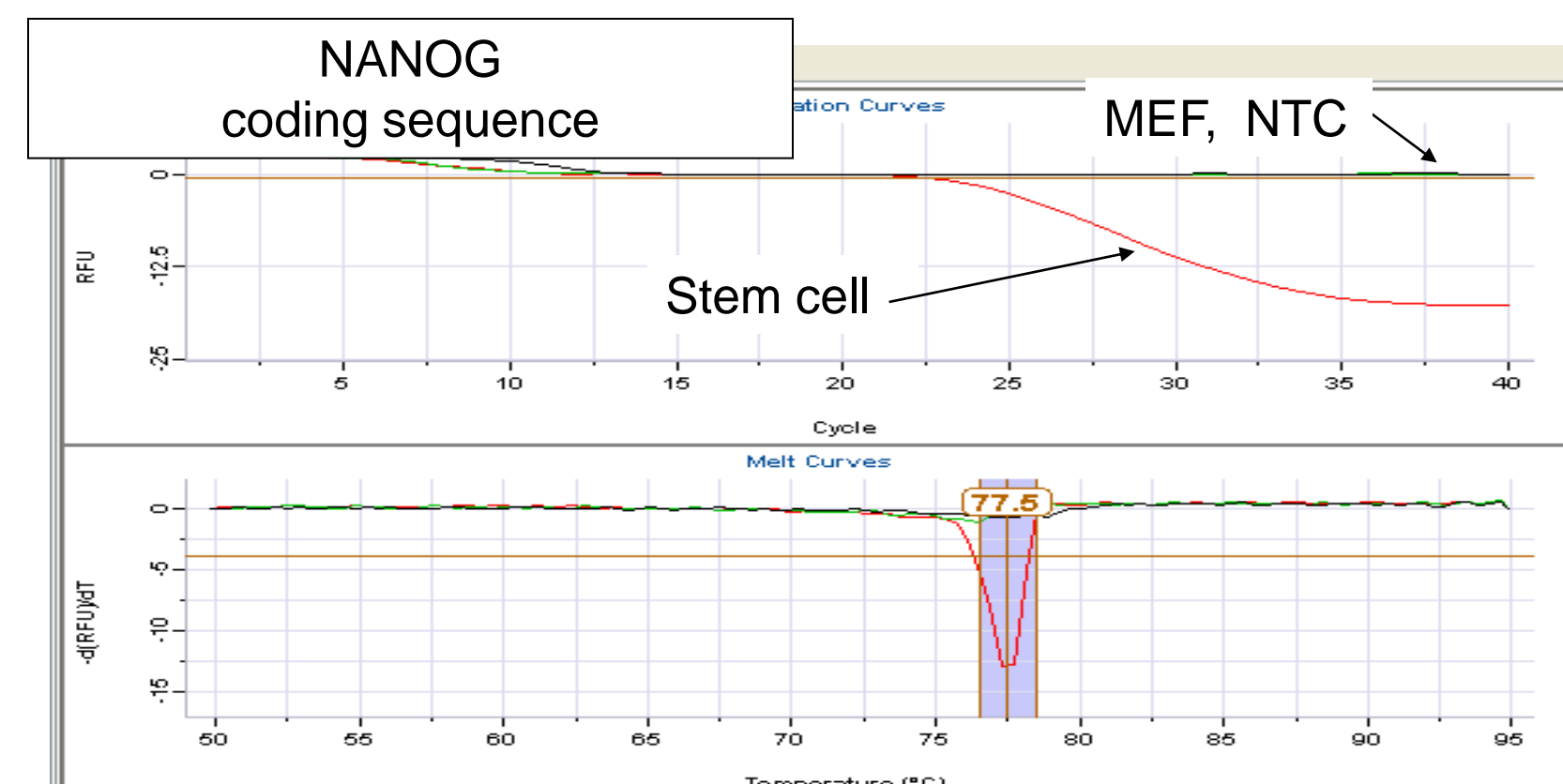
- LIN28 is expressed at high levels in the pluripotent stem cells. As the cells begin to differentiate, expression of LIN28 decreases.
- In contrast, the level of GAPDH expression remains constant.



	LIN28 C <sub>T</sub>	GAPDH C <sub>T</sub>	$\Delta C_T$
stem cell	19.5	18.6	0.9
10 day differentiation	20	18.7	1.3
30 day differentiation	25.5	18.9	6.6
differentiated control	35.4	19.3	16.1

## Species Specificity of Optimized Primer Pairs

- Human embryonic stem cells are often grown on a layer of mouse embryonic fibroblasts (MEFs) to promote growth and prevent differentiation. Consequently, it is critical that the primers do not detect mouse sequences.
- Primers are human specific, as shown by the absence of amplification in RNA from the MEF cells.

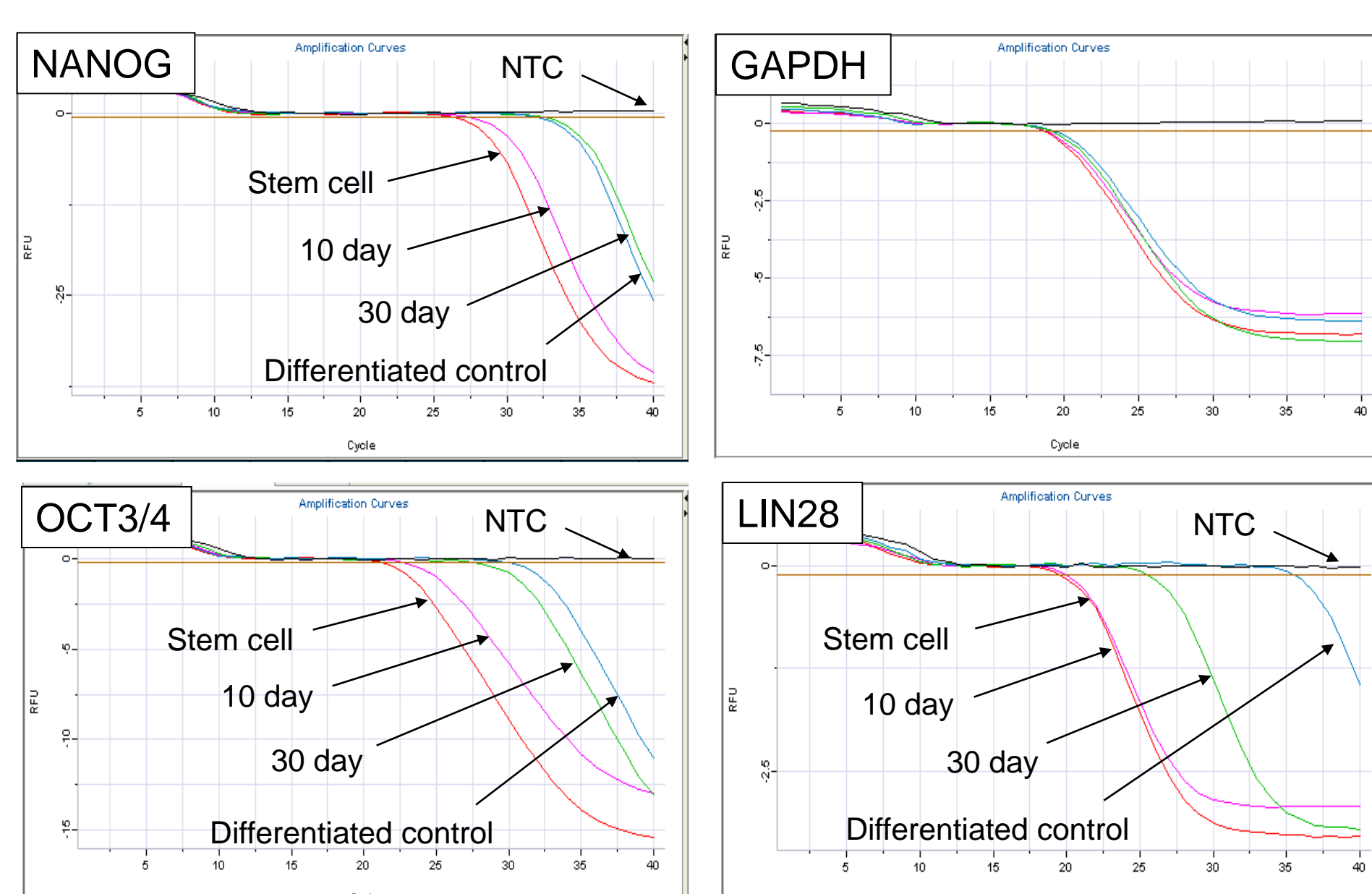


## Monitoring Expression of Pluripotency Transcripts

The first set of optimized primer pairs to be made available will focus on transcripts associated with stem cell pluripotency: NANOG, SOX2, OCT3/4, LIN28, KLF4, and cMYC, each in a duplex with GAPDH.

As stem cells differentiate, expression of pluripotent transcripts decreases.

- Stem cells were treated with growth factors to stimulate neuronal differentiation.
- Cell samples were collected after 10 days and 30 days of differentiation; gene expression is compared to undifferentiated stem cells and a differentiated control sample.
- Plexor® amplification of the pluripotency transcripts shows the dynamic change in expression of those genes as the cells differentiate.

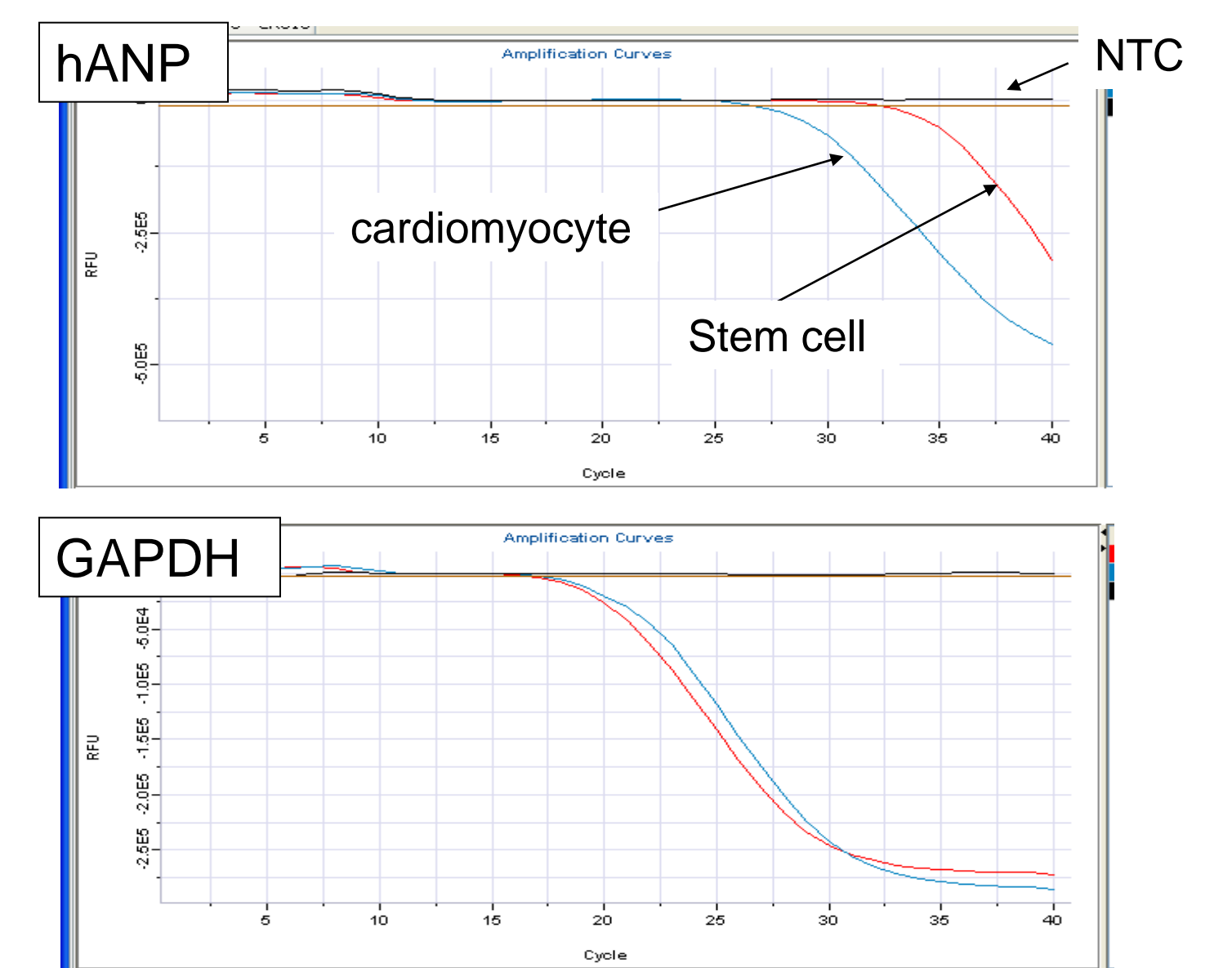


## Potential Future Primer Sets Could Include Differentiation Markers

### Assessing Gene Expression Following Cardiomyocyte Differentiation

As stem cells are induced to differentiate into cardiomyocytes, genes which are associated with the formation of heart tissue are expressed. Plexor® amplification can be used to monitor the level of expression relative to undifferentiated cells.

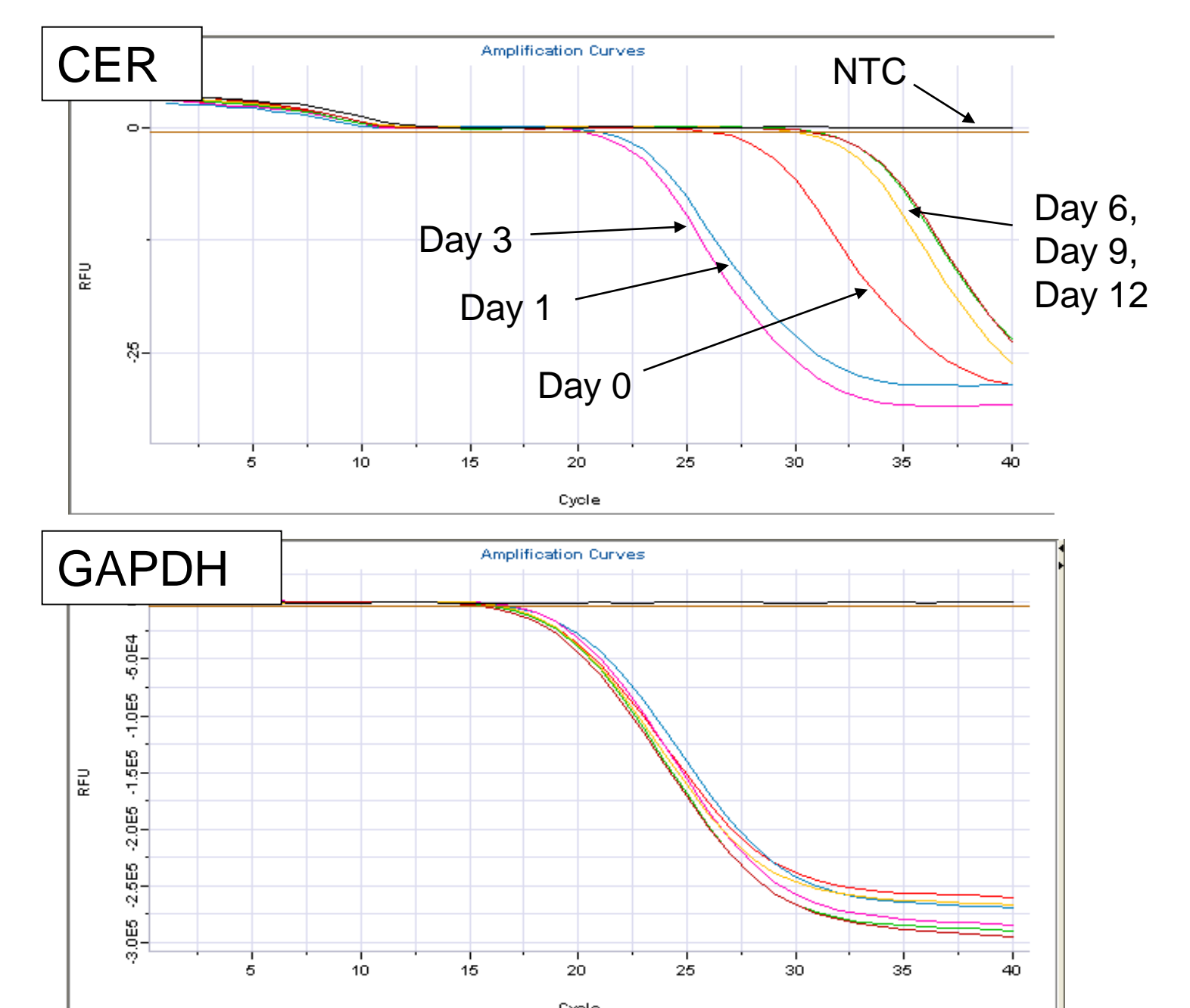
- Stem cells were treated with growth factors to stimulate cardiomyocyte differentiation.
- Cell samples were collected after 40 days of differentiation; beating cardiomyocytes were observed.
- Plexor® amplification of the hANP transcript shows the increase in expression of that gene after the cells have differentiated. The level of GAPDH expression remains constant.



### Monitoring Gene Expression During Pancreatic Differentiation

As stem cells are induced to differentiate into pancreatic cells, genes which are associated with the formation of pancreas tissue are expressed. Expression of these transcripts can change over time, as the cells differentiate. Plexor® amplification can be used to monitor these temporal changes in gene expression, relative to undifferentiated cells.

- Stem cells were treated with growth factors to stimulate pancreatic differentiation.
- Cell samples were collected after 1, 3, 6, 9, and 12 days of differentiation.
- Plexor® amplification of the CER transcript shows the transient expression of that gene as the cells differentiate.



## Summary

The Plexor® Differential Expression System is optimized to quantitatively amplify a two-color duplex, allowing the user to amplify a transcript of interest as well as a reference transcript (GAPDH) in a single reaction.

Optimized duplex primer pairs will be available for specific transcripts of interest. Initial primer offerings include human-specific primers which amplify transcripts associated with stem cell pluripotency.

Possible future sets of optimized primer pairs may include primers which target transcripts associated with human cardiomyocyte differentiation, human pancreatic differentiation, human neuronal differentiation, or mouse-specific pluripotency transcripts.