Assessing heterogeneity at single cell level in a clonal population of transformed epidermal keratinocytes

Teisanu RM[1], Richter H[2], Moore J[3], Paillusson A[2], Hagenbüchle O[2], Weber J[2], Harshman K[2], Barrandon Y[1]

1. Laboratoire de Dynamique des Cellule Souches, Institute of Bioengeneering, Ecole Polytechnique Fédérale de Lausanne
2. Genomics Technologies Facility (GTF), Center for Integrative Genomics, Université de Lausanne
3. Fluidigm Corporation, South San Francisco, CA USA

Human epidermal keratinocytes can be extensively cultured *in vitro*, resulting in the formation of epithelial colonies maintained by a stem cell hierarchy similar to that of normal epidermis. Due to technical limitations, efforts to determine the molecular identity of epidermal stem cells and their progeny generally rely on assessing gene expression levels in large populations of cells. The major caveat of this approach is the averaging of gene expression in a functionally non-homogenous population of cells, thus preventing the identification of individual signatures. The purpose of this study was to optimize methods that will be further employed in determining gene expression signatures of epidermal stem cells and to determine the level of heterogeneity in a clonal population of immortalized human epidermal keratinocytes (GMA 24). The expression level of 32 genes involved in proliferation, differentiation and stemness maintenance was determined in individual GMA24 cells bearing a fluorescent reporter of the S, G2 and M phases of the cell cycle (the *fluorescence ubiquitination cell cycle indicator* (*FUCCI*)). To this end we employed a novel technology based on microfluidics chips (Fluidigm's BioMark system) that allows the assessment of 96 genes in 96 individual samples. Data analysis allowed the classification of genes according to their variability from cell to cell in low, intermediate and high variability genes. Non-expressed genes were not included in the analysis. According to this classification, stable genes represented 30.4% of the expressed genes. Importantly, 56.5% belonged to the highly variable genes, demonstrating that despite the clonal origin of these cells, considerable gene expression variability exists at single cell level. Further studies are required to assess whether this heterogeneity results in functional differences between cells. In conclusion, novel technology enabling the simultaneous analysis and comparison of gene expression in single cells reveals a previously unrecognized heterogeneity in a clonally derived population of cells.

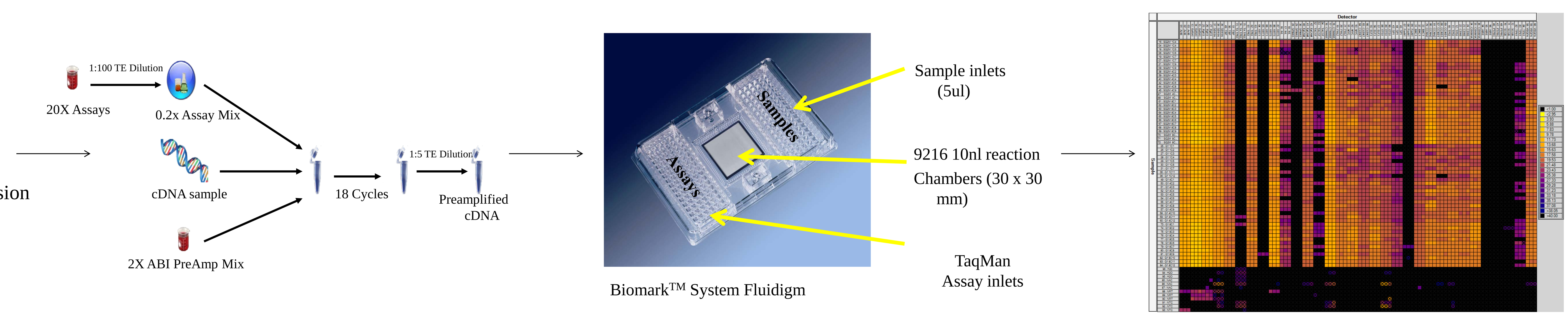
Material and methods:

Genes analyzed: 32 TaqMan Assays on Demand

ActB	KRT1	FoxN1	TP53	ActB	KRT1	FoxN1	TP53	ActB	KRT1	FoxN1	TP53
Eef1A1	IVL	FHL2	CDK6	Eef1A1	IVL	FHL2	CDK6	Eef1A1	IVL	FHL2	CDK6
Rpl13	SPINK5	BNC1	CDKN1A	Rpl13	SPINK5	BNC1	CDKN1A	Rpl13	SPINK5	BNC1	CDKN1A
SDH1A	EpCAM	SP1	PTPRC	SDH1A	EpCAM	SP1	PTPRC	SDH1A	EpCAM	SP1	PTPRC
TBP	TACSTD2	ELF3	VEGFA	TBP	TACSTD2	ELF3	VEGFA	TBP	TACSTD2	ELF3	VEGFA
TP63	CLDN7	CCND1	SCGB1A1	TP63	CLDN7	CCND1	SCGB1A1	TP63	CLDN7	CCND1	SCGB1A1
TA TP63	CTNNB1	CCNA2	TPH1	TA TP63	CTNNB1	CCNA2	TPH1	TA TP63	CTNNB1	CCNA2	TPH1
KRT5	ETS1	MYC	AIRE	KRT5	ETS1	MYC	AIRE	KRT5	ETS1	MYC	AIRE

Samples: sorted single cells according to AG Geminin expression

1cell	1cell	1cell	1cell	1cell	1cell	1cell	1cell	1cell	1cell	1cell	1cell
1cell	1cell	1cell	1cell	1cell	1cell	1cell	1cell	1cell	1cell	1cell	1cell
1cell	1cell	1cell	1cell	1cell	1cell	1cell	1cell	1cell	1cell	1cell	1cell
1cell	1cell	1cell	1cell	1cell	1cell	1cell	1cell	1cell	1cell	1cell	1cell
1cell	1cell	1cell	1cell	1cell	1cell	1cell	1cell	1cell	1cell	1cell	1cell
1cell	1cell	1cell	1cell	1cell	1cell	1cell	1cell	1cell	1cell	1cell	1cell
1cell	1cell	1cell	1cell	1cell	1cell	1cell	1cell	1cell	1cell	1cell	1cell
1cell	1cell	1cell	1cell	1cell	1cell						



Results:

Fluorescence ubiquitination cell cycle indicator (FUCCI):

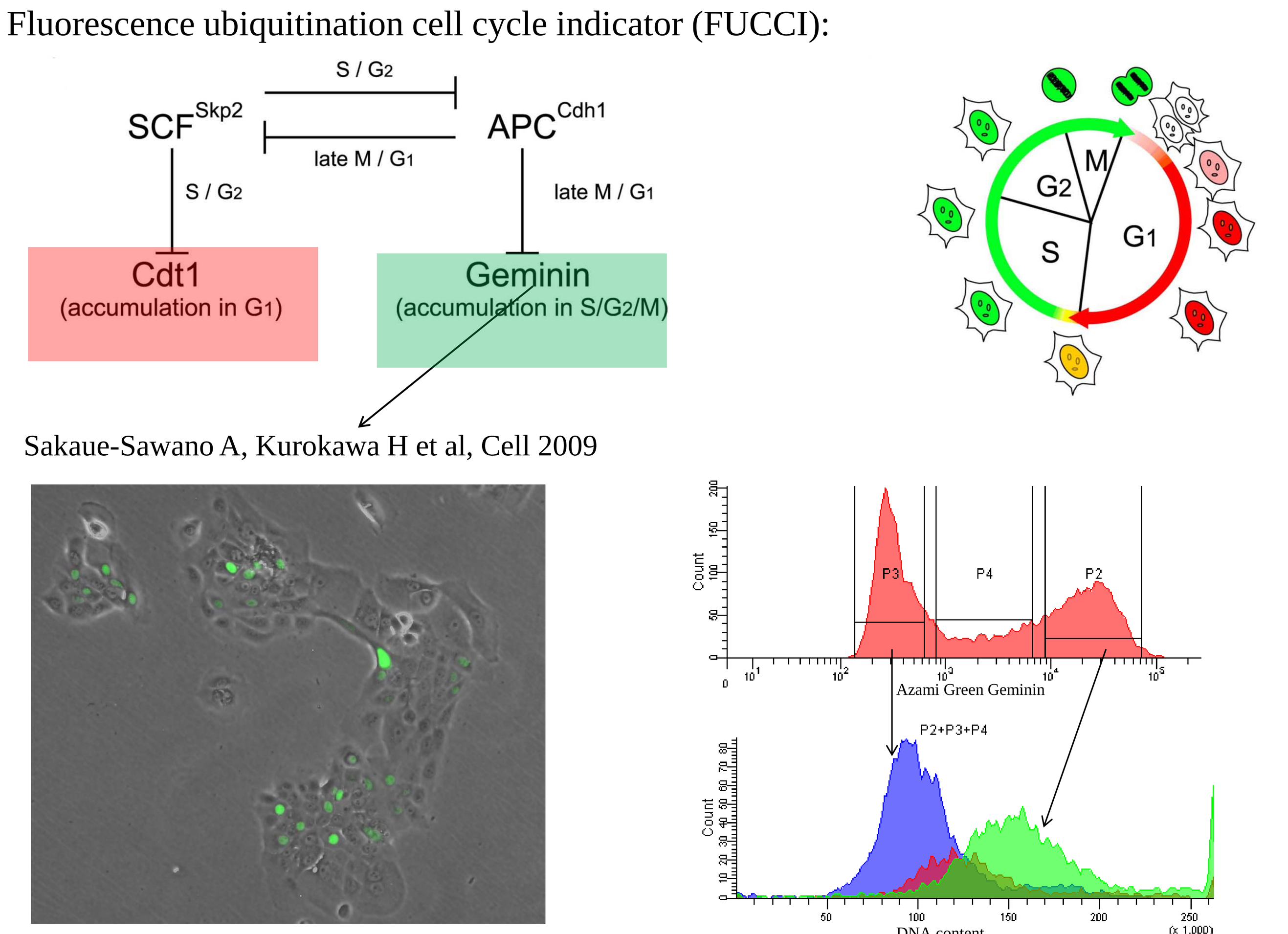


Figure1. Characterization of GMA 24 FUCCI cells
GMA24 cells (immortalized human skin keratinocytes) were transduced with Azami Green Geminin, a reporter of the S/G2/M phases of the cell cycle. A clonally derived population was used for further gene expression analysis.

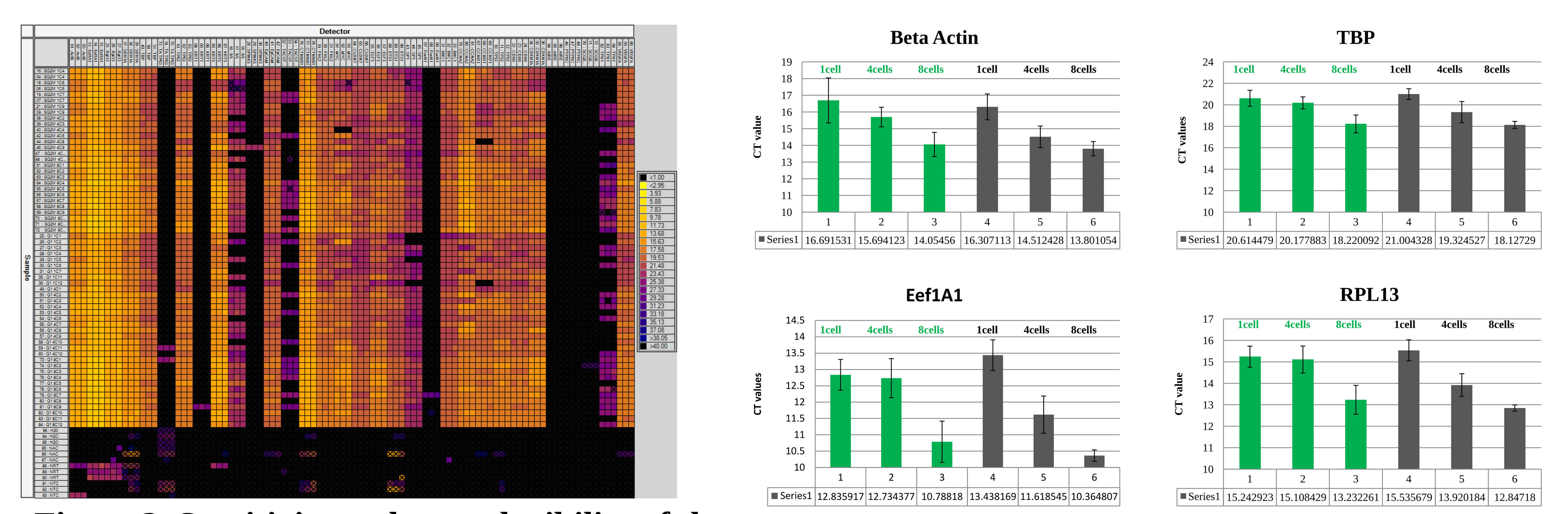


Figure 2. Sensitivity and reproducibility of the system
Cells were sorted in a 96well plate at 1, 4 and 8 cells per well in either in S/G2/M phase (green bars) or G0/G1 phase (grey bars) and gene expression levels were analyzed following amplification

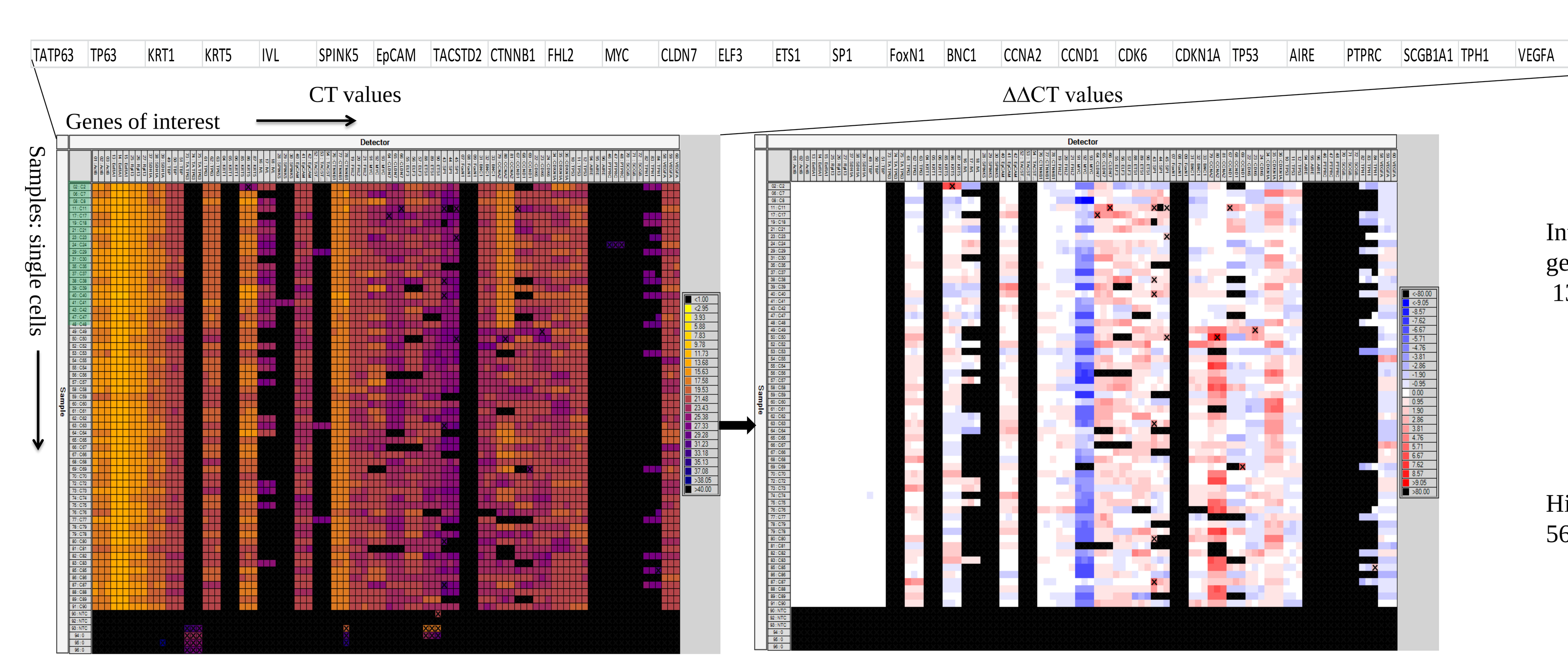


Figure 3. Gene expression levels in 96 individual cells:

- Samples lacking housekeeping genes signal were discarded from the analysis
- To obtain the DDCT values, samples were normalized to SDH1 levels and calibrated against a random sample (Sample 28, single cell in S\G2\M)
- Notice the different level of variability in both normalized and non-normalized data

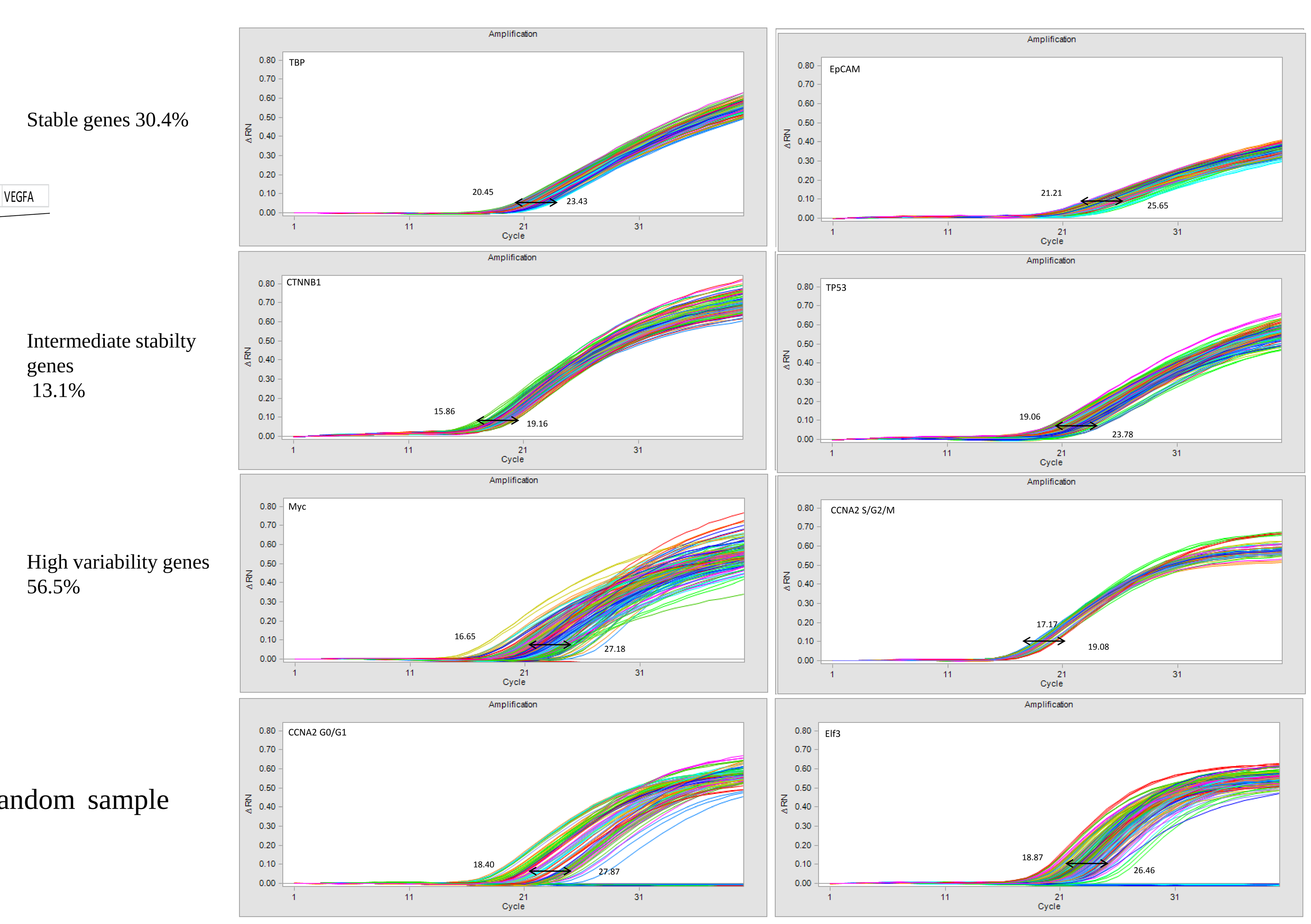


Figure4. Gene classification according to the level of variability:
Genes were classified into three different categories: stable genes (uppermost row) with a variability less than 3 CTs amongst all single cells analyzed; genes with intermediate level of stability (second row) with a variability between 3 and 7 CTs; and highy variable genes (bottom two rows), with a variability higher than 7 CTs. The bottom row shows one cell-cycle specific gene with a bi-modal behaviour. It behaves like a stable gene in S/G2/M phases of the cell cycle and becomes highly variable in the G0/G1 phase.

Conclusions:

In conclusion, our data shows that despite the clonal origin of these cells, considerable variability exists at single cell level. Further studies will be required to assess whether this gene expression heterogeneity results in functional differences between cells. In conclusion, novel technology enabling the simultaneous analysis and comparison of gene expression in a high number of samples reveals a previously unrecognized heterogeneity in a clonally derived population of cells.