Digital PCR to determine the number of transcripts from single neurons after patch-clamp recording

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Summary

Individual cells exhibit a large degree of variability in their gene expression profile. Whole-cell patch-clamp recording enables detecting electrophysiological signals from neurons, and perisomatic RNA can be harvested into the patch pipette from the same cells. As far as now only QRT-PCR has been used to detect the expression of genes in single neurons. However, RNA profiling experiments based on sample amplification protocols on single cells, including traditional QRT-PCR lack exact quantitation due to experimental variations caused by the limited amount of nucleic acids. We have optimized a digital PCR protocol for determining exact mRNA or miRNA copy numbers in single neurons after patch-clamp recording by using digital PCR based on high-density nanocapillary PCR technology. With our method one can identify individual genes participating in the establishment and maintenance of particular neuronal phenotypes, deconvolve different neuronal cell types and discover the exact distribution or variability of gene expression profiles of the electrophysiologically phenotyped cells more precisely than classical single cell QRT-PCR could achieve.

We also provide comparative information on the applicability and sensitivity of other digital PCR technologies (droplet- and chip-based digital PCR methods) for single cell genomic analysis.

We used our methods to profile single neurons from live brain slices prepared from rats, transgenic mice, as well as from human surgical specimen.

Results & Discussion

To circumvent the shortcomings of single cell QRT-PCR sensitivity, we used high-throughput nanocapillary QRT-PCR, the OpenArray® system (Life Technologies) to record digital signals from single cell mRNA in a dPCR setup (Figure 1). To verify the sensitivity and dinamic range of the protocol at first we recorded the presence of individual, synthetic target molecules (s_rps18 having the amplicon sequence of rat rps18) in a digital way in a serial dilution study (Figure 2). Synthetic rps18 template was partitioned evenly among hundreds of individual reactions and an absolute readout of total copy number was obtained after QRT-PCR in the OpenArray® plate format. We obtained good correlation with the theoretically calculated template molecules in the reaction mixture with the recorded positive hits (Figure 2). When we used end-point, droplet digital PCR (BioRad) to detect synthetic, spike-in templates we obtained pronounced differences at the lower end of the dilution (below 30) and weak correlation (Figure 3). Similar sensitivity could be obtained by using the 3D end-point dPCR from Life Technologies (data not shown). Therefore, we suggest to apply quantitative real-time PCR system (either traditional QPCR or digital QPCR) to detect low copy number messages. To profile single cells in a sensitive and reproducible manner digital QPCR is the best choice. With this approach even multiple genes and miRNA can be analyzed (Figure 5).

The approach described here can be also exploited to isolate and evaluate single abnormal neurons independently in various disease models of the CNS. In a human study surgical specimen were obtained, and cortical slice preparations submerged into artificial cerebrospinal fluid. Pyramidal cells were harvested by patch-clamp technique from non-diseased and diseased brain regions. Intracellular content was aspirated into the recording pipette containing 1x SingleCellProtectTM (Avidin Ltd. Cat.#SCP-50) solution in order to prevent nucleic acid degradation. The buffer is compatible with direct RT reaction. dPCRs were run on OpenArray® plates to determine the relative mRNA copy number changes of a transporter and an ion channel gene. We could record that disease state did not alter the number of the house-keeping rps18 transcripts, but significantly elevated the number of mRNA molecules of the ion channel and down-regulated the expression of the transporter gene (Figure 4).

References

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2. Molnár G. et al GABAergic neurogliaform cells represent local sources of insulin in the cerebral cortex. *J Neurosci*. 2014; **34**:1133-1137.

Figure 1. Technical background of Gene expression analysis of single neurons by real-time digital QRT-PCR method

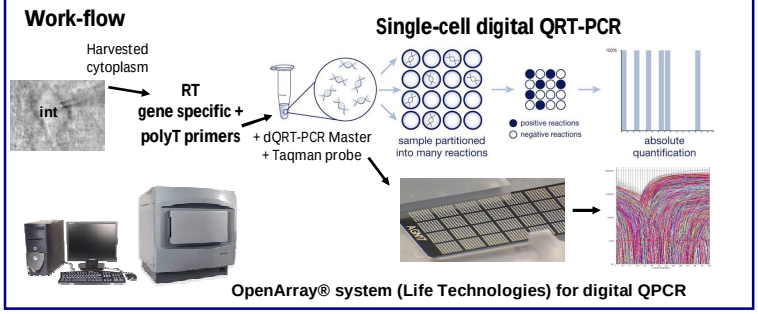


Figure 2. Determination of synthetic DNA copies with dQRT-PCR technology

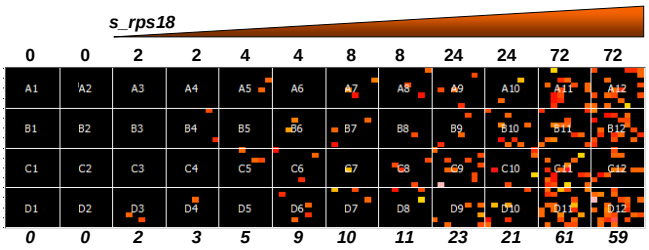


Figure 3. Comparison of sensitivity of endpoint dPCR & QRT-PCR technologies

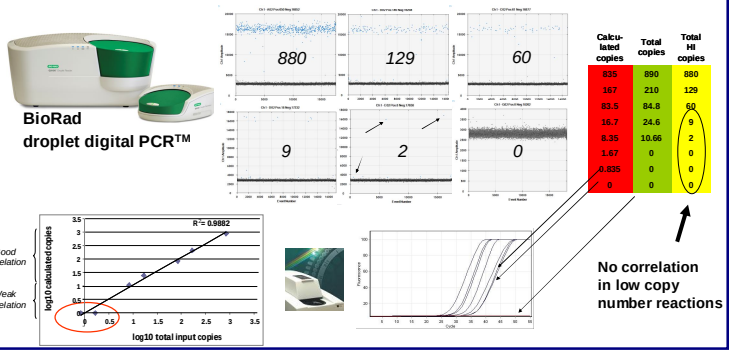
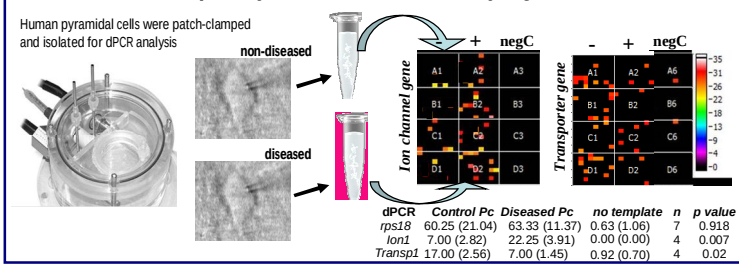
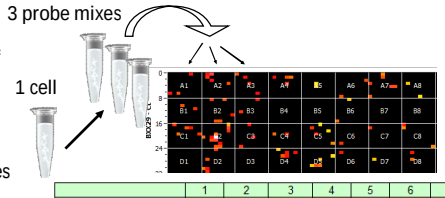


Figure 4. Gene expression analysis of single neurons after *ex vivo* patch-clamp study of human brain slices by dQRT-PCR



dPCR	Control Pc	Diseased Pc	no template	n	p value
rps18	60.25 (21.04)	63.33 (11.37)	0.63 (1.06)	7	0.918
Ion1	7.00 (2.82)	22.25 (3.91)	0.00 (0.00)	4	0.007
Transp1	17.00 (2.56)	7.00 (1.45)	0.92 (0.70)	4	0.02

Figure 5. Multiple gene expression analysis from single neurons by dQRT-PCR



Expression analysis of 2 miRNA and 1 house-keeping gene from 1 neuron

Possible only for genes with medium/high expression

	1	2	3	4	5	6	7	8
cell	PC	PC	PC	PC	PC	PC	PC	PC
template amount	1/5	2/5	2/5	1/5	2/5	2/5	2.5/5	2.5/5
probe	Rps18	miR-132	miR-127	Rps18	HMOX1	HSPB1	miR-127	miR-204
mRNA/miRNA normalized	27	53	24	28	29	15	25	21

Acknowledgements

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