Droplet Digital PCR confirms copy number variation in the segmental duplication region of titin

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NEB and *TTN*

Large neuromuscular disorder genes

Code for structural muscle proteins

Both genes contain expressed segmental duplication (SD) regions

- Variation in the *NEB* SD may cause myopathy
- Variation in *TTN* SD?

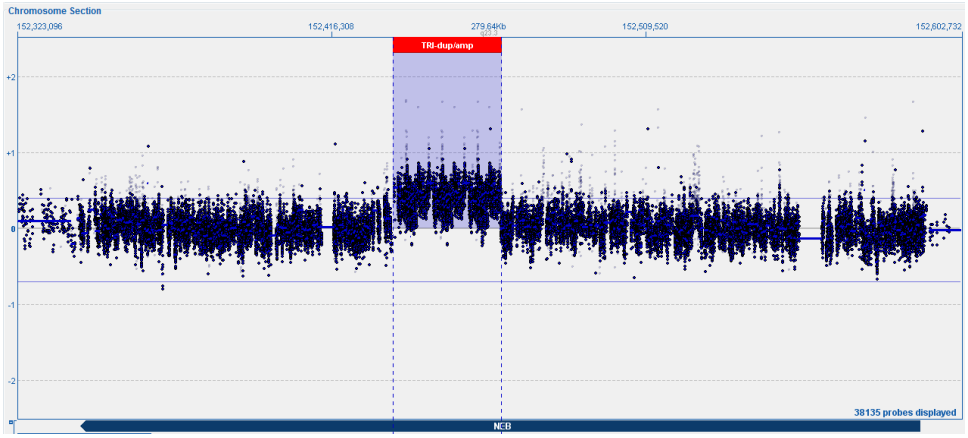
aCGH solved variation in *NEB*

Reliable CNV detection requires a lot of probes

Probe design possibilities are limited by sequence length

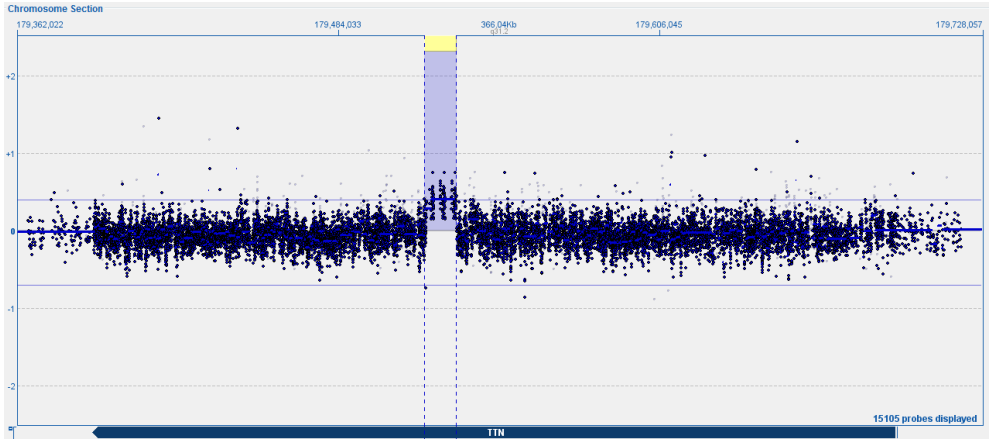
CGH-arrays relatively expensive and time-consuming for the analysis of a single region

NEB SD ~30 kb (3x10kb)



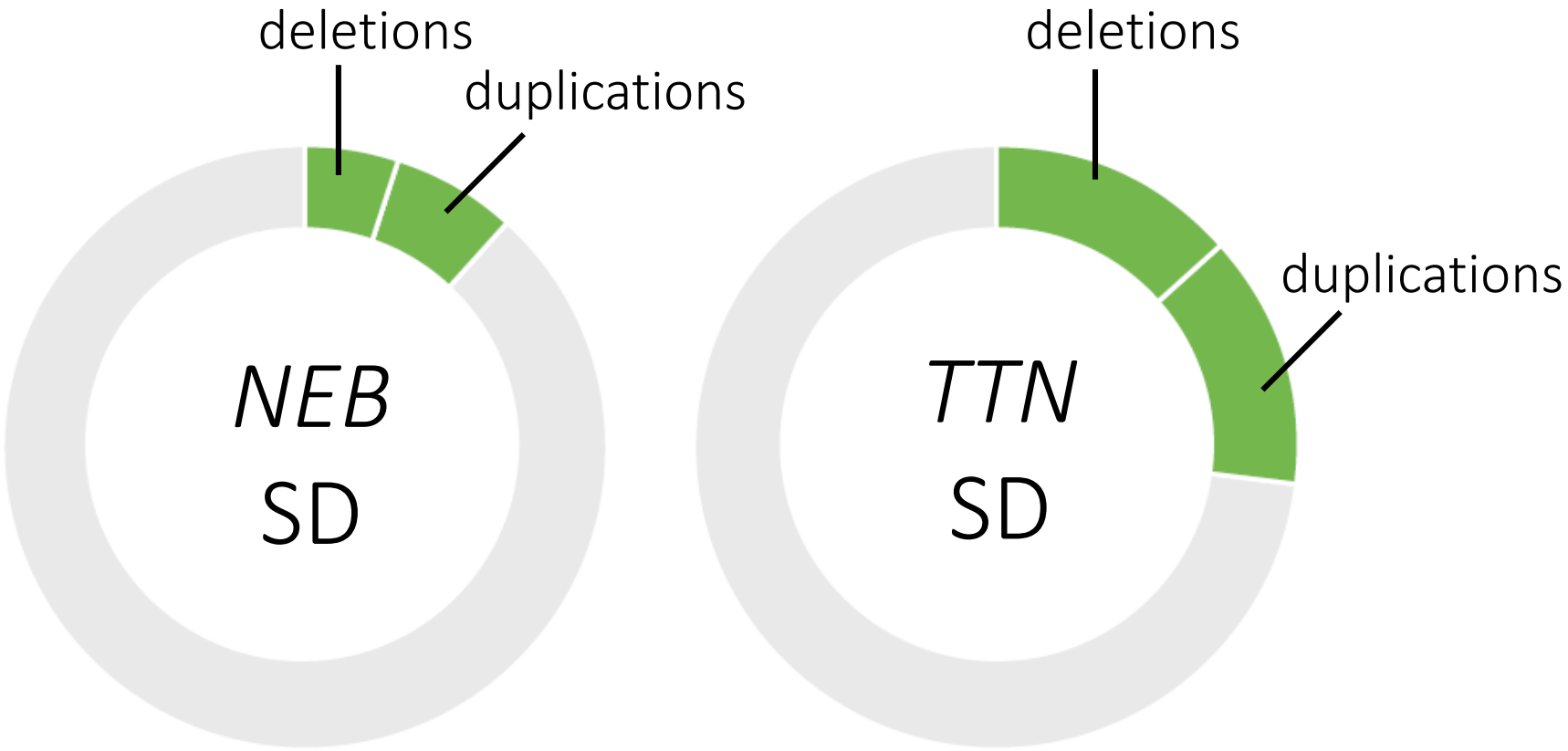
NEB SD 8/6

TTN SD ~11.5 kb (3x1.6 kb + large introns)



TTN SD ?/6, ?/8

Variation in SD regions as per aCGH



Based on ~430 samples run on the NM- and/or the NMD-CGH arrays (Kiiski et al. 2013, Sagath et al. 2018).

Droplet Digital PCR

A partitioned PCR reaction (>10k droplets/reaction)

The reaction contains primers and hydrolysis probes for

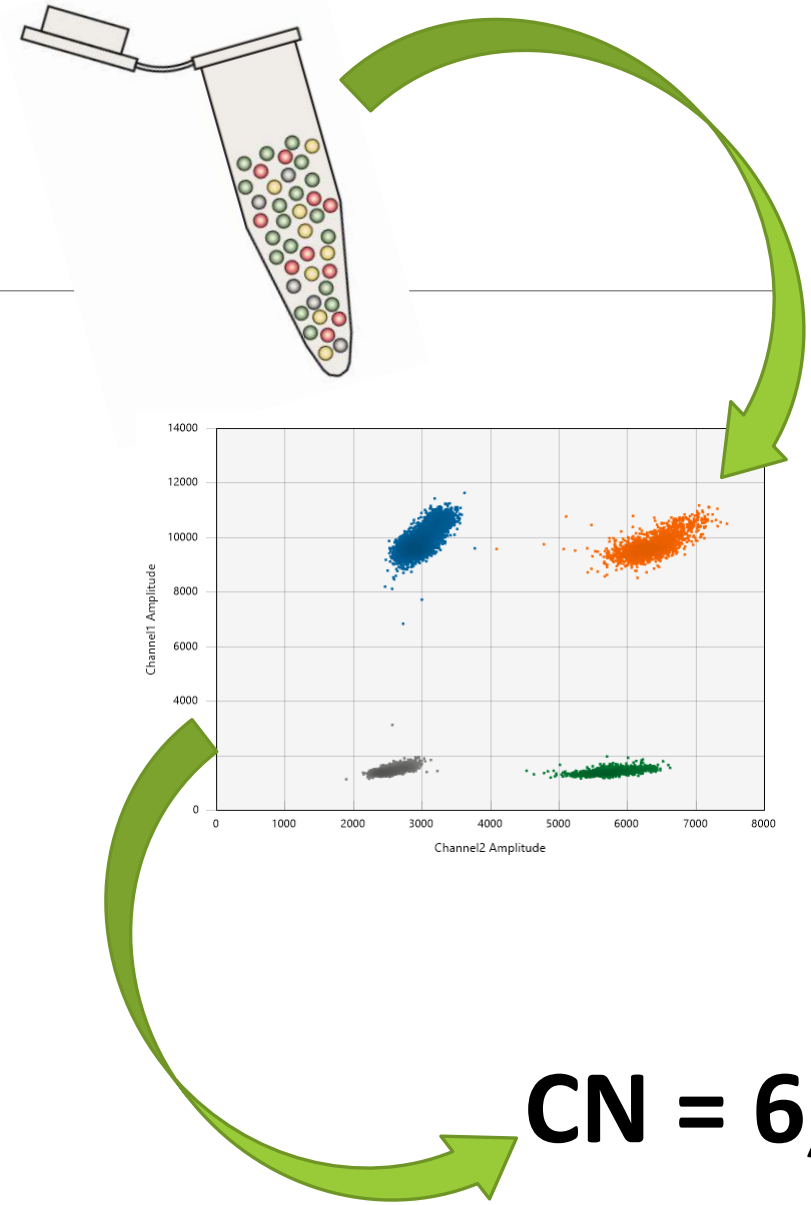
- The region of interest
- A diploid reference gene

The partitioned sample is PCR'd

Individual droplet intensities measured

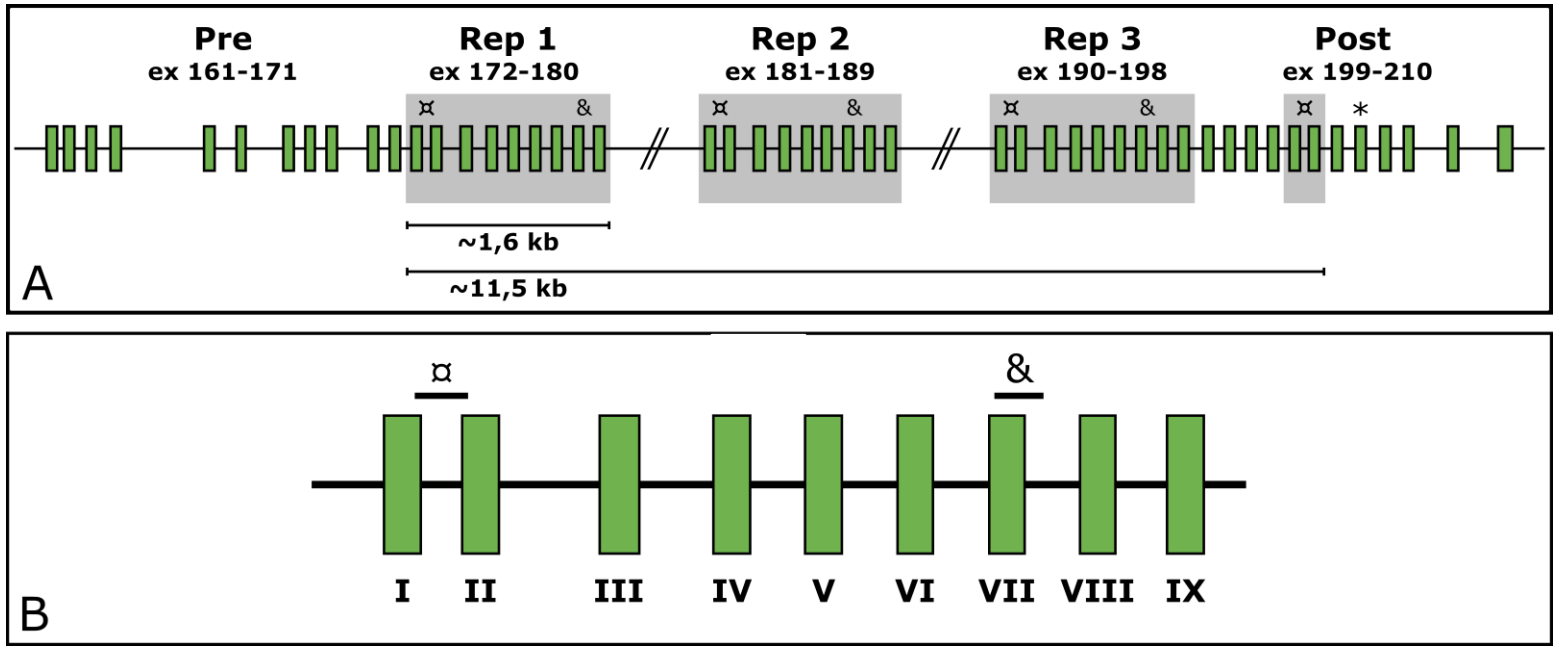
Droplets categorized according to fluorescence intensity

Poisson statistics applied to calculate copy number of ROI



CN = 6,98

TTN segmental duplication structure

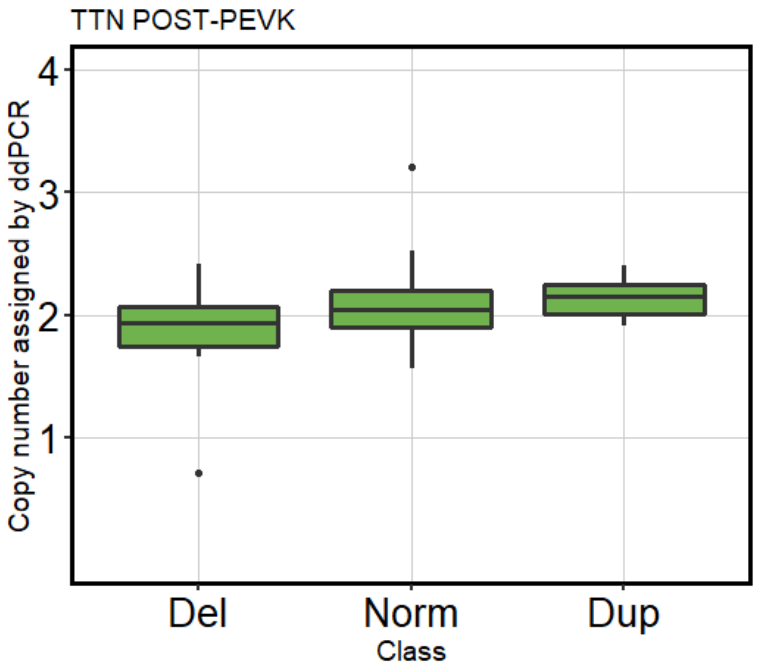


¤ *TTN* SD Ex I normal CN = 8

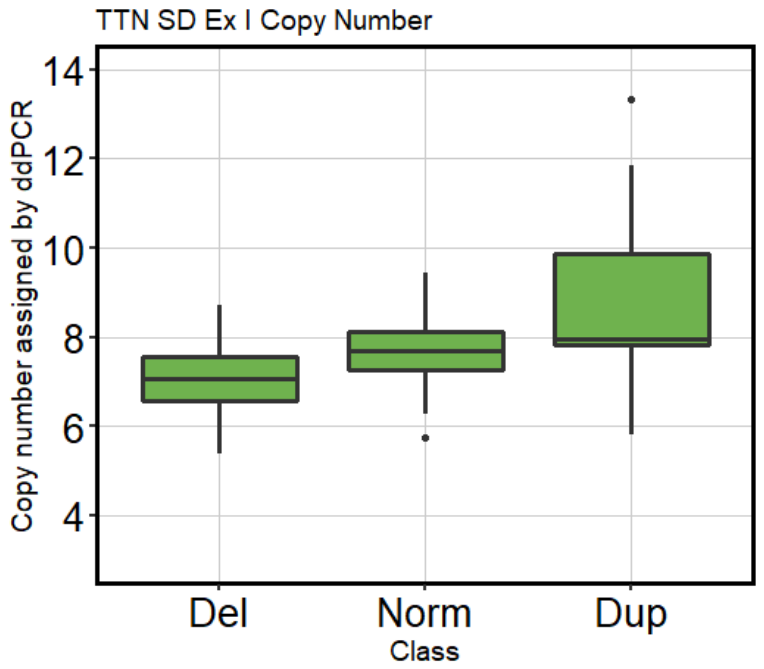
& *TTN* SD Ex VII normal CN = 6

* *TTN* POST-SD normal CN = 2

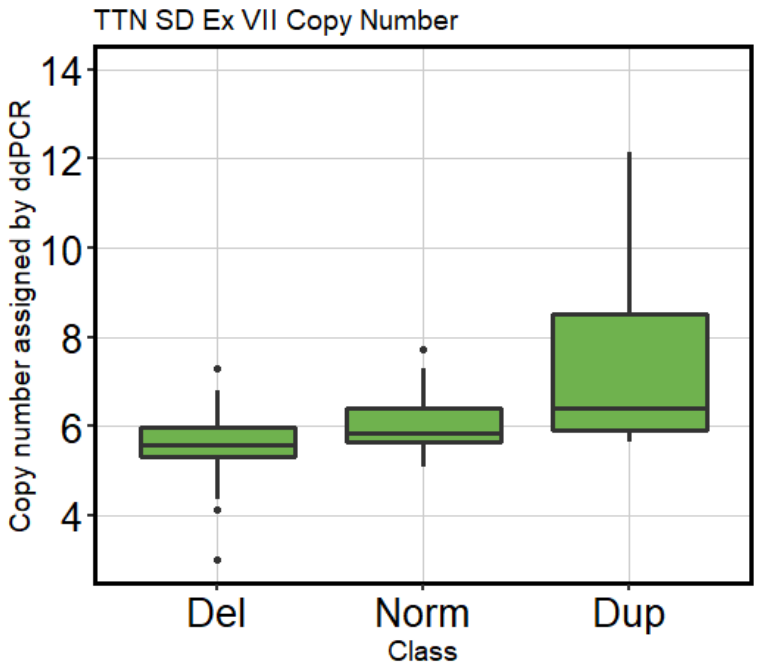
Preliminary results



ANOVA $p > 0.05$
Expected normal CN = 2
n = 60



ANOVA $p < 0.005$
Expected normal CN = 8
n = 61



ANOVA $p < 0.0005$
Expected normal CN = 6
n = 61

Conclusions & future plans

Our ddPCR-based system for CN detection of SD regions is transferrable

Inexpensive, rapid and applicable to large sample cohorts

The results indicate true normal CN variation of the *TTN* SD

- Can we define exact CN?
- Is there a pathological threshold?

Long-read sequencing without amplification is a putative option

Our trials so far have been unsuccessful -> exploring options

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