

# Thermostable FGF2-G3 supports muscle stem cell expansion at ten-fold lower concentration in fully animal-free, chemically-defined conditions

A collaborative study between Media City Scientific and Qkine

## Introduction

Skeletal muscle stem cells (MuSCs), also known as satellite cells, are among the most challenging primary cell types to culture *ex vivo*. MuSCs exist in a deeply quiescent state *in vivo* and are exquisitely sensitive to their culture environment. Upon isolation, they rapidly activate and begin to differentiate. Maintaining the self-renewal capacity required for meaningful expansion without triggering terminal differentiation demands precise control over media composition and growth factor delivery [1].

Fibroblast growth factor 2 (FGF-2, basic FGF) is a critical mitogen for MuSC proliferation and self-renewal. However, standard recombinant FGF-2 is thermolabile under standard culture conditions, losing ~65% of its activity within 3 hours and ~80% within 24 hours at 37°C [2]. This instability means that high working concentrations are required to maintain effective levels throughout a feeding interval, which becomes a practical and economic challenge when scaling MuSC culture. It also drives frequent medium changes that can themselves destabilize these sensitive cultures.

Qkine manufactures animal origin free, thermostable variants of FGF-2 (FGF2-G3), which retain substantially greater bioactivity at physiological temperatures, with a half-life of >7 days at 37°C [3]. FRS™ Pioneer (Media City Scientific) is a fully chemically defined, animal-origin-free serum replacement formulated for broad cell type compatibility and lot-to-lot consistency. While FRS Pioneer is conventionally used for 100% replacement of FBS for the culture of standard immortalized

cell lines, here we evaluate FRS™ Pioneer supplemented with Qkine growth factors as a serum replacement for MuSC culture. We compare the performance of standard FGF-2 and thermostable FGF2-G3, demonstrating equivalent expansion at a ten-fold reduction in working concentration of the thermostable variants.

## Methods

### Culture conditions

MuSCs were thawed into FBS-containing medium. After 24 hours, cells were directly adapted into DMEM/F12 supplemented with one of six conditions: (1) no supplement; (2) 20% FRS™ Pioneer; (3) 19% FRS™ Pioneer + 1% FBS; (4) 20% FBS; (5) 20% FRS™ Pioneer + 2 ng/ml TGF-β1 (Qk010) + 50 ng/ml FGF-2 (Qk025 or Qk027); or (6) 20% FRS™ Pioneer + 2 ng/ml TGF-β1 (Qk010) + 5 ng/ml FGF2-G3 (Qk052 or Qk053). Cells were maintained across three passages over 11 days.

### Adhesion strategy

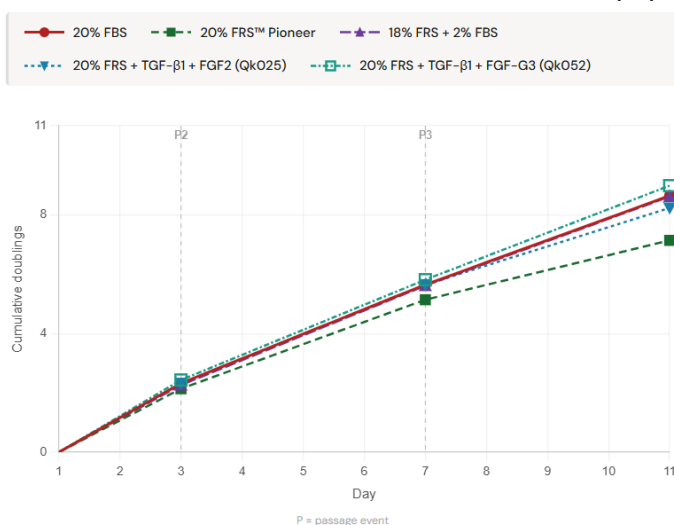
MuSCs do not produce sufficient endogenous ECM for conditioned-medium surface conditioning. Standard TC-treated plates were pre-coated with 0.5 µg/cm<sup>2</sup> recombinant vitronectin (Qk120) for 2 hours at 37°C prior to seeding in all conditions.

Primary cells are inherently variable between donors and isolates; the conditions described here represent a validated starting point, and minor optimization of growth factor concentrations or seeding density may be beneficial for specific cell sources.

## Results

### **FRS™ Pioneer supplemented with growth factors supports MuSC expansion comparable to FBS**

MuSCs cultured in 20% FRS™ Pioneer supplemented with TGF-β1 and either standard FGF-2 or thermostable FGF2-G3 achieved cumulative population doublings comparable to the 20% FBS



**Figure 1. Cumulative population doublings of skeletal muscle stem cells at day 11.** Defined medium (FRS™ Pioneer + TGF-β1 + FGF-2 or FGF2-G3) achieved expansion equivalent to the 20% FBS control. Basal media alone failed to sustain MuSC proliferation past 72 hours. Dashed vertical lines indicate passage events. Points indicate measured values; lines represent interpolated growth between passage events. GFs: 2 ng/ml TGF-β1 + 50 ng/ml FGF-2 (Qk025) or 5 ng/ml FGF2-G3 (Qk052).

At the practical level, this means effective FGF-2 exposure between feeds is far more consistent when using FGF2-G3, reducing the need for frequent medium changes to maintain mitogenic signal. For MuSC culture — where frequent disturbance can itself trigger differentiation — this improved stability has implications for protocol design as well as reagent cost.

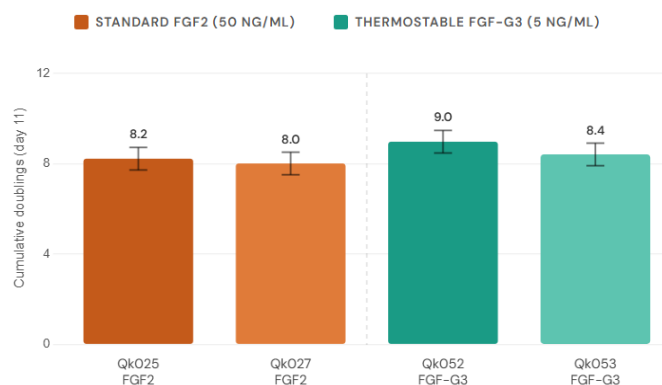
### **Isoform comparison: 145 aa FGF2-G3 and 154 aa FGF2-G3**

Among the thermostable variants, the 145 aa isoform of FGF2-G3 (Qk052) may drive slightly increased MuSC proliferation relative to the 154 aa variant (Qk053) at the same 5 ng/ml concentration. This trend is consistent with previously published data from collaborative work between Qkine and the University of Turin [4]. While the difference was modest, it may be worth considering for applications where maximizing expansion yield is the primary objective.

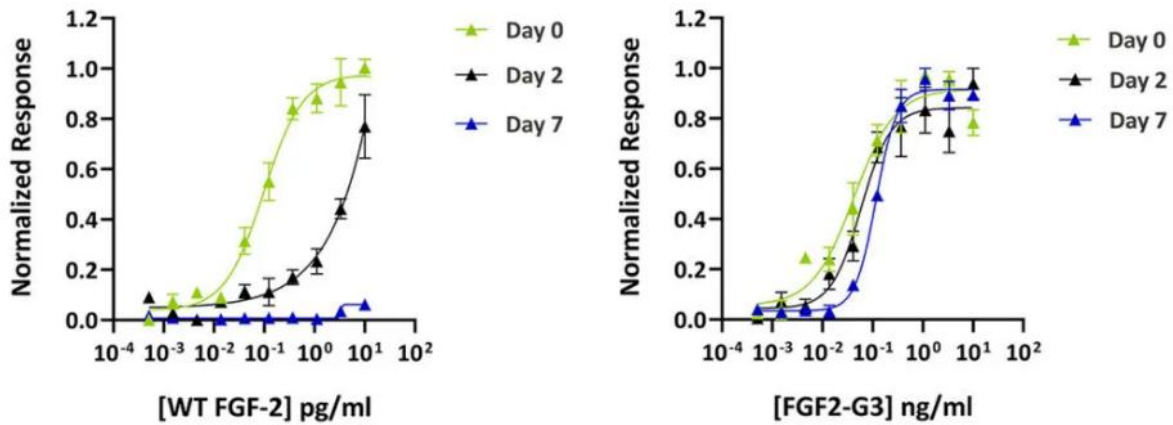
positive control over 11 days and three passages. 18% FRS™ Pioneer / 2% FBS hybrid condition also performed comparably to the FBS control.

### **FGF2-G3 delivers equivalent expansion at 10x lower concentration**

The standout finding of this study is the concentration advantage of thermostable FGF2-G3. Equivalent MuSC expansion was achieved with 5 ng/ml FGF2-G3 compared to 50 ng/ml standard FGF-2 — a ten-fold reduction in working concentration. This advantage arises from the enhanced thermal stability of FGF2-G3: whereas standard FGF-2 degrades substantially within hours at 37°C, FGF2-G3 retains bioactivity throughout the feeding interval.



**Figure 2. Thermostable FGF2-G3 delivers equivalent performance at a 10x lower concentration in MuSC expansion.** Cumulative population doublings of primary muscle stem cells (MuSCs) at day 11 comparing four growth factor formulations within the 20% FRS™ Pioneer culture system. All conditions supplemented with 2 ng/ml TGF-β1 (Qk010). Standard FGF-2 conditions: Qk025 (dark orange) and Qk027 (light orange), each at 50 ng/ml. Thermostable FGF-G3 conditions: Qk052 (dark teal) and Qk053 (light teal), each at 5 ng/ml. FGF-G3 at 5 ng/ml delivers equivalent or superior expansion to standard FGF-2 at 50 ng/ml. Data from a single experiment; n = 3.



**Figure 3. Thermostable FGF2-G3 145 aa maintains bioactivity for >7 days under cell culture conditions.**

Bioactivity was determined using the Promega serum response element luciferase reporter assay in transfected HEK293T cells. FGF2-G3 145 aa or WT FGF-2 154 aa (Qk027) were incubated in media at 37°C for 0, 2 or 7 days then cells were treated in triplicate with a serial dilution for 3 hours. Firefly luciferase activity was measured and normalized to the control Renilla luciferase activity.

## Conclusion

FRS™ Pioneer supplemented with TGF-β1 and FGF-2 supports MuSC expansion equivalent to FBS over 11 days without the batch variability inherent to serum-supplemented culture. The thermostable FGF2-G3 variants deliver equivalent proliferative performance at one-tenth the working concentration of standard FGF-2. This finding has direct implications for reagent cost, protocol frequency, and the practical feasibility of scaled MuSC culture.

Together, these results support a defined, animal-origin-free culture system for muscle stem cells that is both biologically effective and practically optimized for scale, with potential applications in muscle disease research, cell therapy, and cultivated meat production.

## References

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- [3] Dvorak P, Bednar D, Vanacek P, et al. Computer-assisted engineering of hyperstable fibroblast growth factor 2. *Biotechnology and Bioengineering*. 2018; 115: 850–862. doi.org/10.1002/bit.26531
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## Media City Scientific

Media City Scientific manufactures FRS™ Pioneer, a fully chemically defined, animal-origin-free serum replacement engineered for broad primary cell type compatibility and lot-to-lot consistency. FRS™ Pioneer is available directly and through Qkine.

<https://www.mediacityscientific.com>

## Qkine

Qkine are committed to raising the standards of growth factors, cytokines and related proteins to better support long-term and complex stem cell culture.

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