Group Meeting

8/8/2007

Agenda

- Cell culture (C2C12 regular and 4R-CAT)
- Sonication
- ChIP preps and DNA concentration
- Solexa

Large Cell Culture Effort

Goal: collect chromatin to be used in a Solexa ChIP analysis

Setup: 36x 15 cm plate and 30x 6 cm plate

15 cm plates harvested at 0, ~12, 24 and 60 hrs

6 cm plates fixed at 0, 6, 12, 18, 24, 36, 48, 60, 72, 96 hrs

More Cell Culture

As Biran and others have noted, cell density increases after the removal of serum and addition of differentiation medium.

To compensate, 2x 15 cm plates were used per sample for the 0 hrs timepoint

1x 15 cm plate was used per sample for all post-differentiation timepoints (for ease of comparison)

Even More Cell Culture

Actual timepoints harvested:

C2C12 (WT): 0, 15, 24, 61

C2C12 (4R): 0, 12, 25, 60

Small plates fixed as planned (but there was always a fix accompanying the collection of chromatin)

Sonication

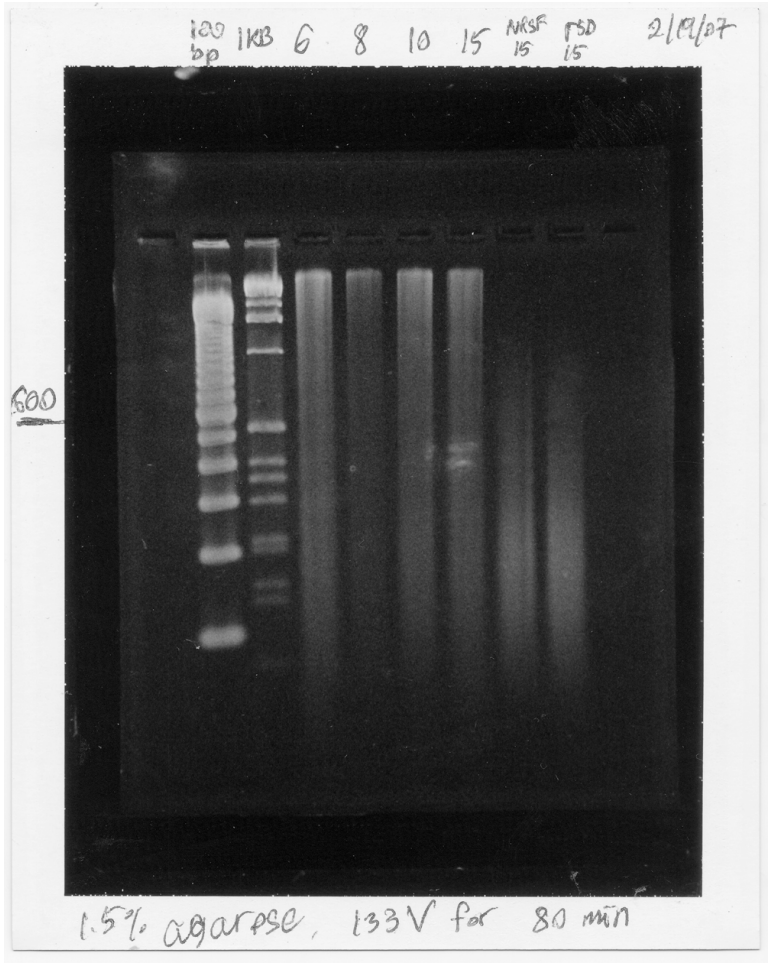
Which parameters are best?

Ali and Gordon get good results with 1 ml volume

Brian gets good shearing with ~200-300 µl volume

What is a “good result”?

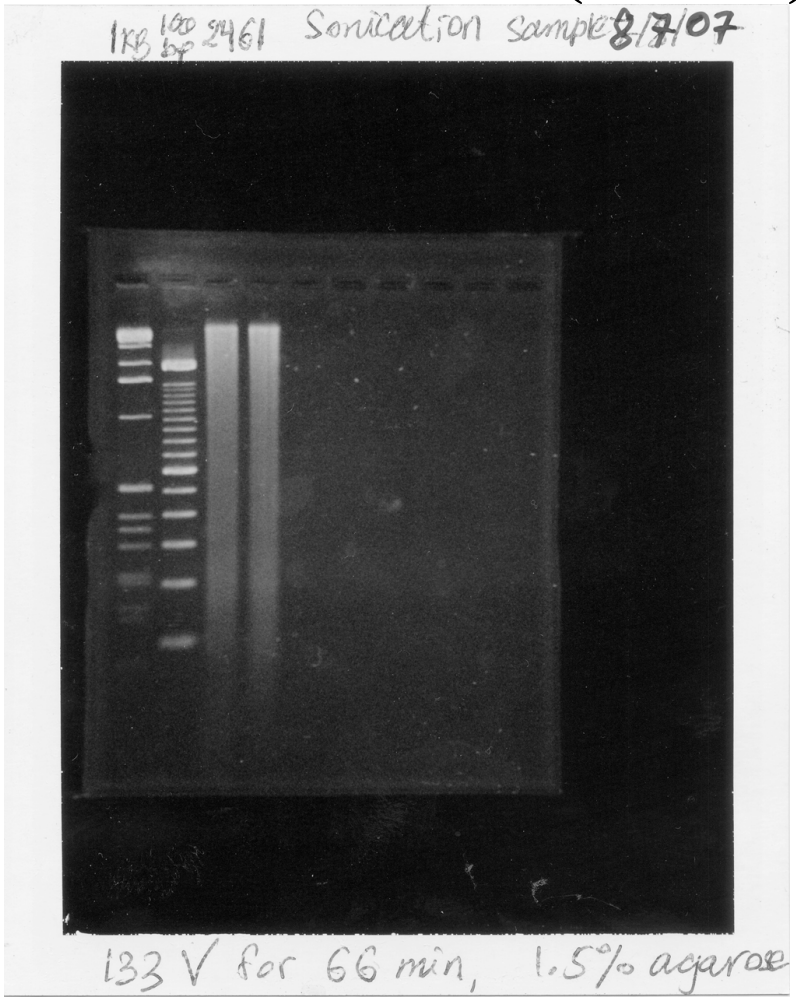
Sonication (5 months ago)



each lane = 1/2 of a 3x 10 cm plate sample

Lanes 3-6 are sonicated using the number of cycles shown (2.0)
Lanes 7-8 are the product from lane 6 (15 cycles of sonication) that was purified using the QIAquick purification kit / column.

Sonication (now)



Lanes 1-2 are the 1 KB and 100 bp ladders, respectively
Lanes 3-4 are sonication products of the 24 and 61 hrs diff time points from the C2C12 (wt) t.c. (12 cycles @ 2.5, 300 µl)

Sonication

Switching back to using 1 ml now after having used 200-300 μl for the last several months

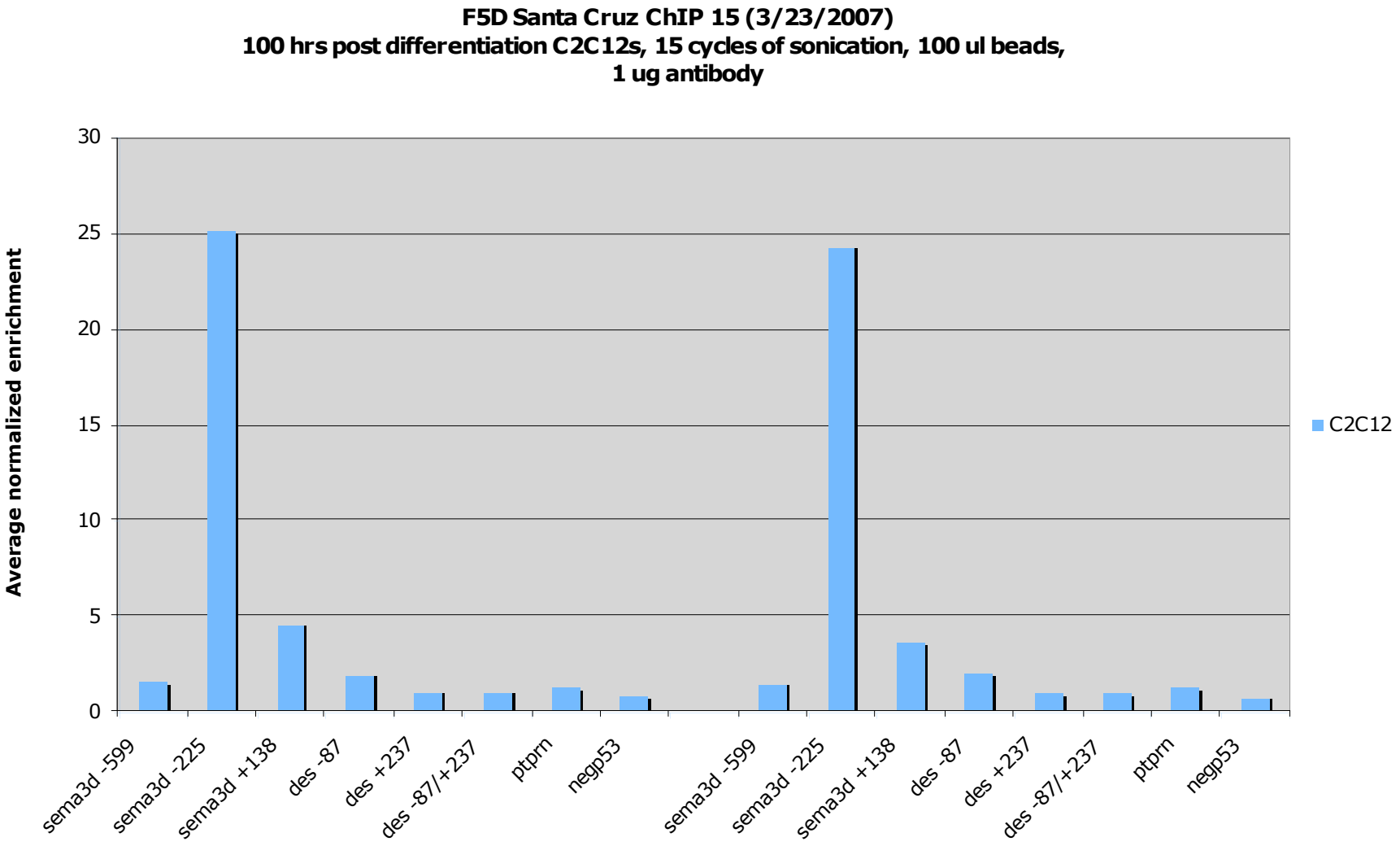
Ali and Gordon both use 1 ml

Did a batch of 8 sonications last night using Gordon's parameters

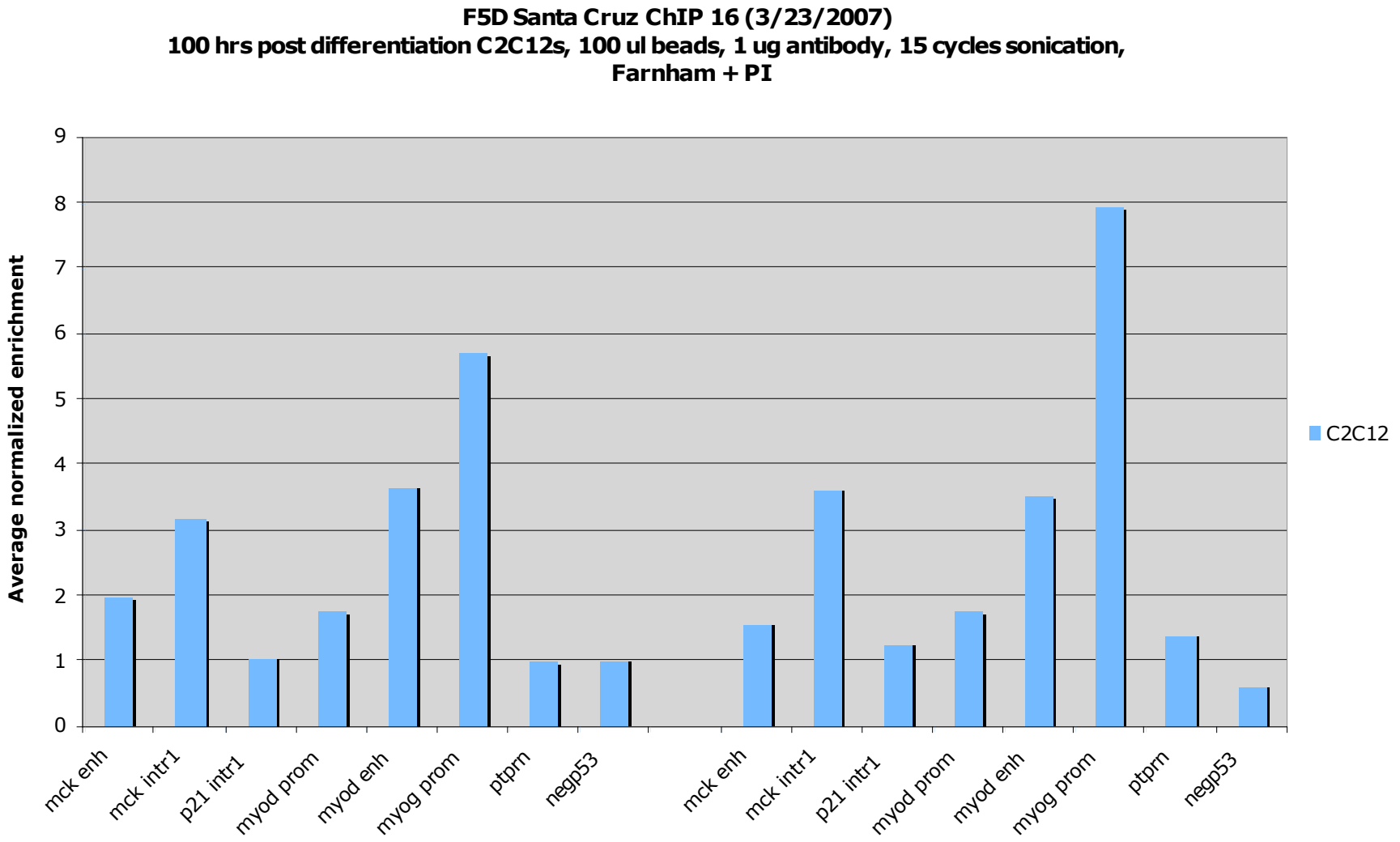
Foaming is a problem

Results pending

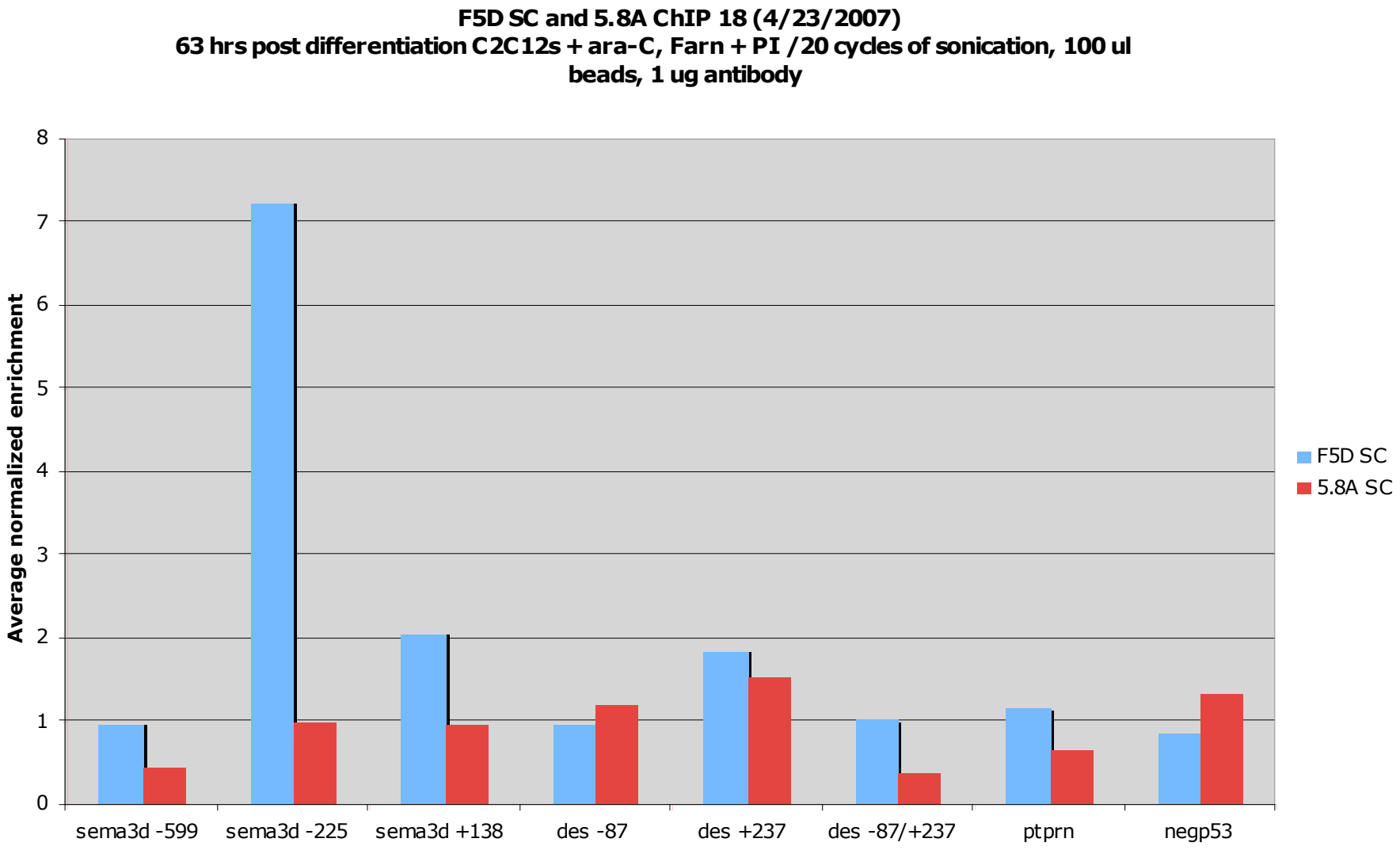
F5D ChIP (new primers)



F5D ChIP (old primers)

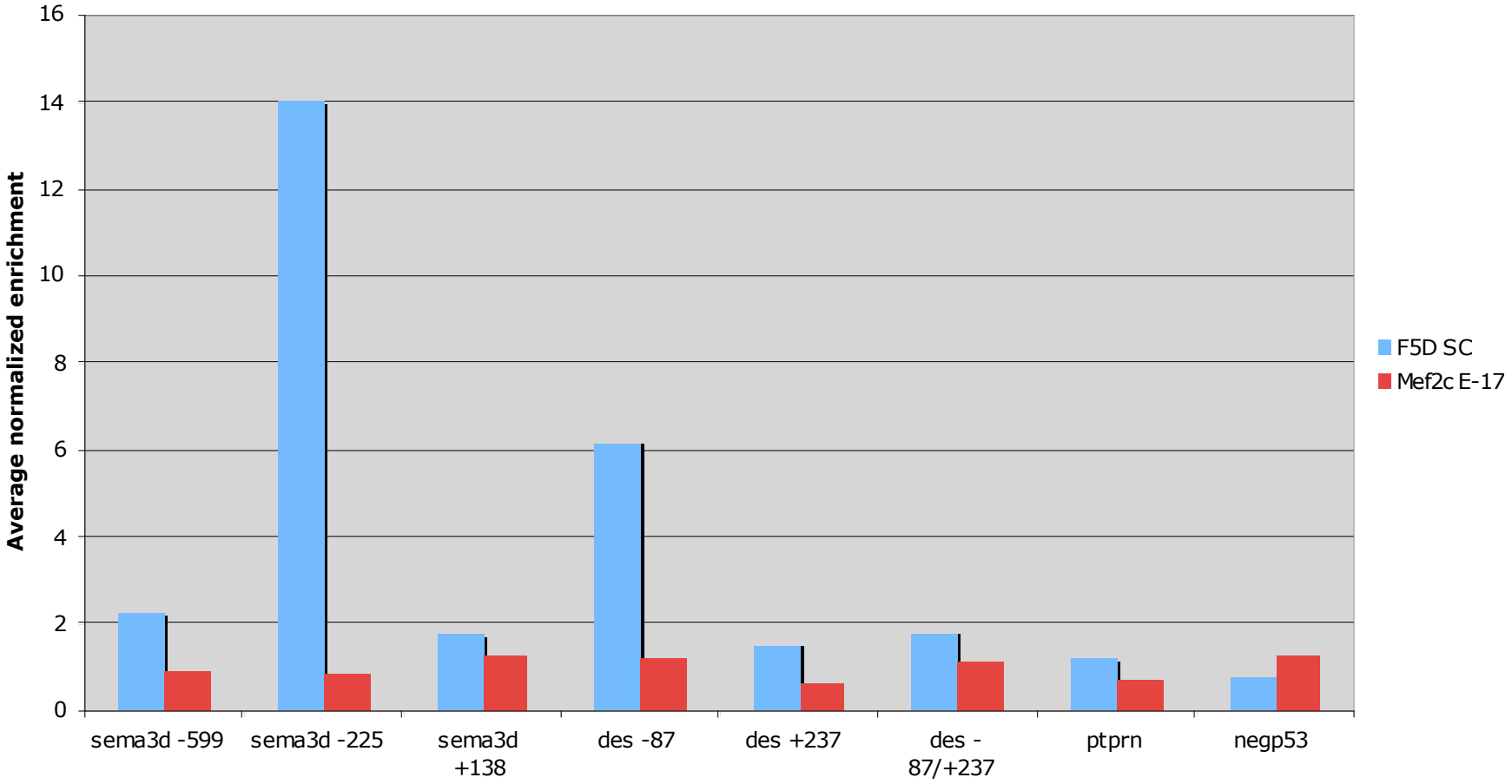


F5D and 5.8A ChIP (new primers)

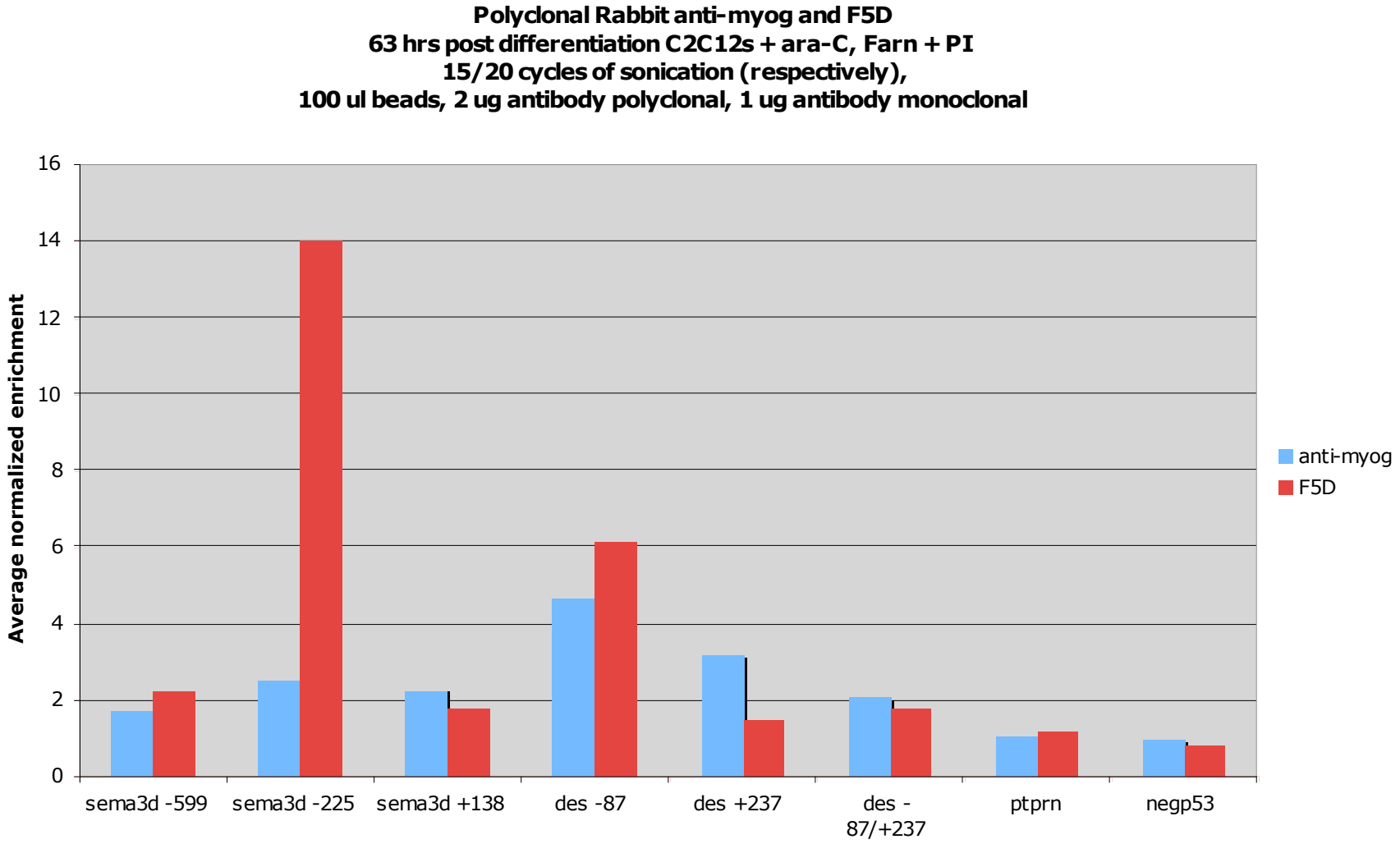


F5D and Mef2c - E17 ChIP (new primers)

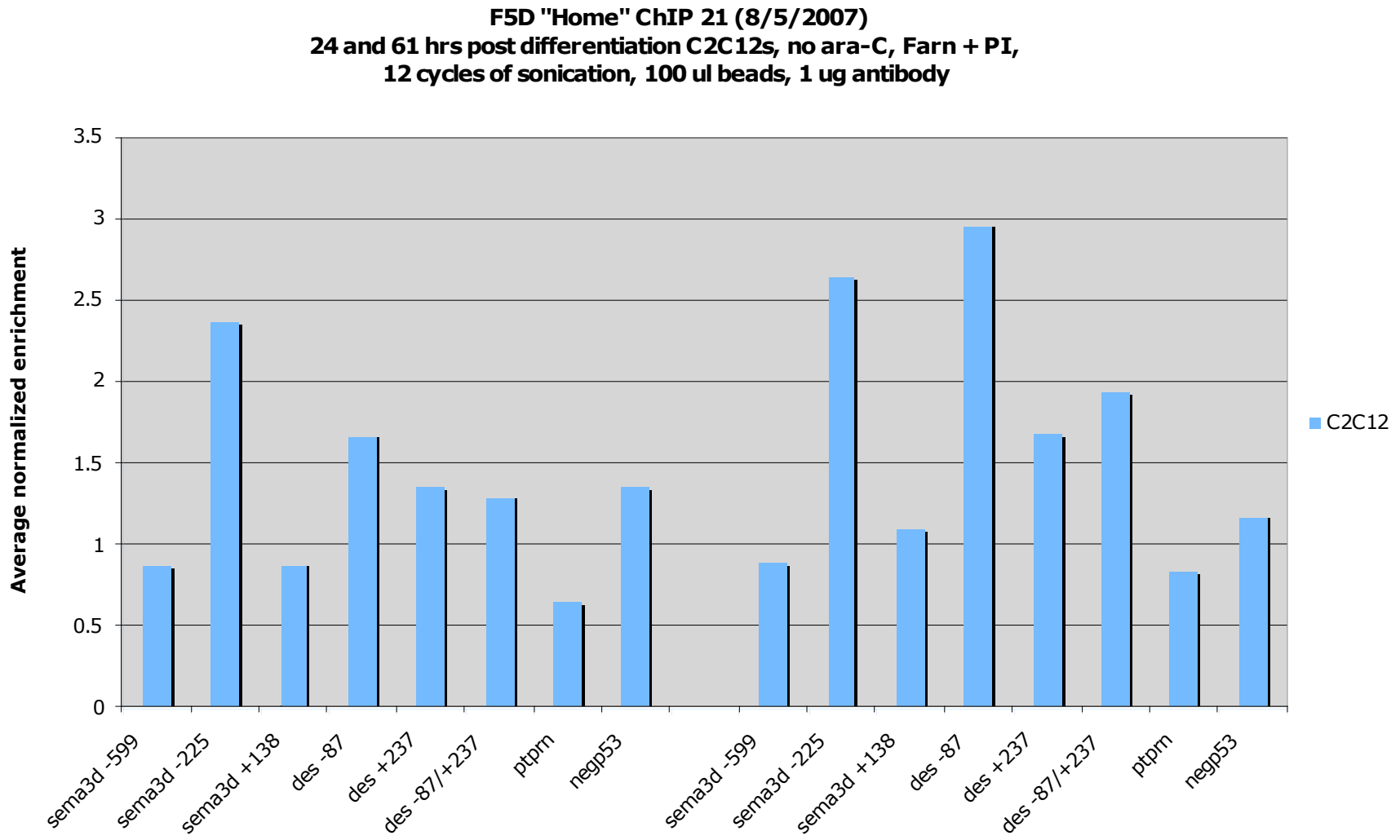
F5D Santa Cruz and Mef2c E-17 ChIP 19 (4/25/2007)
63 hrs post differentiation C2C12s + ara-C, Farn + PI /20 cycles of sonication, 100 ul beads, 1 ug antibody monoclonal / 4 ug polyclonal



F5D SC and anti-myog polyclonal ChIP (new primers)



F5D Home ChIP (new primers)



Cell Density/Growth

Cells (aka cell body) grow during diferentiation → larger pellet at later timepoints

However, amount of DNA appears to increase as well

Will test the effect of ara-C on post-differentiation growth (results pending)

Cell Density/Growth

Density after 24 hrs diff: 197 ng/μl

Density after 61 hrs diff: 308 ng/μl

Extrapolated total chromatin:

- 24 hrs = 325 μg
- 61 hrs = 508 μg

Factors Affecting Final DNA Conc.

- Time
- Scraping efficiency
- Transfer efficiency during washes
- Phenol/chloroform extraction efficiency
- ara-C

Solexa

Myogenin Solexa run pending completion of:

- Test ChIPs
- Sonication tests
- Immunostaining

The End

RNA Timecourse Data (from Brian)