

Internal Reference Scaling (IRS) is a Critical Component in Analyses of Biological Studies with Multiple TMT Experiments

Phillip A. Wilmarth, Ashok P. Reddy, John E. Klimek, Jennifer M. Cunliffe, and Larry L. David
Proteomics Shared Resources, Oregon Health & Science University, Portland, Oregon USA

Background:

- Many biological studies have more than 11 samples and need more than one TMT labeling experiment.
 - Existing normalization methods are inadequate.
 - Novel experimental designs and normalization methods are required.¹
- Data and Analysis:**
- Publicly available developing mouse lens data.²
 - Six time points (E15, E18, P0, P3, P6, and P9) per isobaric tandem mass tag (TMT) experiment, repeated 3 times.
 - Thermo Lumos using SPS MS3 for reporter ion intensities.
 - 3155 proteins identified and quantified in all 3 experiments by Proteome Discoverer (v2.1).
 - Reference channels were not available and were simulated by averaging all channels in each TMT experiment.
 - Exported data analyzed with R (v3.4.3) in Jupyter notebooks.
 - See https://github.com/pwilmart/IRS_normalization.git

Normalization Methods:

- Since same amount of protein digest is labeled in each channel, the reporter ion totals for each channel should be the same. Sample loading (**SL**) normalization equalizes reporter ion totals.
- The trimmed mean of M values (**TMM**) normalization³ corrects for compositional bias and is commonly used in RNA-Seq analyses.
- Internal Reference Scaling (**IRS**)¹ uses pooled standard channels to correct random MS2 sampling and adjusts multiple TMT experiments to the same intensity scale.

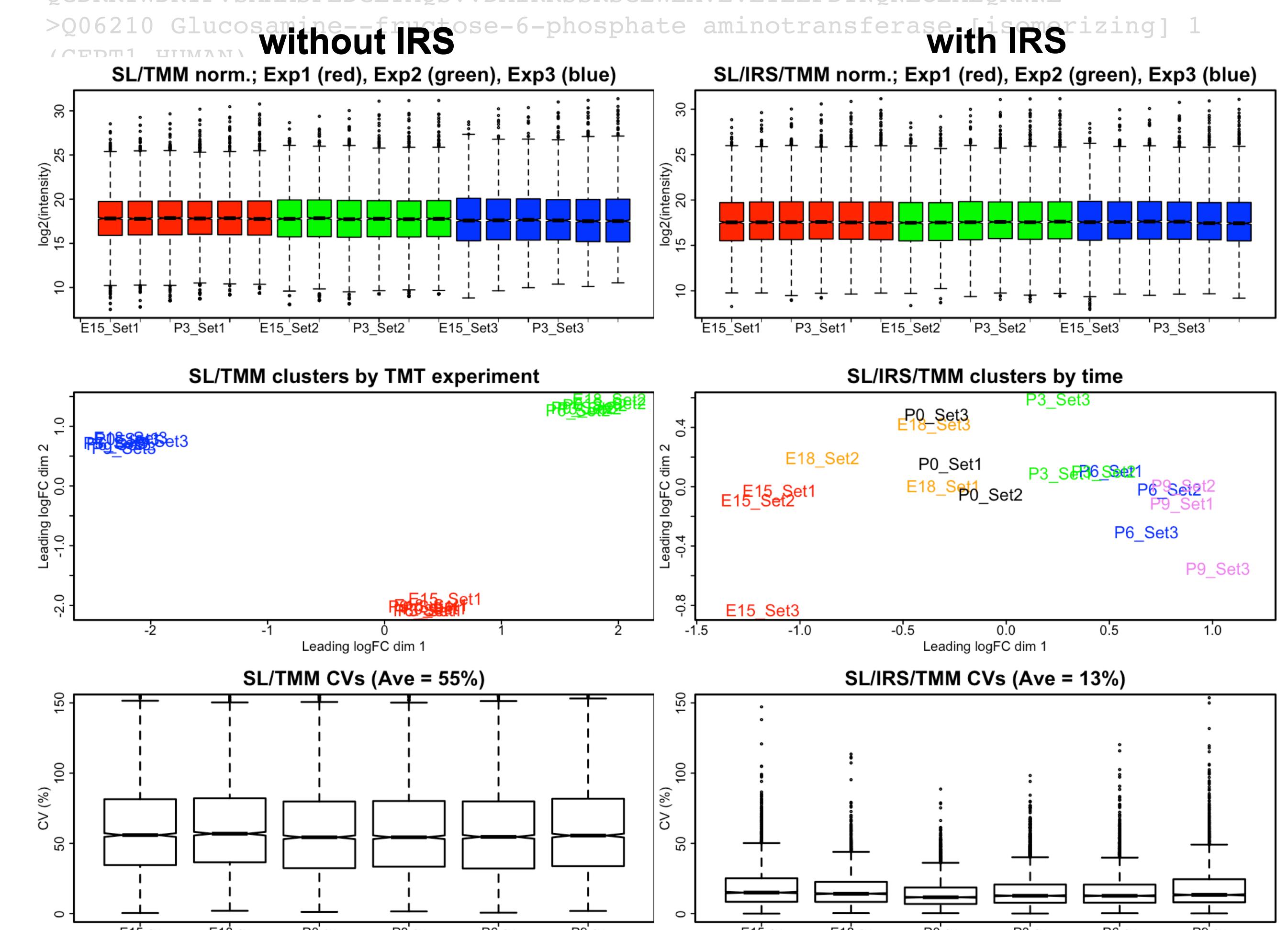
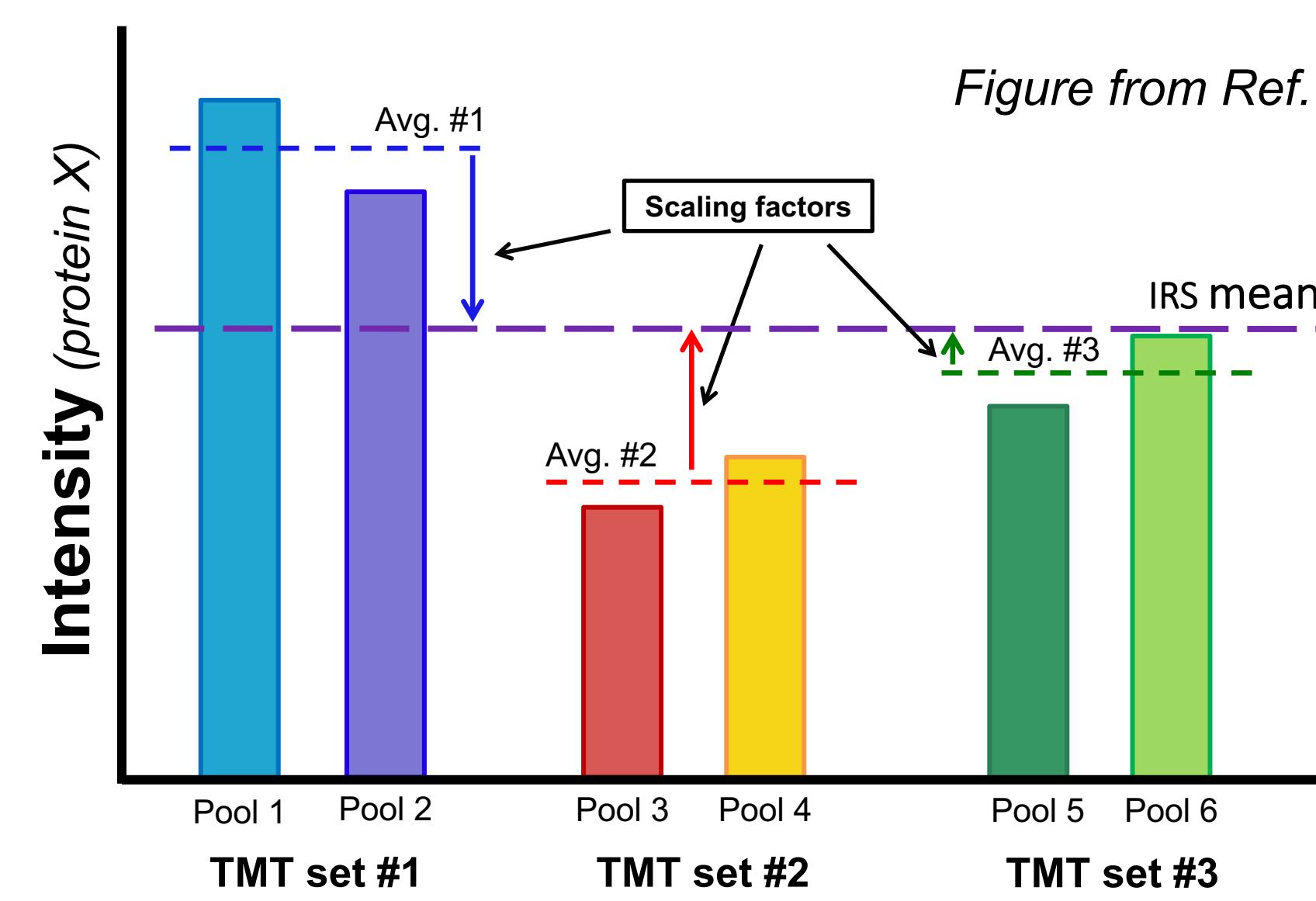


Figure 2. After SL and TMM normalization, reporter ion intensity distributions are aligned (top left). However, samples cluster by TMT experiment and CVs are large (left). Samples cluster correctly by developmental time after IRS and CVs are dramatically lower (right).

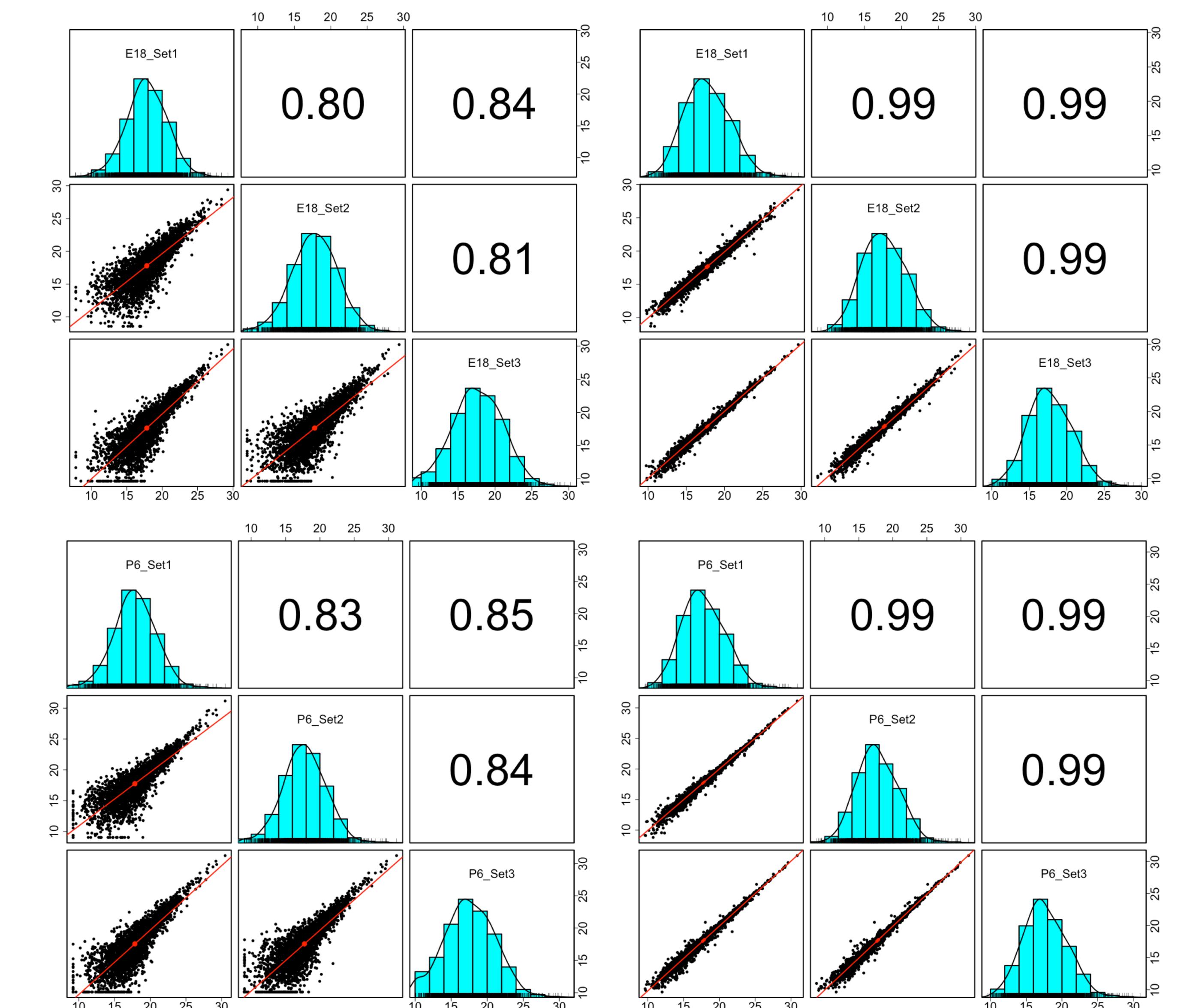
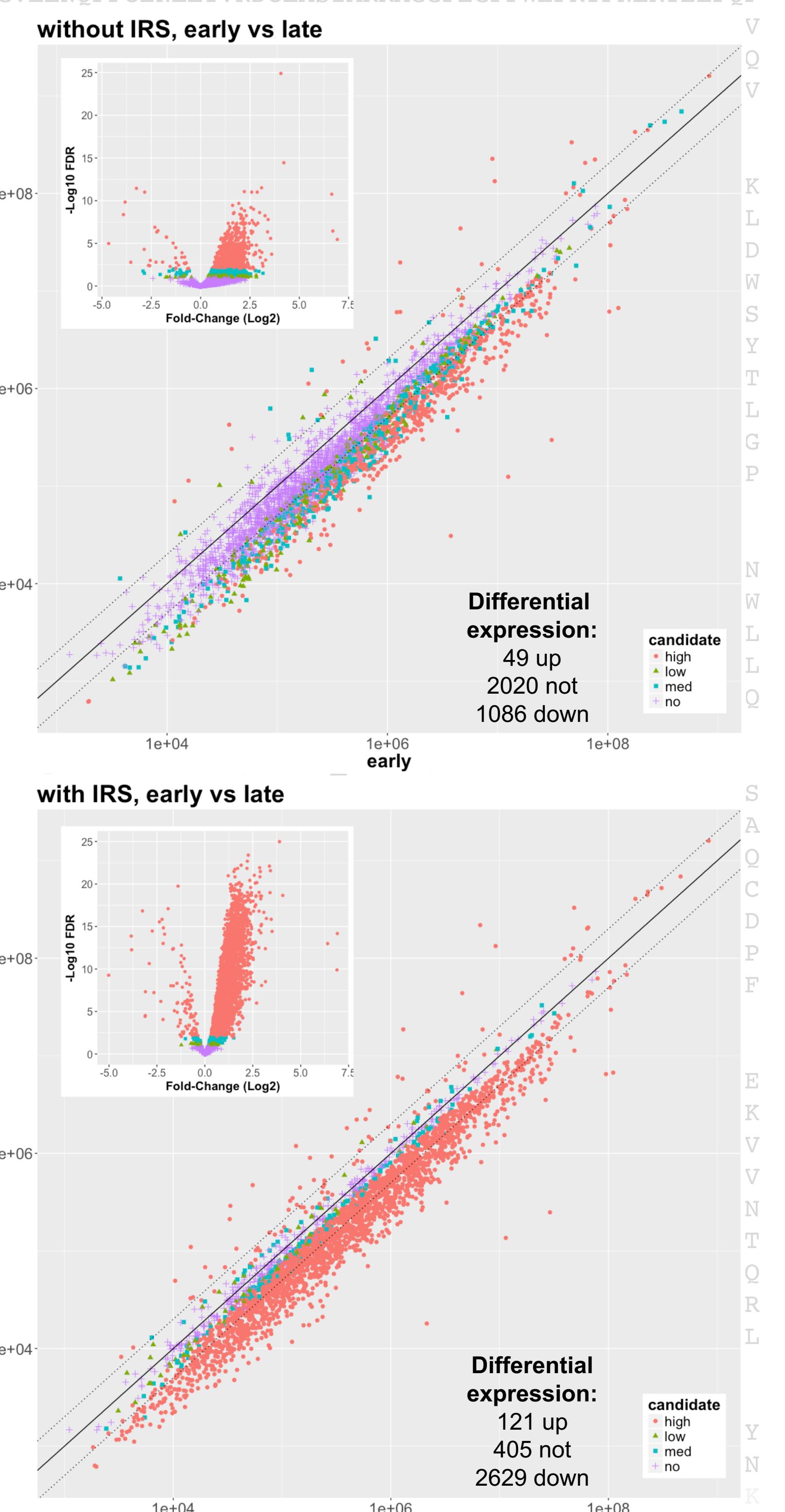


Figure 3. Intra-replicate scatter plots without IRS (left) and with IRS (right) for embryonic 18d (top) and postnatal 6d samples (bottom).



Summary:

- Internal Reference Scaling is a unique normalization method that corrects random MS2 sampling.

- IRS is a critical component in multi-TMT experiments.

References:

1. Plubell, Deanna L., et al. "Extended multiplexing of tandem mass tags (TMT) labeling reveals age and high fat diet specific proteome changes in mouse epididymal adipose tissue." *Molecular & Cellular Proteomics* 16.5 (2017): 873-890.
2. Khan, Shahid Y., et al. "Proteome Profiling of Developing Murine Lens Through Mass Spectrometry." *Investigative ophthalmology & visual science* 59.1 (2018): 100-107.
3. Robinson, Mark D., and Alicia Oshlack. "A scaling normalization method for differential expression analysis of RNA-seq data." *Genome biology* 11.3 (2010): R25.
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