**Notes on NIH planning meeting: 7/2/13**

AIM 1. TF Dynamics

AIM 2. TF Interactions

AIM 3. In planta

AIM 4. Modeling

**DENNIS: TRUMP card is TARGET which allows high through data on TF function that:**

* 1. Is rapid and accurately predicts what happens in planta
	2. Provides novel insights into in vivo function (e.g. direct TF targets) –including ones that don't stably bind to target
	3. This approach could be applied to any system (animals, yeast), plants is just an example.

 **Dream of Systems Bio is to predict out of sample data**

1. Show tools/pipeline
2. Test on demand in high through put system

**Show more examples of overlap of protoplast and in planta**

 Current: ABI3, bZIP1

 More?: CCA1 protoplasts vs cca1/lhy mutant

Additional: HRS1?; GLK1?

Dennis thinks the whole grant could be simpler than shown below. We hunt down an important network or two using our TARGET method as well as time series on protoplast as Kranthi suggests. (I don’t really see what more we gain from clustering and co-expression. It’s just not our comparative advantage.) This is of interest to NIH because it shows (i) that DEX is a faster but still accurate assay compared to constituitive over-expresson (which may not be possible in humans for example). (ii) that we can rapidly learn an important network. That is we are presenting an experimental/analytical toolbox.

**AIM 1: TF DYNAMICS**

**Kranthi:**

Exp. 1. Nitrogen time-course in protoplasts: RNA Seq & ChIP Seq (either RNA pol or H3K4)

Exp. 2. Example; TF time course: RNA Seq & ChiP Seq

Exp. 3. Set of TFs; Perturbations (either dynamic or not)

**Possible models:**

* N-treat protoplasts data can be used to identify waves of N-regulated clusters and associated TFs a la Regev model.
* DREM model for kinetic data- (like Ecker) need bZIP1 time course data.

**ISSUES:**

**Do we need time course on all TFs?**

* Will time course on one TF tell us if we need time course on all TFs or help define a subset of time-points?
* No, we might need to try two extreme time course points and see if the target information changes a lot. If not, then maybe no need to do intermediates. Data-driven experimentation.
* Ying needs fine scale time course on TFs to use the Aviv Regev method (What time points does she need)?

**Problems with overexpression and off target gene activation**

* level of TF expression and lack of cell-specificity
* Use **cell specific expression** data in roots to refine the protoplast
	+ E.g. only consider regulation of genes in the cell-types they are normally expressed in. This deals with cell-specificity but does not deal with effects of overexpression.
	+ Use cell-specific data to identify TFs that are ubiquitous
	+ Use cell specific data to identify TFs that are only in specific cell types
	+ Try TARGET on GFP marked cell types

**Networks based on regulation? (compared to ones based on DNA binding)**

 Try expression vs DNA binding separately to see how they compare

 Try a layered pipeline: (+/- N; +/- DEX)

 Step 1: +CHX to identify primary targets

 Step 2: Fill in with -CHX data to then identify secondary targets

 Step 3; Introduce bound vs no bound

Do you get different results for bound vs not bound?

**AIM 2: TF INTERACTIONS**

1. 2 different inducers: DEX vs Estradiol
2. 2 different tags:
3. Sequential Chip- possible with small amount of material from protoplasts?
4. Dynamics of each? Expression and Chip seq – to determine FFLs

**Possible experiment:**

Use CCA1 recombineered line (CCA1 tagged but expressed under native promoter). Use transgenic line as host for bZIP1 DEX. This would tell how does bZIP1 activation affect CCA1-target binding.

* This would test whether bZIP1 recruits CCA1 to a promoter (in vivo pull down).

**AIM 3: In Planta**

1. Recombineering hard, maybe you only have to use Native promoter with tagged version
2. Try native promoter in DEX protoplast system?

**AIM 4 Modeling**

1. Whole genome dynamic modeling: Ying- Try Regev, DREM, others?
2. Small network dynamic modeling; predict out of sample outcome
	1. Dennis test NetProphet
	2. Test yeast data from NetProphet in Jesse pipeline