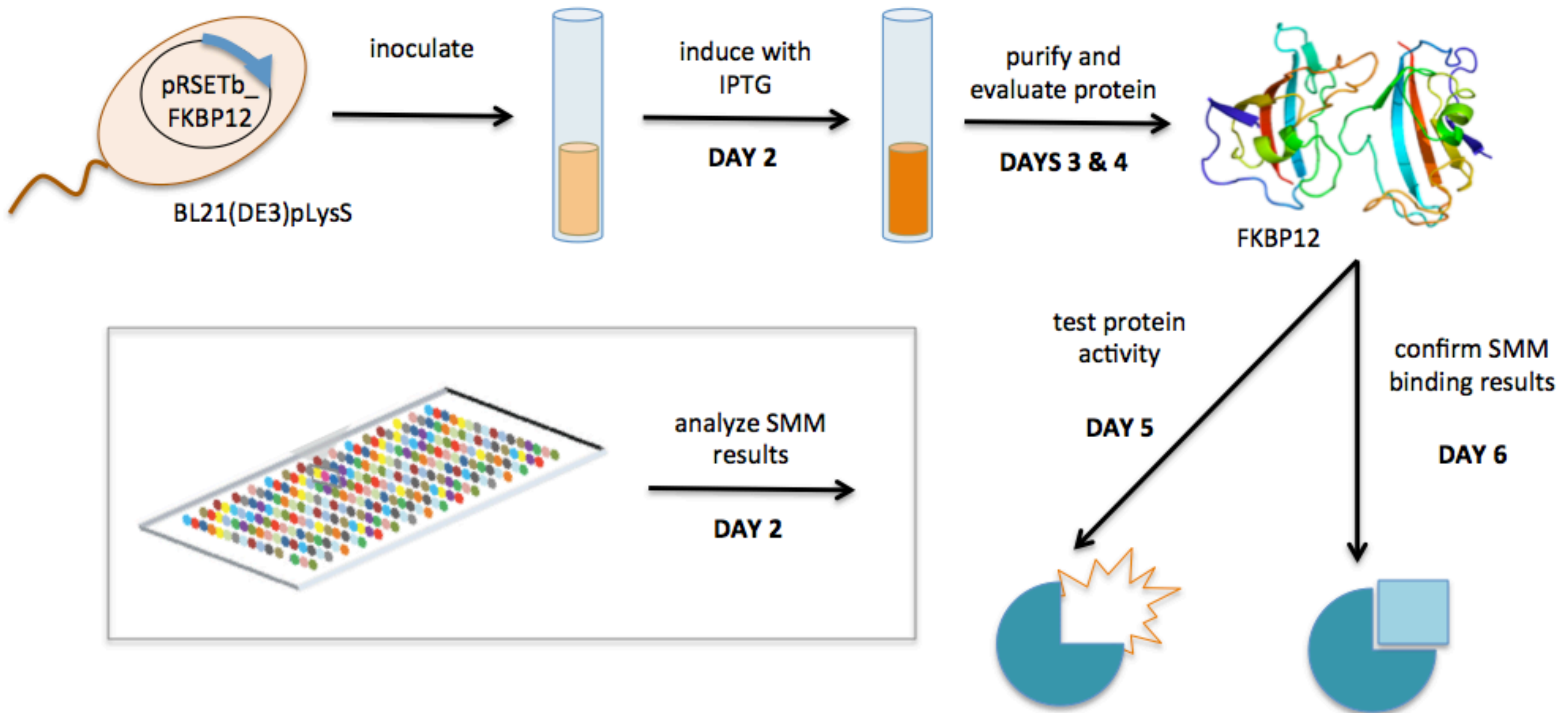
M1D2: Complete small molecule microarray analysis and induce protein expression

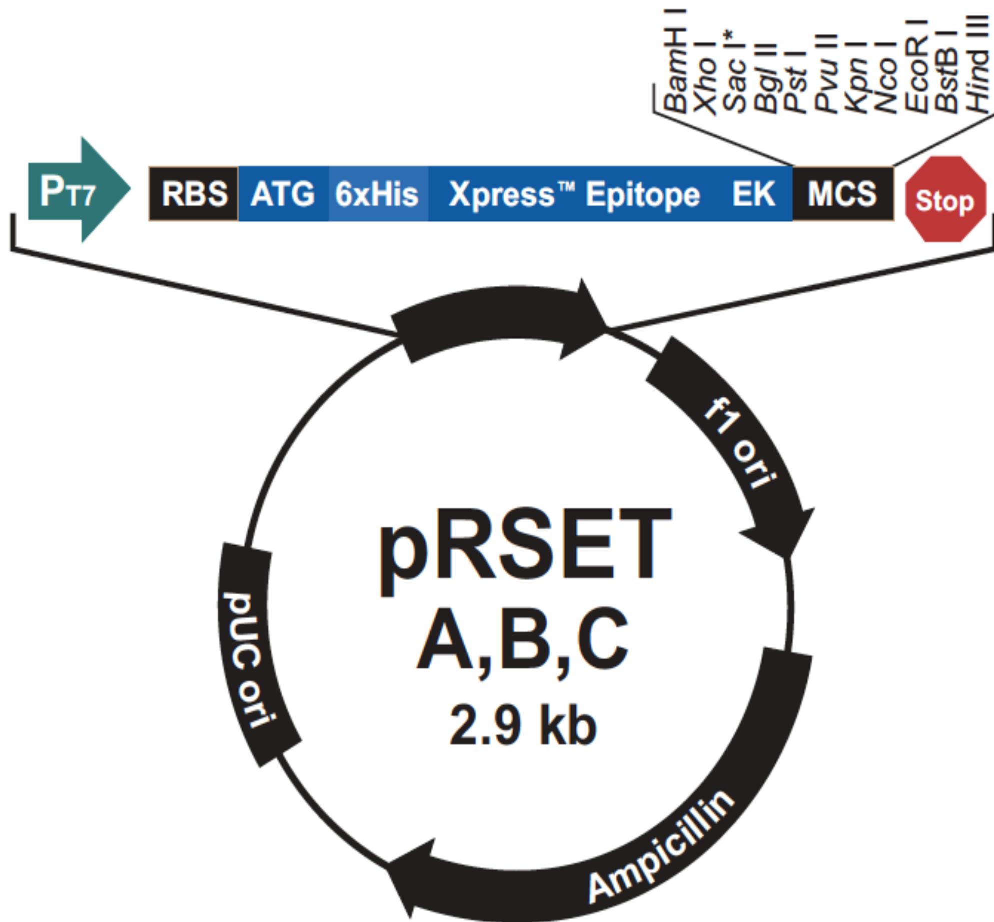
1. Pre-lab discussion
2. Induce FKBP12 expression
3. Gel electrophorese confirmation digests
4. Complete SMM data analysis

Prepared with help & slides from Rob Wilson

Overview of Mod1 experiments



But first, a review of cloning



- P_{T7}

  IPTG-inducible

- 6xHis

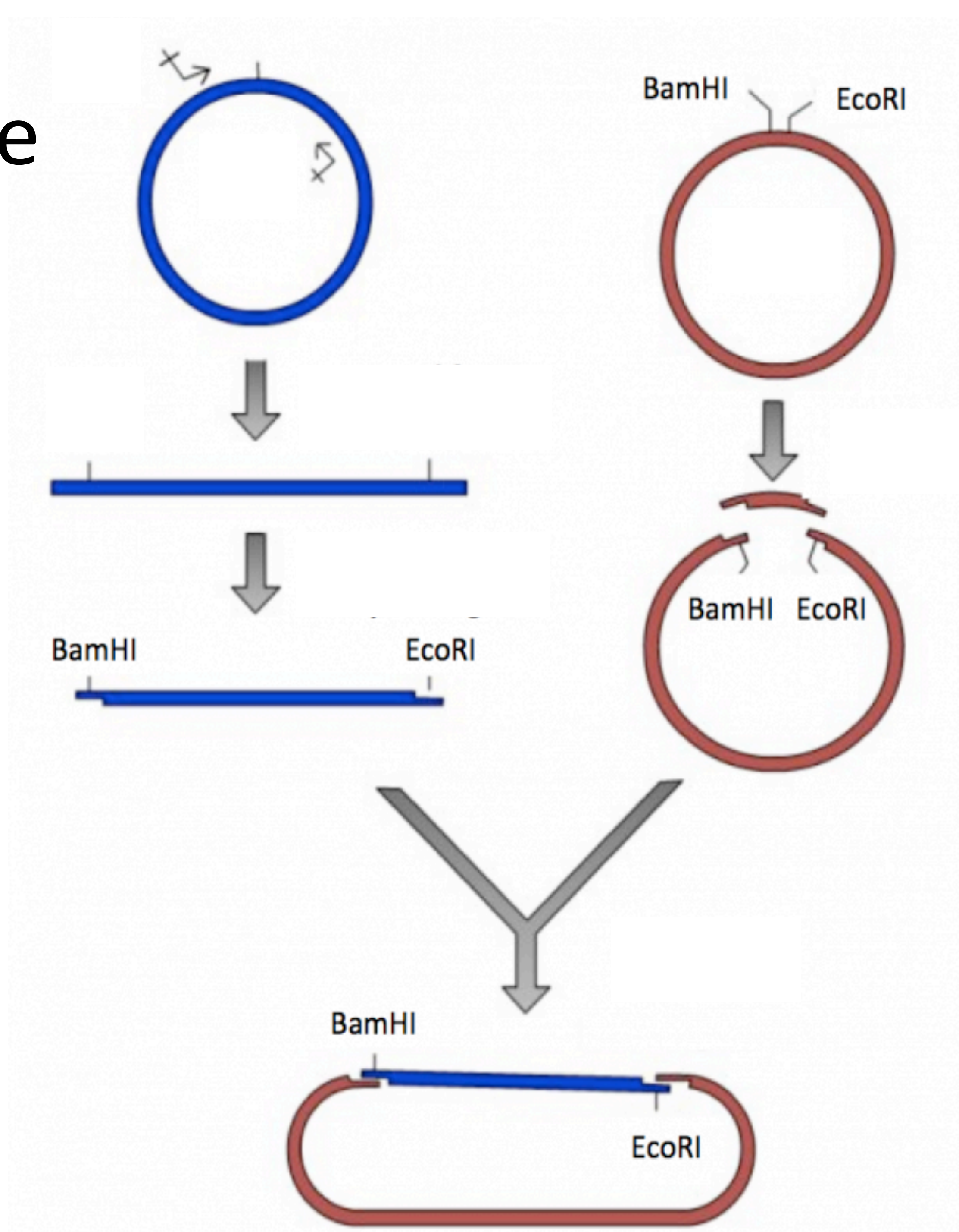
  used in purification - binds to Ni

- MCS

  mulitple cloning site

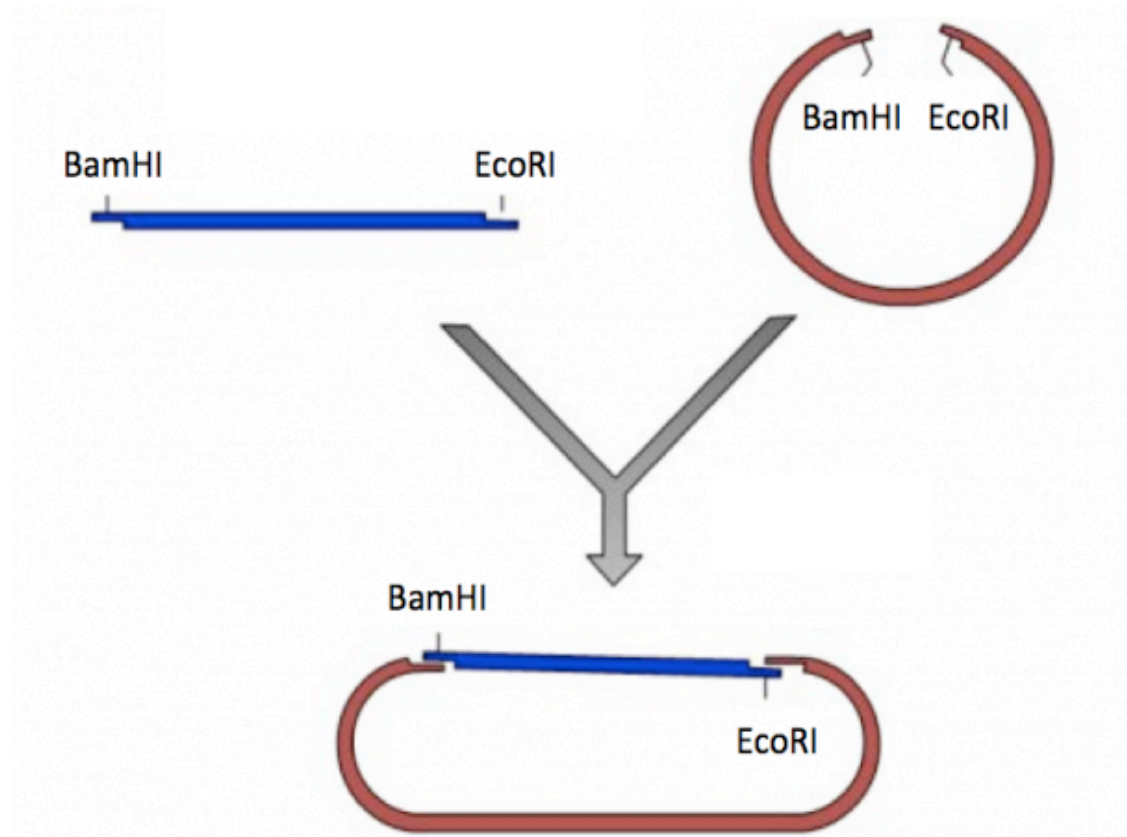
How did we clone our insert?

- Amplification
- Digestion
- Ligation



How did we check our product?

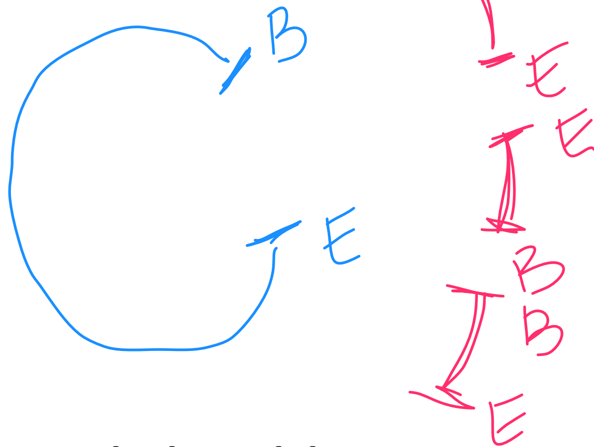
- Transformation
- Purification
- Digestion



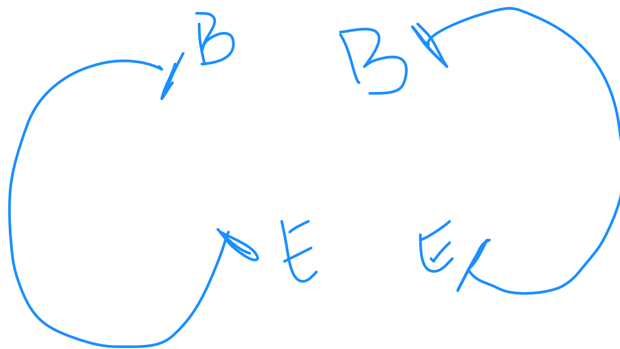
Ideally, 4:1 **molar** ratio of insert:backbone

Why perform confirmation digests?

- Too much insert

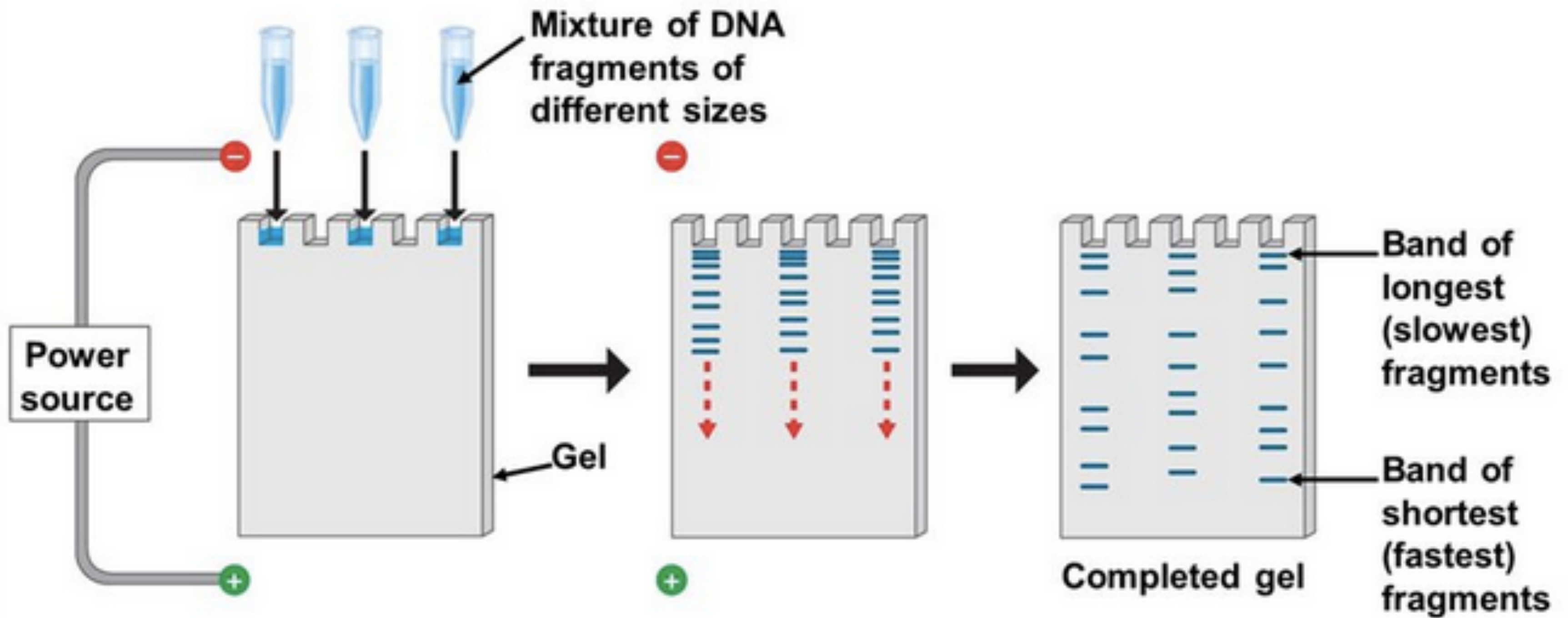


- Too much backbone

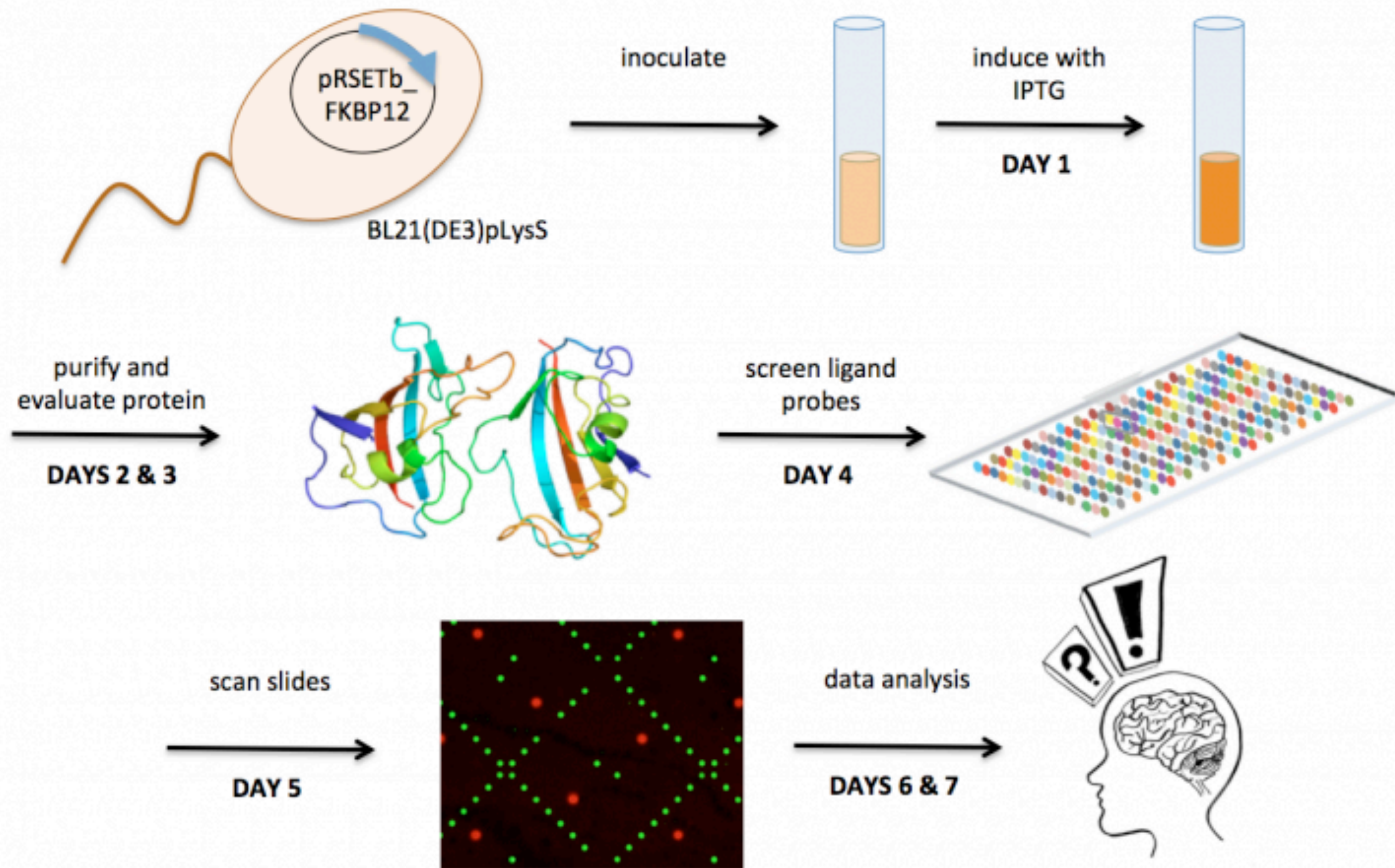


How will we visualize gel results?

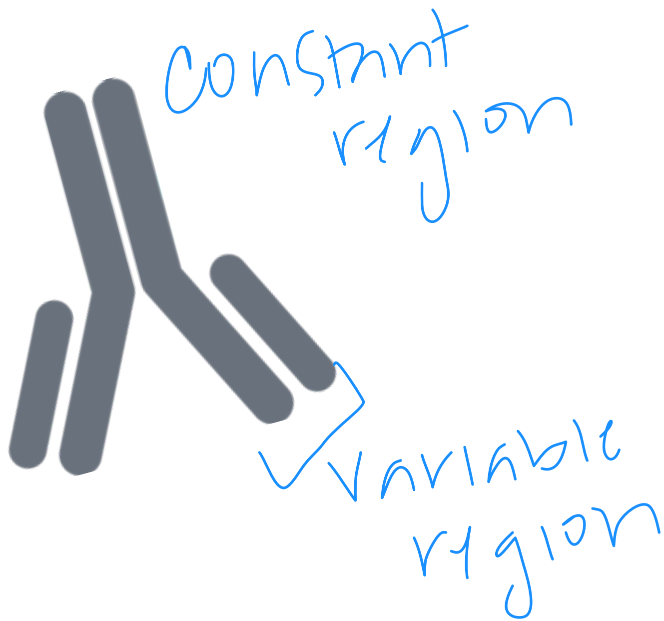
DNA fragments resolved using 1% agarose gel

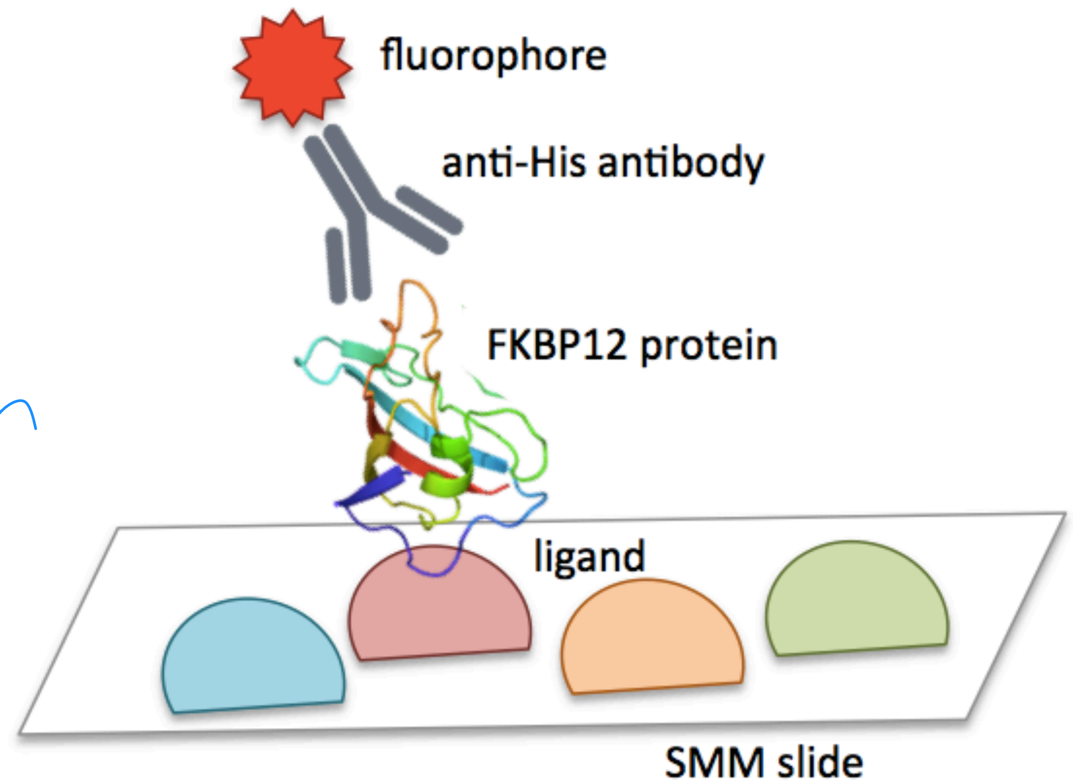


Quick recap of Sp17



Using immunofluorescence to detect ligand binding



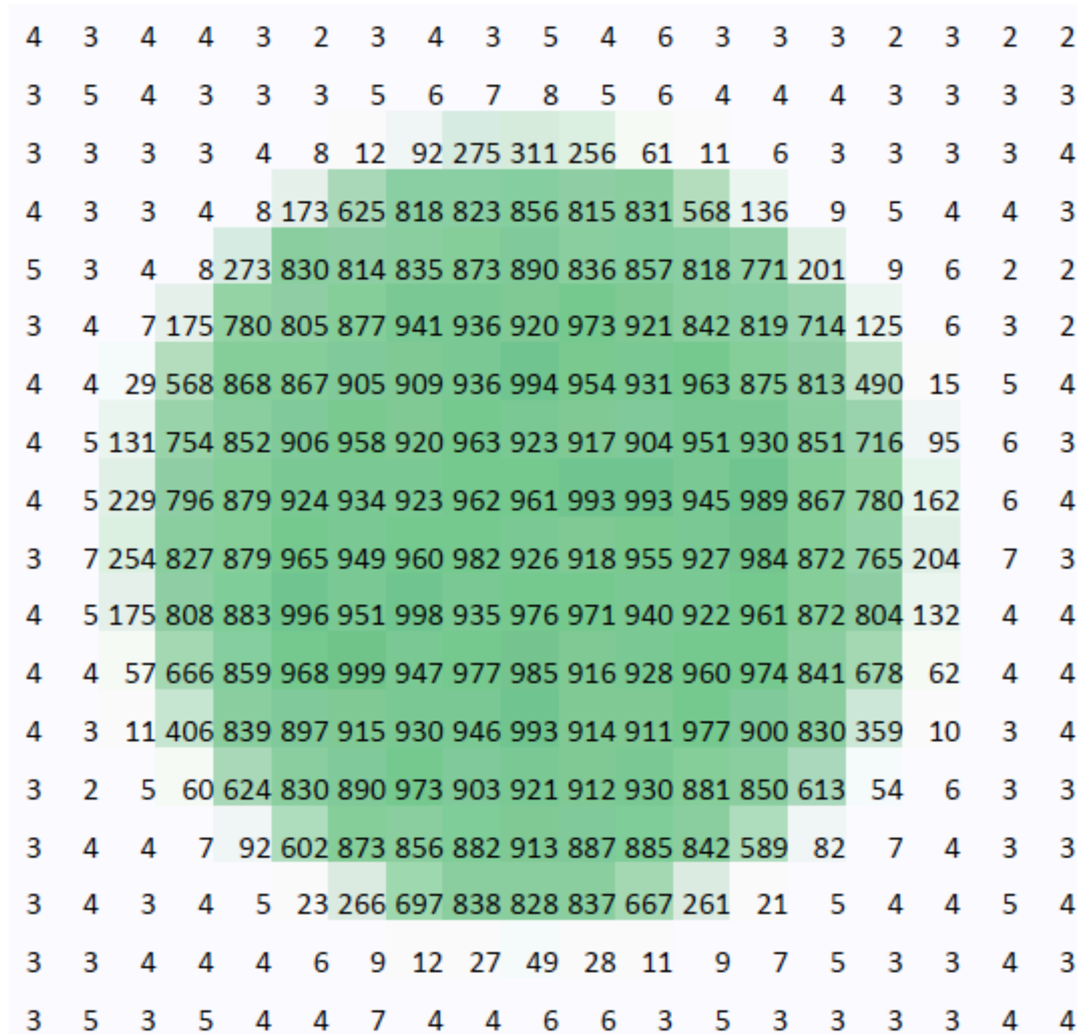


SMM quantification steps

1. Align GAL file to fluorescence on 532 nm channel (sentinel spots)
2. Quantify fluorescence on 635 nm channel
3. Identify ‘hits’ with improbably high fluorescence
4. Identify compounds that hit repeatedly
5. Compare top hits to common binders list

Images represent arrays of numbers

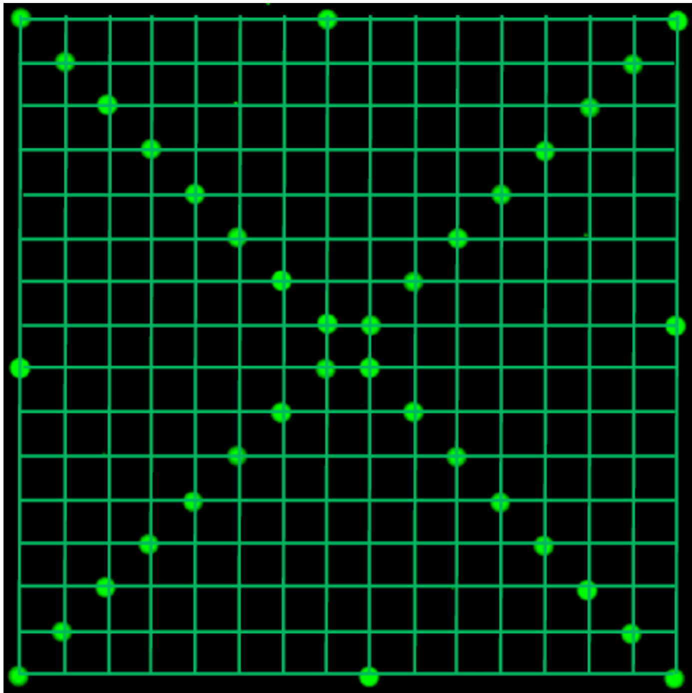
- Each pixel is a 16-bit number that represents intensity
- Computational analysis used to define ‘hits’

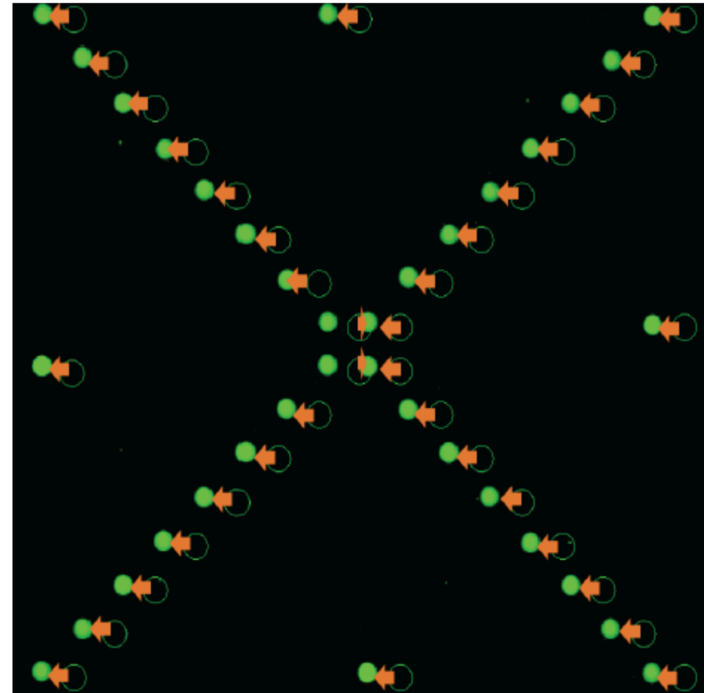


4	3	4	4	3	2	3	4	3	5	4	6	3	3	3	2	3	2	2
3	5	4	3	3	3	5	6	7	8	5	6	4	4	4	3	3	3	3
3	3	3	3	4	8	12	92	275	311	256	61	11	6	3	3	3	3	4
4	3	3	4	8	173	625	818	823	856	815	831	568	136	9	5	4	4	3
5	3	4	8	273	830	814	835	873	890	836	857	818	771	201	9	6	2	2
3	4	7	175	780	805	877	941	936	920	973	921	842	819	714	125	6	3	2
4	4	29	568	868	867	905	909	936	994	954	931	963	875	813	490	15	5	4
4	5	131	754	852	906	958	920	963	923	917	904	951	930	851	716	95	6	3
4	5	229	796	879	924	934	923	962	961	993	993	945	989	867	780	162	6	4
3	7	254	827	879	965	949	960	982	926	918	955	927	984	872	765	204	7	3
4	5	175	808	883	996	951	998	935	976	971	940	922	961	872	804	132	4	4
4	4	57	666	859	968	999	947	977	985	916	928	960	974	841	678	62	4	4
4	3	11	406	839	897	915	930	946	993	914	911	977	900	830	359	10	3	4
3	2	5	60	624	830	890	973	903	921	912	930	881	850	613	54	6	3	3
3	4	4	7	92	602	873	856	882	913	887	885	842	589	82	7	4	3	3
3	4	3	4	5	23	266	697	838	828	837	667	261	21	5	4	4	5	4
3	3	4	4	4	6	9	12	27	49	28	11	9	7	5	3	3	4	3
3	5	3	5	4	4	7	4	4	6	6	3	5	3	3	3	3	4	4

Align SMM results to using sentinel spots

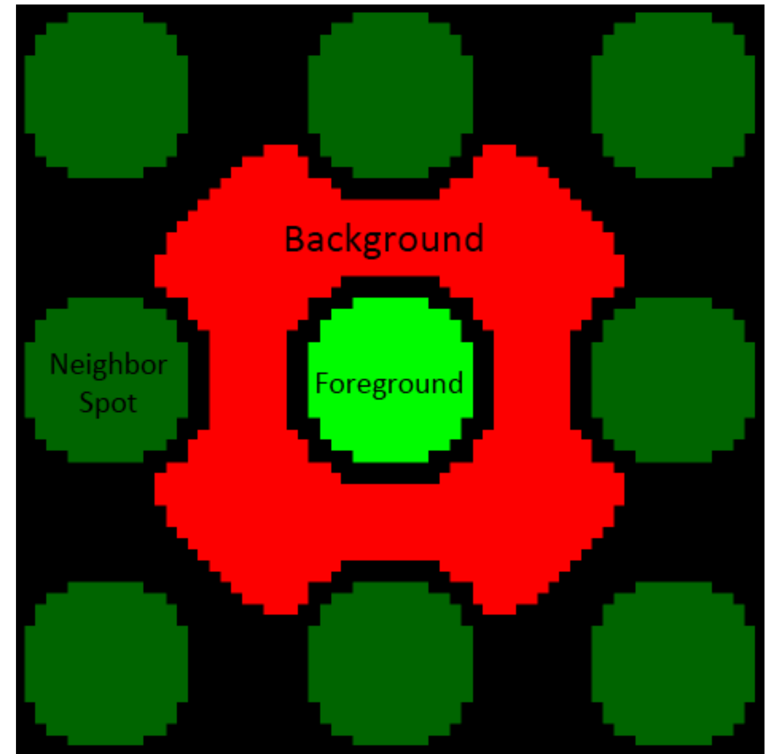
- Every spot can be located using intersecting lines between sentinels





Quantify fluorescence to identify hits

- Foreground
- Background

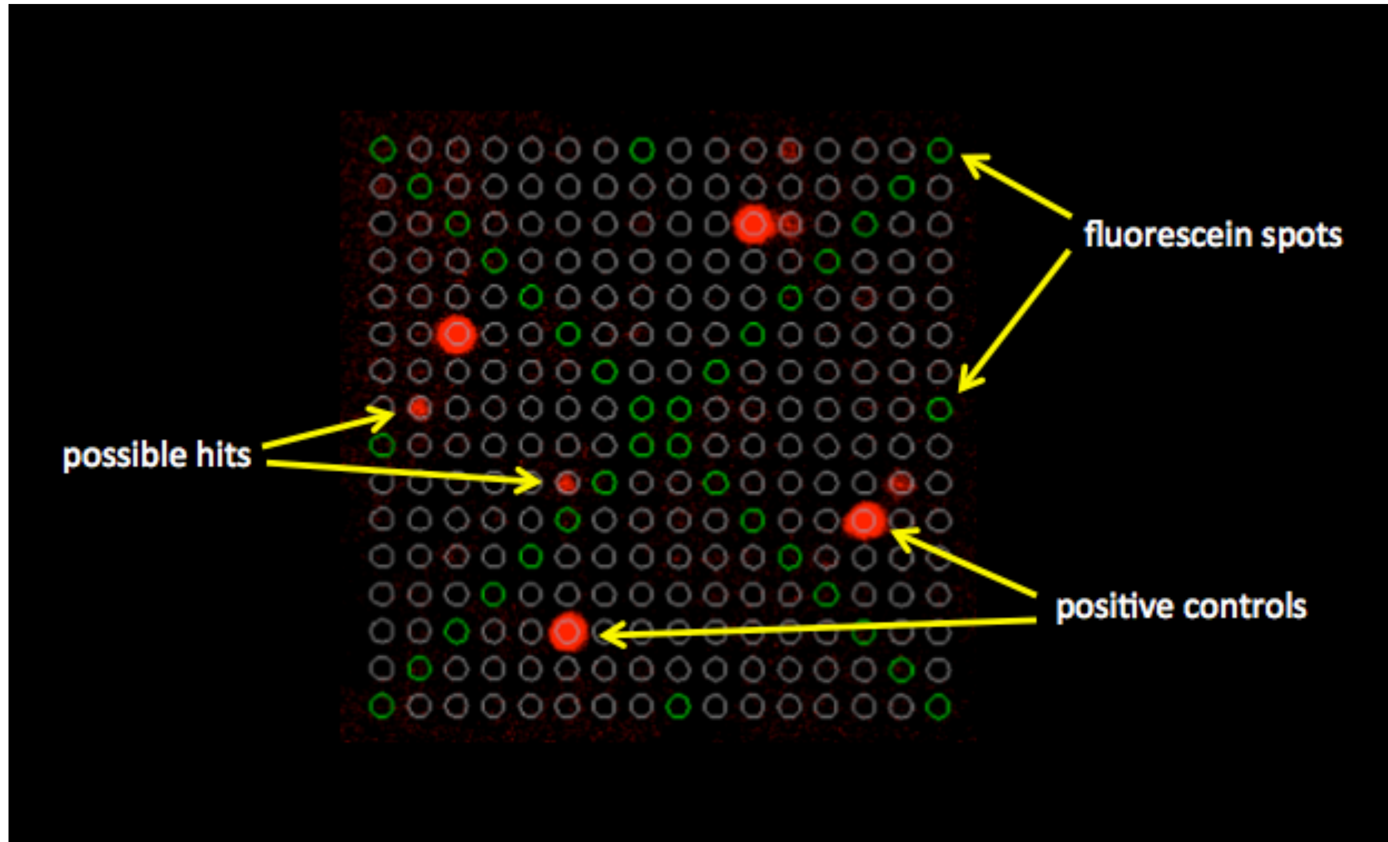


$$\text{Signal-to-noise ratio (SNR)} = \frac{\mu_{foreground} - \mu_{background}}{\sigma_{background}}$$

How to evaluate the SMM results

- Is the background noisy?
- Are the positive controls easily recognized?
- Do any areas appear strange? Damaged?
  - Manufacturer or handling defects
- Are the hits aligned with printing spots?
- Do you trust the data?

What do we expect to see?



Factors that influence hit identification

- How many false positives are expected?
  - More hits needed if confidence is low
- How many chemical ‘patterns’ are evident?
  - Repeated patterns between compounds may increase confidence
- Are the hits unique to the screen?
  - Promiscuous binders may decrease confidence

Today in lab…

- Wipe down bench with 70% EtOH before and after wetlab work

For next time…

- Draft a figure with your confirmation digest results for your Data summary
  - Include a title and caption

Notes on figure making:

- Image should not be the entire page
  - Only needs to be large enough to be clear
- Title should be conclusive
  - Don't include what you did, rather include what you found
- Caption should not detail the methods
  - Define abbreviations, symbols, etc.