

Unleashing high-throughput reaction screening

Introduction

A successful high-throughput approach to reaction screening requires rational and systematic exploration of a broad set of variables to achieve optimization expeditiously. There are many types of potential variables that can be screened. Even with the aid of high-throughput screening tools, chances are that it's still unlikely to screen all the possible combinations of reaction variables a process chemist desires. A well-planned high-throughput approach enables scientists to more clearly see the "big picture", quickly abandon disadvantaged routes and rapidly pinpoint advantageous and impactful conditions to focus on.

Let's talk about high-throughput reaction screening

Where to start

Before a particular set of reaction variables can be assembled for screening, let's first look back at different scenarios where the decision to conduct high-throughput screening (HTS) comes into the picture.

The goal of a HTS approach generally falls into two categories. It might be at the exploratory or discovery phase of the research where a certain transformation is not known but highly desired. A HTS approach enables discovery scientists to scout out potential conditions more systematically so they can evaluate its feasibility. But more likely, HTS gets called in much later when a process needs to be optimized to produce higher yields and/or eliminate impurities while examining alternative strategies to avoid costly, toxic or patented reagents.

With the project goal in mind, a comprehensive review of the knowledge base pertaining to this particular transformation comes next. This review typically consists of studying what's known about this specific reaction and similar work or transformations that can be found in patents and literature. If the project has never gone through HTS, there might be only several known conditions that have been attempted with varying success, partially due to limits in available resources. In short, at this early stage of understanding, the overall picture of potentially impactful variables might still lack focus and be somewhat jagged.

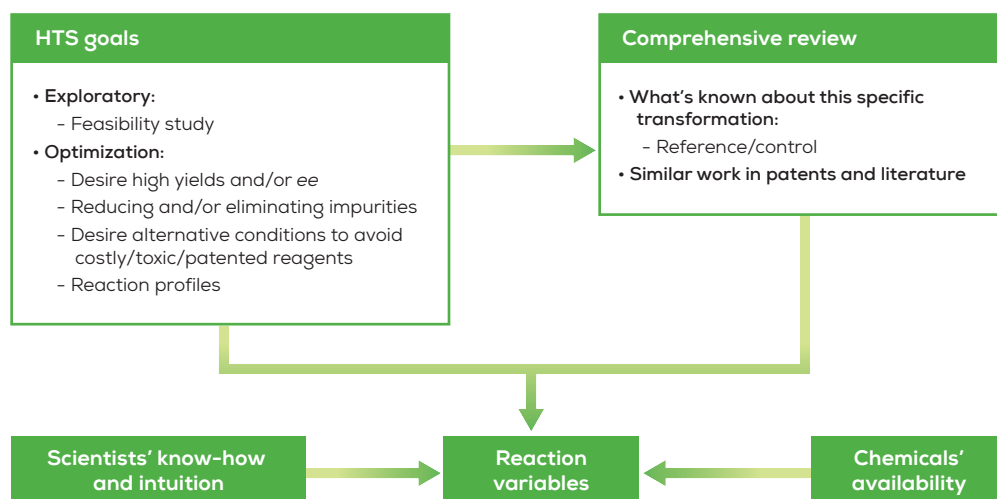


Figure 1: Defining reaction variables to screen.

Before the design for that first screening plate can be decided, two additional factors also need to be considered. First, availability of relevant structures or chemicals is crucial. In other words, there is no point in including a cool ligand with great activity that appeared in JACS in your screening if it was synthesized in a university lab and is still not commercially available. A comprehensive evaluation of available and relevant chemical space is a prerequisite to HTS planning.

Second, project scientists' know-how and intuition also play an important role in deciding what to screen. Instead of being limited to only screen more familiar or known conditions, HTS opens up the possibility for scientists to test more out-of-the-box ideas (Figure 1).

Discrete vs. continuous

Normally, we will end up with a big list of potential variables to screen after the above exercise. For example, in typical C-X coupling chemistry, we might have gathered individual lists for different catalyst precursors, ligands, bases, solvents and additives in addition to variables such as equivalents, concentrations, temperatures and reaction duration (time). So how do we distill them into an 8x12 plate with 96 different conditions?

Generally, different variables are categorized into two groups: discrete (such as different ligands and bases) and continuous variables (such as temperature and time). Initial screening should

almost always be focused on discrete variables while fixing continuous variables, which tend to be based on known conditions (such as equivalents and temperature).

Even with the focus on discrete variables during initial broad screening, the decision on which specific reagents to screen within each variable can still be daunting. In general, the guiding principle for initial screening should be broad-based with project goals, structural diversity and their availability in mind.

Experimental design

One common screening approach after having decided on an initial set of discrete variables is *via* full factorial experimental design (Figure 2). This approach would test interaction of every combination, teasing out interesting permutations that are not obvious, thus encouraging non-intuitive discoveries. This design approach also helps with mapping out impactful variables and/or combinations, discovering clues and observing trends. One or several known conditions should also be included as reference or control.

Under a different scenario, it might be more desirable to include more variables or reagents at once. A fractional factorial design can be used in this instance to test out more unique combinations.

Generally, it's not advisable to screen more than one plate at a time, especially at the beginning of this process. Knowledge from these early plates

3 Catalyst precursors 4 Ligands 8 Solvents		1	2	3	4	5	6	7	8	9	10	11	12
		Catalyst precursor 1				Catalyst precursor 2				Catalyst precursor 3			
		Lig 1	Lig 2	Lig 3	Lig 4	Lig 1	Lig 2	Lig 3	Lig 4	Lig 1	Lig 2	Lig 3	Lig 4
A	Solvent 1												
B	Solvent 2												
C	Solvent 3												
D	Solvent 4												
E	Solvent 5												
F	Solvent 6												
G	Solvent 7												
H	Solvent 8												

Known condition as reference/control

Figure 2: Discrete variables in full factorial design.

tends to point to obvious “no-nos”. Doing parallel screens without this knowledge can lead to a much higher chance of wasted efforts and materials.

Execution of a screen

With final experimental design in hand, we are now ready to execute our first screening plate (Figure 3). All aspects of a design, from chemicals needed to experimental conditions for each of the conditions (96 conditions in our current example), would be entered into Unchained Labs Library Studio® software. A unique experimental ID will be generated at this stage which would follow this experiment throughout the whole screening process.

While Library Studio focuses on experimental details, Automation Studio™ translates that Library design into actionable instrument control, where either Big Kahuna or Junior would do all the heavy lifting (Figure 4). Actions include introducing all the necessary chemicals *via* solid, slurry, liquid and viscous liquid dispense, to heating, cooling and stirring to preform process conditions based on experimental design.

There are also more specialized technologies such as the Optimization Sampling Reactor (OSR) and the Screening Pressure Reactor (SPR). The OSR gives researchers the capability to add reagents and withdraw samples at different pressures and temperatures during the course of the reaction, thus reaction profiles and other continuous variables can be looked into much more easily. The SPR on the other hand, provides a high-throughput solution under even more challenging pressure and temperature conditions. At the end of this process, HPLC or GC analysis would typically follow to evaluate every reaction including reference.

Linked by one unique experimental ID, all experimental data that spans from initial reaction design to processing conditions and finally analytical results (such as HPLC from third party equipment) can be viewed, analyzed and shared from anywhere, either immediately or years from now.

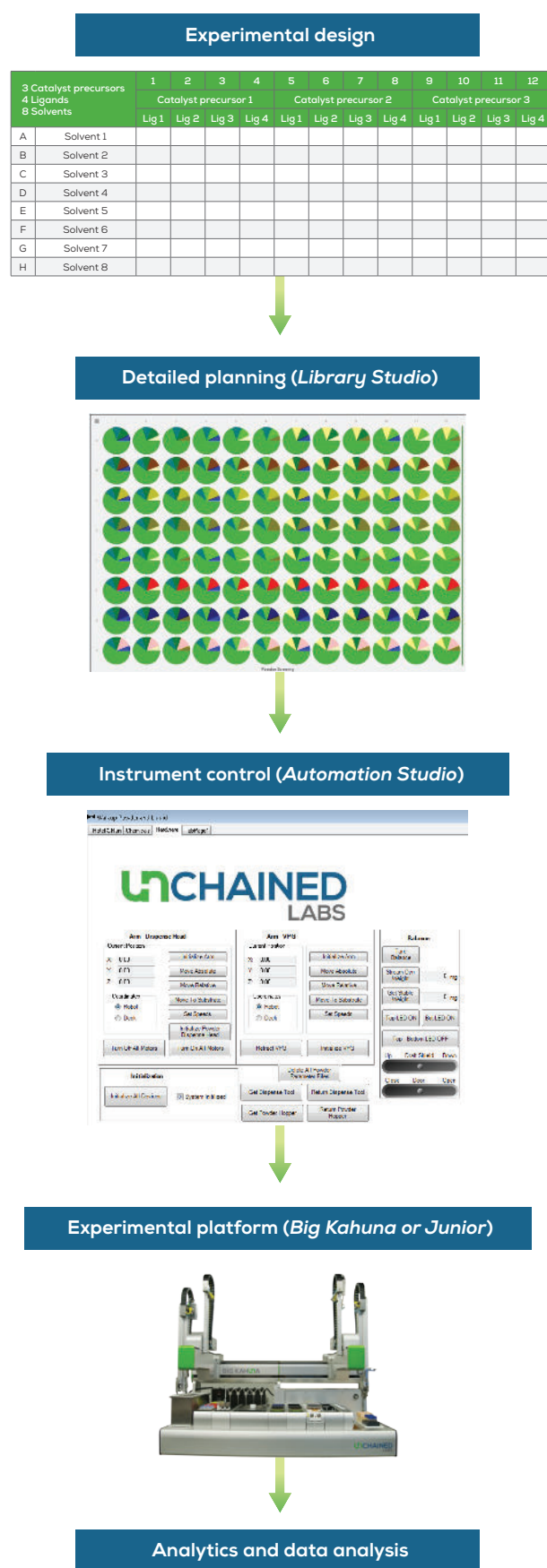


Figure 3: Reaction screening workflow.

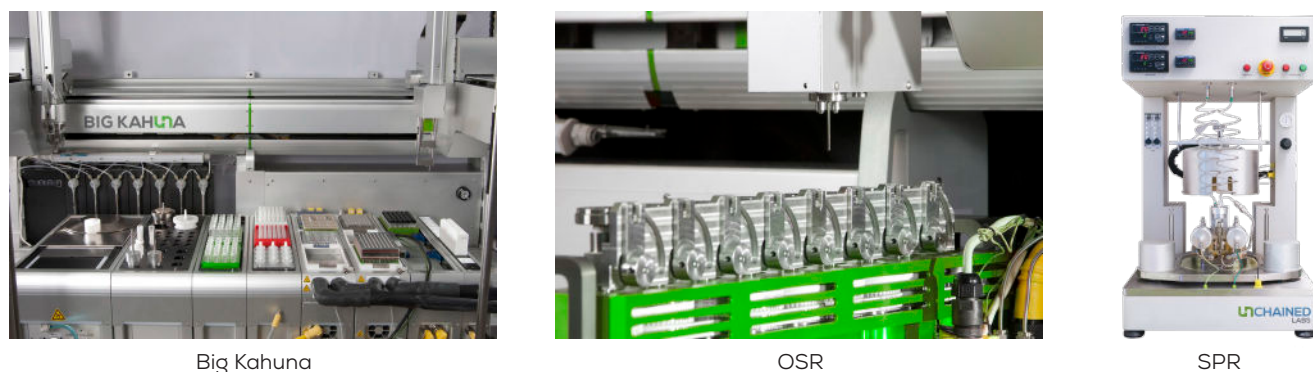


Figure 4: The right process chemistry tools.

Follow-up screens

Just like any research program, this process is an iterative one. There are several possible scenarios based on results from our first screening (Figure 5). The results might be a dud and not worth pursuing further. There might be several interesting conditions that are worth scaling up or repeating under normal benchtop conditions to confirm screening results.

More discrete variable screening might be needed to finish up what was left out in earlier designs. Ideally, some of the chosen screening conditions do show promise or hit the mark, so there should be areas where further investigation should be focused. Variables or structural moieties that show

the most promise should get the most attention in follow-up screenings so structural variations within these privileged classes can be examined in greater detail.

If this iterative process to narrow down the most advantageous discrete variables (such as certain combination of catalyst precursor, ligand and solvent) is successful, continuous variables are next. At this stage, an automated high-throughput solution can also be tremendously helpful. The OSR¹ can be utilized to probe continuous variables such as temperature, pressure, reaction progress (time) and concentrations with ease and precision.

¹ Please see application note titled "Accelerating your process optimization: sampling from reactions in-progress means better decisions in less time" for a detailed description.

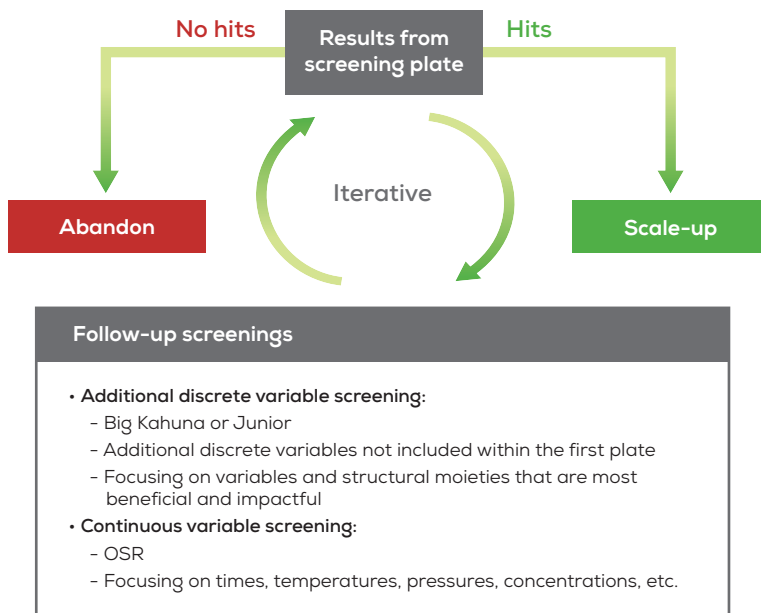


Figure 5: It's an iterative process.

Case study

How crowded can it get?

Let's dive into a case study on an early discovery program involving installing sterically challenging moieties *via* C-C coupling chemistry (Figure 6). Here, the research goal was to install bulky aromatic substitutions at *ortho* position of a phenol – the bulkier, the better. If protecting and deprotecting the phenol group can be avoided, it would be a huge plus as well.

Benchtop approach

Just like any typical project, we started this study in a traditional way by investigating several types of transformations. We first tried Negishi coupling and the coupling was successful (Figure 7). The phenol group was protected by a MOM group and the Mesityl group was the bulkiest group successfully coupled. One big drawback with this approach was the sensitivity of Zn chemistry which led to big batch-to-batch variations.

Next we tried Suzuki coupling and again found success (Figure 8). Because of the more inert

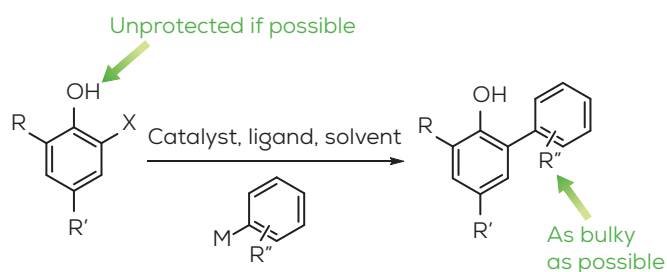
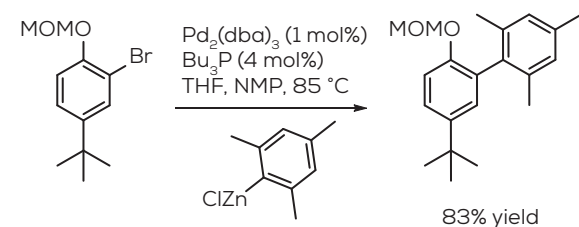


Figure 6: Research goal.



Limitations:

- Protecting group needed
- Sensitive Zn chemistry

Figure 7: Negishi coupling (traditional approach).

nature of this approach, phenol protection was not necessary and the Mesityl group was installed with higher yield and greater consistency.

High-throughput approach

We moved on to couple an even more sterically hindered 2,4,6-triisopropyl phenyl group (Trip group). Anticipating the challenging nature of this coupling, we planned a high-throughput approach to explore Suzuki coupling conditions in a more systematic fashion (Figure 9). We screened two Suzuki coupling-focused plates using Trip boronic acid with varying Pd catalysts, phosphine ligands, bases and solvents, but unfortunately, no promising lead appeared and the Suzuki coupling route was dropped.

The next HTS strategy was to apply more active Kumada coupling chemistry (Figure 10). More basic Grignard reagents would be involved. Instead of using more precious Grignard reagents, we introduced a new basic variable (NaH and MeMgCl in the first plate) to deprotonate the phenol group first, forming corresponding salts. The first screen also included both TripMgBr and PhMgCl to evaluate the desired transformation from both ends of steric spectrum.

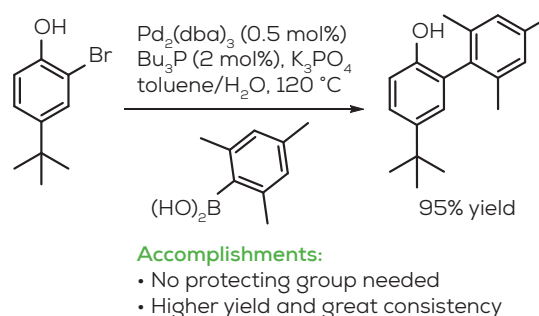


Figure 8: Suzuki coupling (traditional approach).

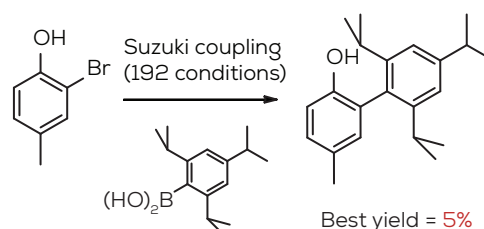


Figure 9: Suzuki coupling (high-throughput approach).

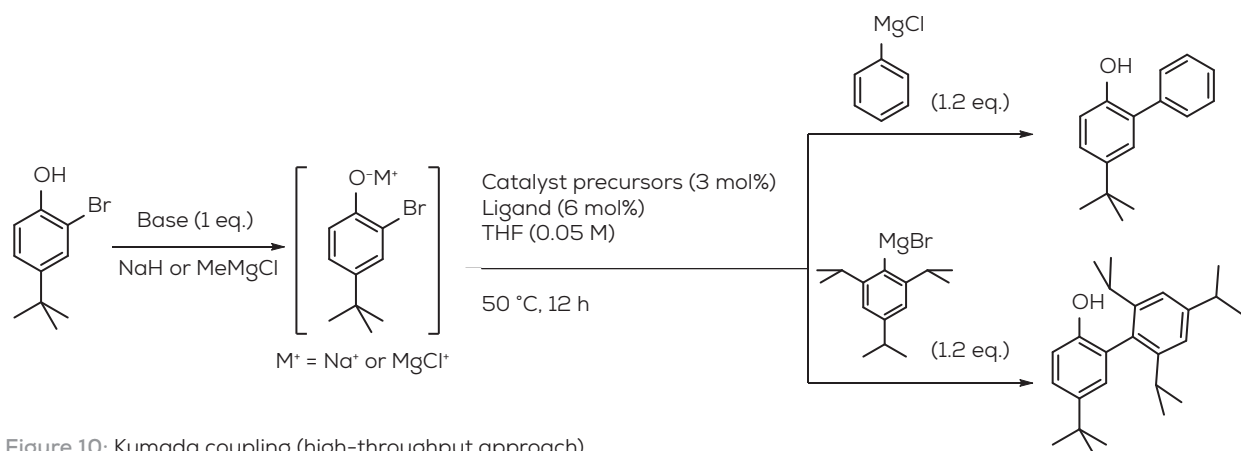


Figure 10: Kumada coupling (high-throughput approach).

Operation details

First screen design:

- 2-Br-4-*t*-Bu-Phenol (limiting reagent): 2.3 mg, 10 μmol
- Deprotonating bases (1 eq., 10 μmol): NaH, MeMgCl
- Grignard reagents (1.2 eq., 12 μmol): PhMgCl, TripMgBr
- Catalyst precursors (3 mol%, 0.3 μmol): Pd1 = Pd(acac)₂, Pd2 = Pd₂(dba)₃, Ni1 = Ni(acac)₂
- Solvent (200 μL , 0.05 M): THF

- Reaction conditions: 12 h and 50 °C
- Ligands (6 mol% for monophosphines and 3 mol% for bisphosphines + ligand free condition) (Figure 11)

We laid out all chosen discrete variables in an 8x12 grid for ease of visualization (Figure 12) and prepared all the chemicals including necessary stock solutions. We then put this information and all experimental procedures into Library Studio (Figure 13) which automatically generated a unique experimental ID. Using that ID, Automation Studio could extract all the required information from Library Studio to execute the screen.

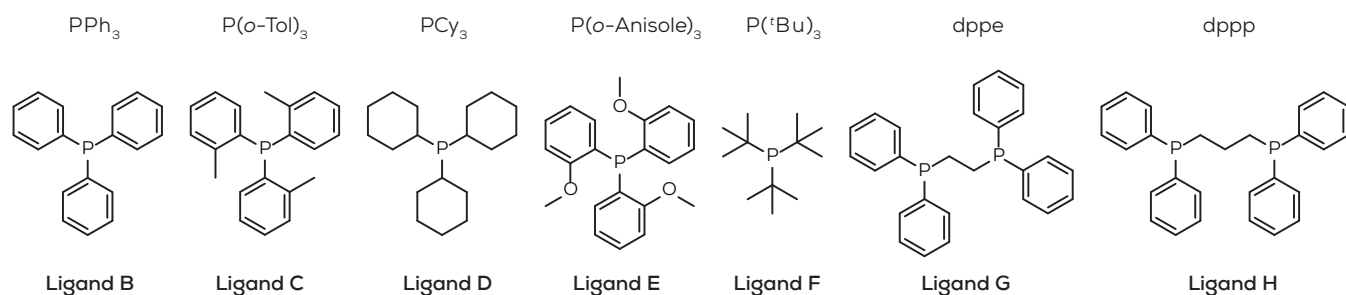


Figure 11: Seven phosphine ligands included in the first round of screening.

		1	2	3	4	5	6	7	8	9	10	11	12
Grignard		PhMgCl						TripMgBr					
Base		NaH			MeMgCl			NaH			MeMgCl		
Catalyst precursors		Pd1	Pd2	Ni1	Pd1	Pd2	Ni1	Pd1	Pd2	Ni1	Pd1	Pd2	Ni1
A	Ligand free	THF											
B	Ligand B												
C	Ligand C												
D	Ligand D												
E	Ligand E												
F	Ligand F												
G	Ligand G												
H	Ligand H												

Figure 12: First plate screening design.

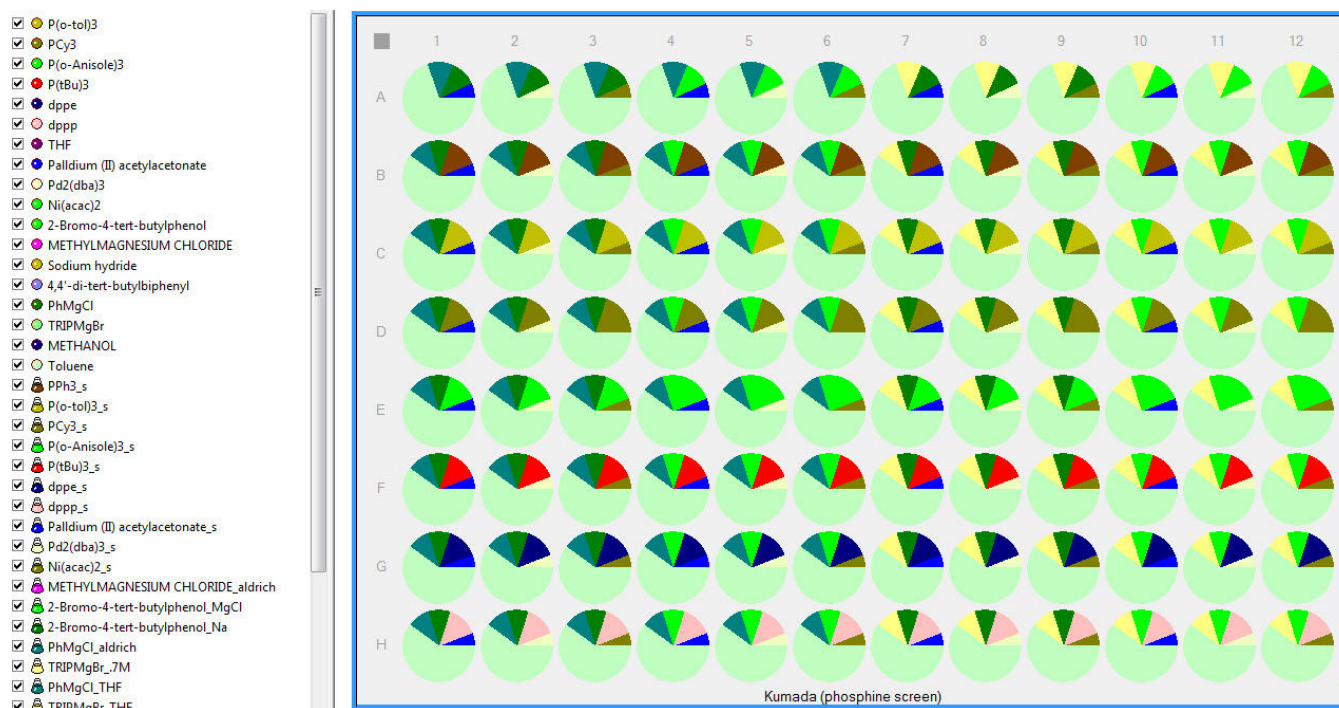


Figure 13: First plate screening design in Library Studio.

Procedure

- Added 3 catalyst precursor/THF stock solutions according to Library design (30 μ L; 0.005 M for $\text{Pd}_2(\text{dba})_3$, 0.01 M for $\text{Pd}(\text{acac})_2$ and $\text{Ni}(\text{acac})_2$)
- Added 7 ligand/THF solutions (70 μ L; 0.0086 M for monophosphines and 0.0043 M for bisphosphines)
- Added preformed Na and MgCl phenolic salts in THF (50 μ L; 0.2 M)
- Added PhMgCl and TripMgBr THF solutions (50 μ L; 0.24 M)
- Stirred the reaction plate at 50 $^\circ\text{C}$ for 12 h after sealing
- Added 50 μ L of MeOH and kept for 30 mins with stirring to quench each reaction
- Opened the plate and removed all solvents
- Added 300 μ L of toluene to redissolve all reaction components
- Transferred 25 μ L of this reaction toluene solution from each reactor to a GC plate followed by 5x dilution (addition of 100 μ L toluene)
- Conducted GC analysis

Data analysis

Still linked by that initial experimental ID, all GC data was saved to a central database through Lab Execution and Analysis (LEA) software suite, Unchained Labs' enterprise data solution (Figure 15). Product conversions were then calculated based on the GC area percentage between product and overall areas (Figure 16 for PhMgCl series and Figure 17 for TripMgBr series). The clear standout was Well A7, which achieved the highest product conversion to form Trip adduct without the use of any ligands.

This result was unexpected, but very exhilarating to see. We immediately ran two follow-up plates to investigate this ligand-free condition further.

Follow-up screens

The first follow-up screening was focused on probing the extent of the ligand-free condition by using different Grignard reagents with different steric requirements (Figure 18). We also investigated other non-Pd based catalyst precursors (Ni- and Fe-based).

Screen design (first follow-up screen):

- Base: NaH
- Catalyst precursors: Pd(acac)₂, Pd(OAc)₂, Pd₂(dba)₃, Ni(acac)₂, Fe(acac)₂, Fe(acac)₃
- Solvent: THF
- Reaction conditions: 3 h and 80 °C
- Grignard reagents (Figure 14)

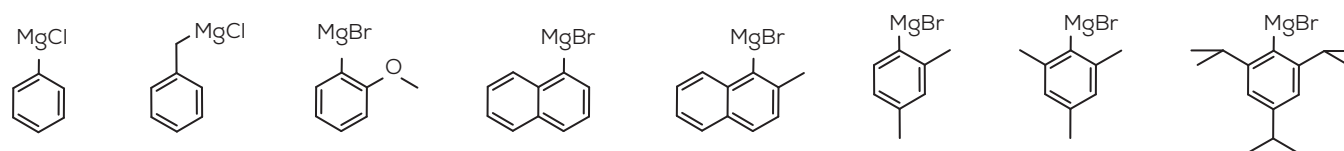


Figure 14: Grignard reagents used in the first follow-up screening with increase in steric bulk.

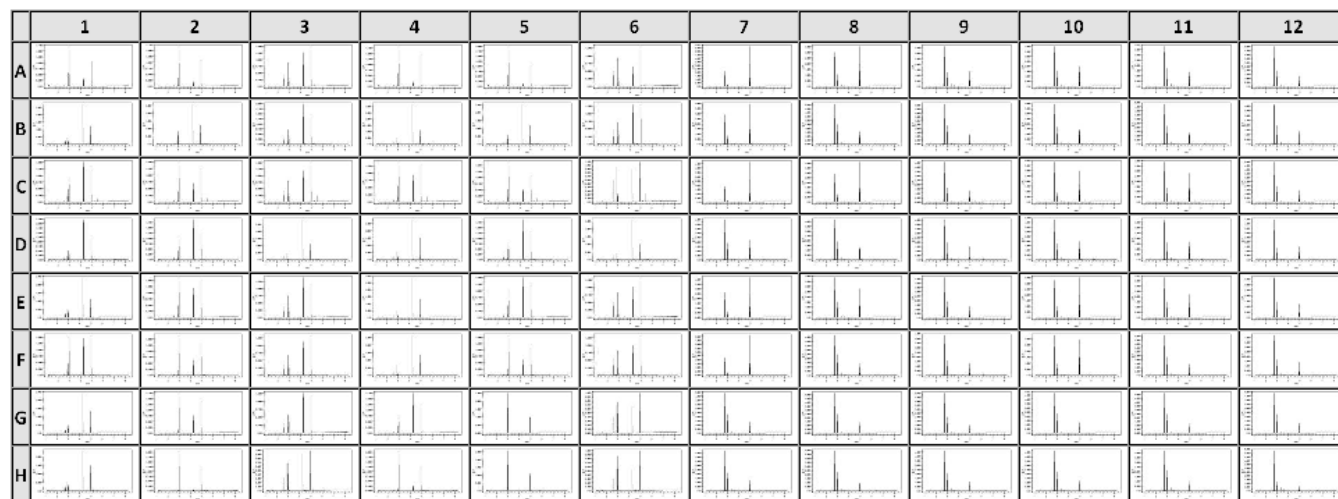


Figure 15: GC data (initial screening plate).

The results clearly confirmed the trend between the size of the Grignard reagents and their ability to successfully couple to *o*-bromo phenol without the presence of any ligands; the bulkier the Grignard (such as Mesityl and Trip groups), the better (Figure 19). Unfortunately, non-Pd based catalyst precursors appeared inferior and Pd-based catalysts are generally required for this transformation.

With the extent of this ligand-free Kumada coupling understood, we planned a second follow-up screen of different discrete variables in search of less expensive catalysts and higher yield (Figure 20).

Screen design (second follow-up screening):

- Base: LiH, NaH, MeMgCl, TripMgBr
- Catalyst precursors: PdCl₂, NiCl₂
- Solvents: Toluene, 1,4-Dioxane, THF
- Grignard reagent: TripMgBr
- Reaction conditions: 12 h and 80 °C

The results clearly show: 1) less costly PdCl₂ can successfully catalyze the desired coupling; 2) NaH, thus Na-salt provides the best starting point and 3) there is little solvent effect when using Na-phenolic salt as starting material (Figure 21).

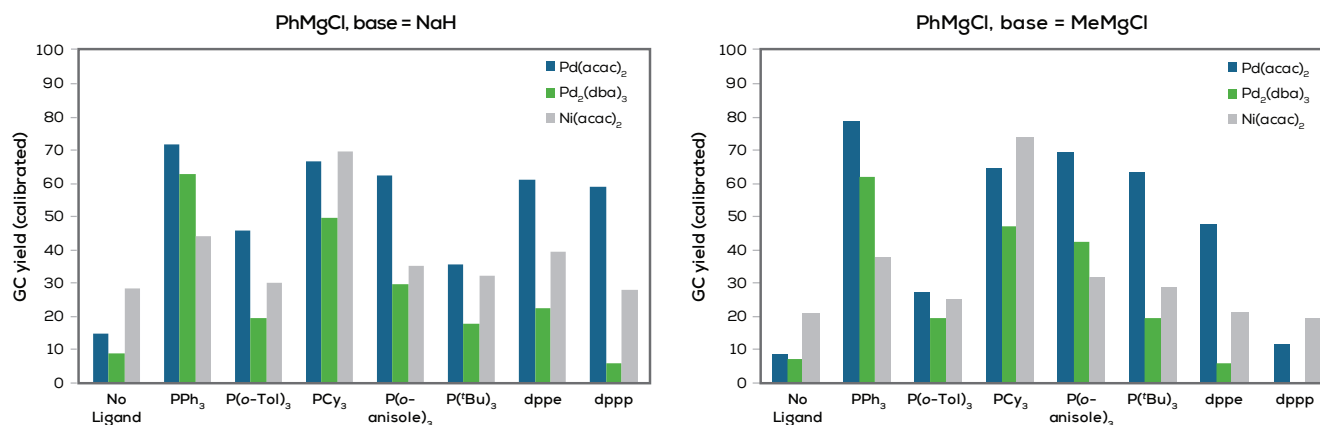


Figure 16: Data analysis - PhMgCl addition.

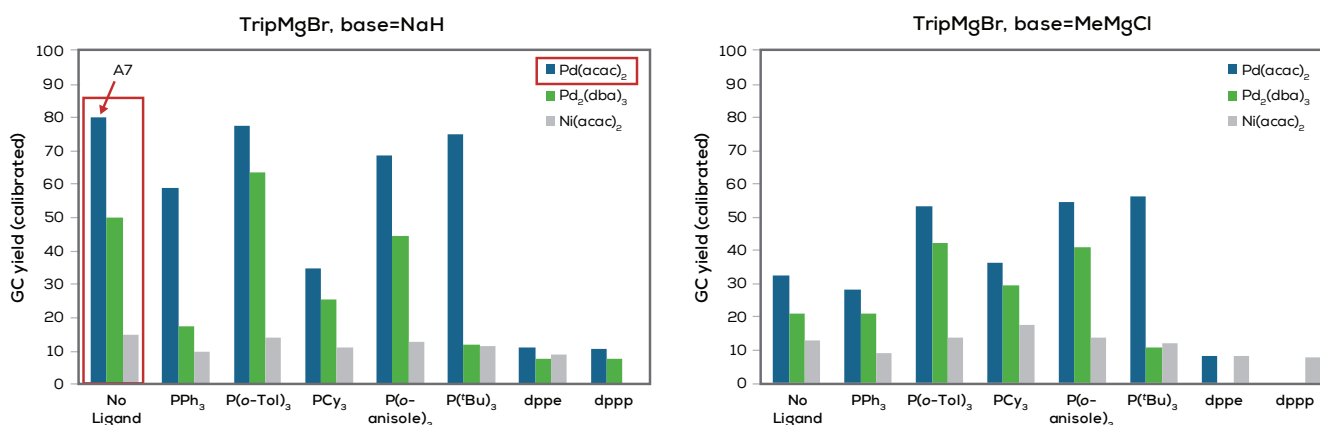


Figure 17: Data analysis - TripMgBr addition.

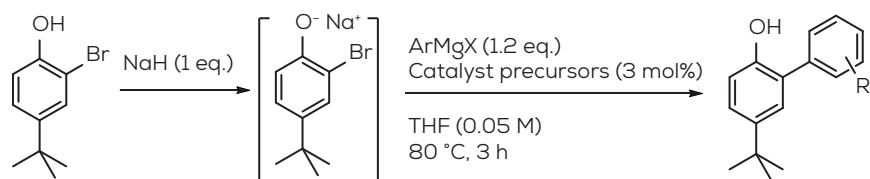


Figure 18: First follow-up screening.

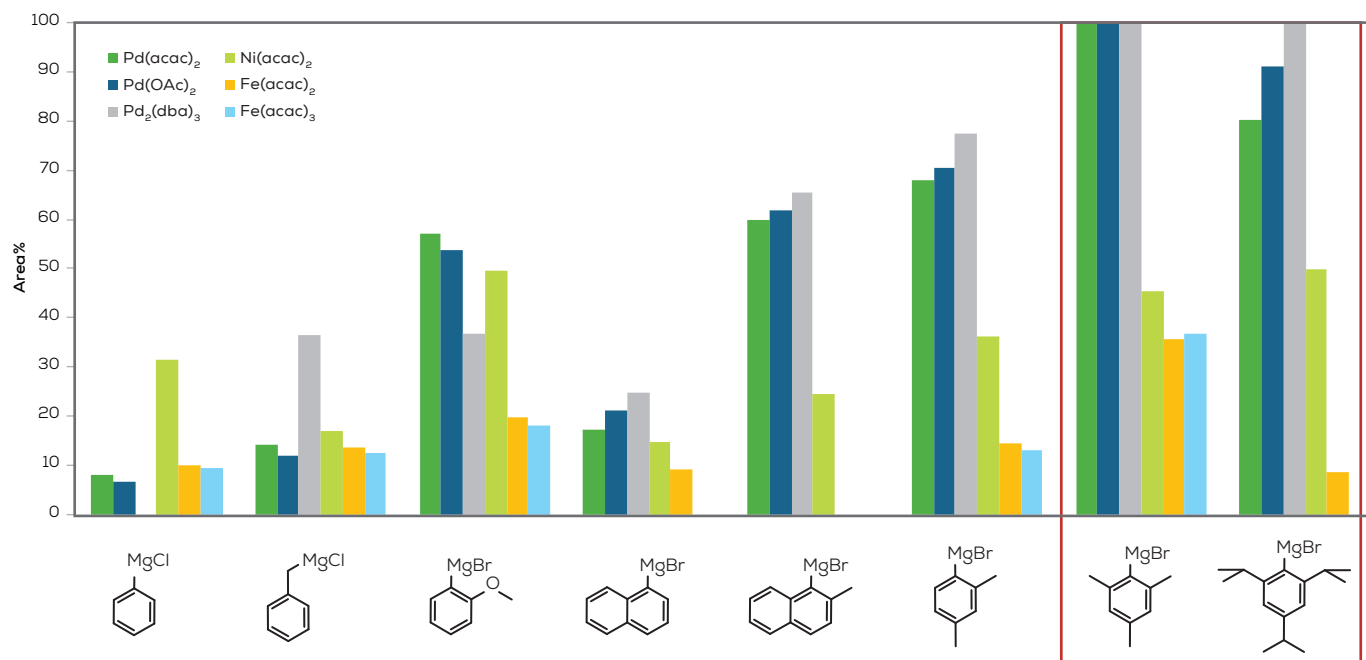


Figure 19: Data analysis - First follow-up screening.

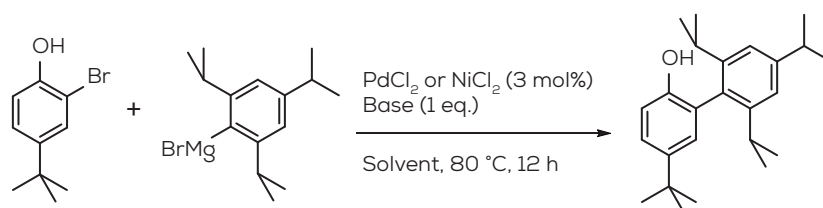


Figure 20: Second follow-up screening.

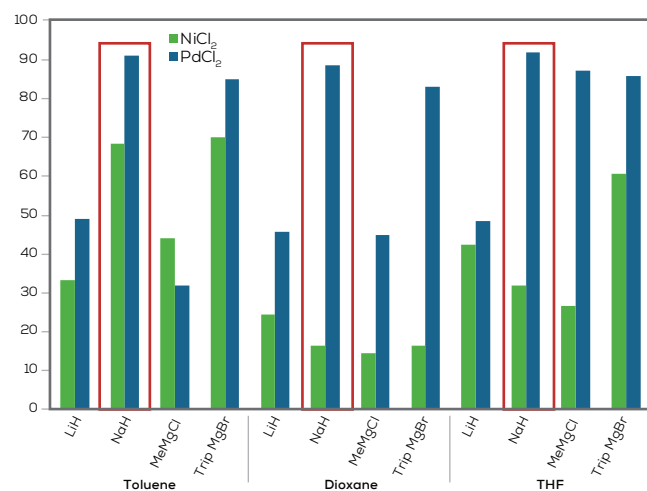


Figure 21: Data analysis – Second follow-up screening.

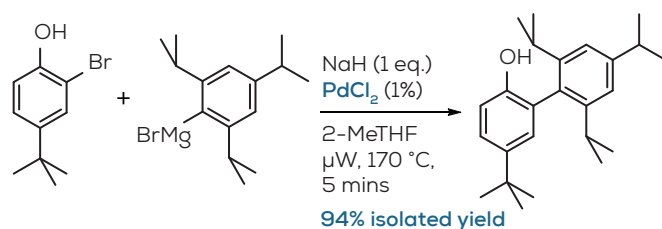


Figure 22: Scale-up of optimized condition.

Scale-up

Finally, the screen results were confirmed by performing benchtop chemistry using NaH as deprotonating base and PdCl₂ as the catalyst, the best discrete variables discovered (Figure 22). The reaction was done in a microwave reactor for a much quicker conversion (5 mins at 170 °C). The isolated yield (at 94%) was even better than what was observed during the secondary screen.

Since the project was in its early discovery stage and because the goal to insert a very sterically hindered aromatic substitute at *ortho* position of a phenol was resoundingly successful, we did not pursue investigation of continuous variables to further refine reaction conditions.



Unchained Labs
 6870 Koll Center Parkway
 Pleasanton, CA 94566
 Phone: 1.925.587.9800
 Toll-free: 1.800.815.6384
 Email: info@unchainedlabs.com

Conclusion

High-throughput reaction screening enables scientists to systematically explore relevant reaction variables and achieve process optimization in an iterative fashion. This approach involves first optimizing with respect to discrete variables, followed by further optimization of continuous variables.

The case study presented here illustrated the importance of approaching a problem in this iterative fashion. Our goal was to install a bulky aromatic group at *ortho* position of a phenol. Initial benchtop chemistry using either Negishi or Suzuki coupling was partially successful. We then introduced an automated high-throughput approach to explore a broad set of relevant discrete variables in order to install a very sterically challenging Trip group. After four rounds of such screening exploring two types of transformations and many different variables, the Trip group was successfully coupled at *ortho*-phenol position without needing a protecting group or any ligands.