Rapid synthesis of off-DNA DEL hits

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1. Introduction

DNA-encoded libraries (DELs), first introduced by Brenner in 1992,[1] have become widely used in drug discovery, with over 1,250 publications to date.[2] DELs enable the simultaneous screening of billions of molecules and are made by performing sequential chemical reactions on DNA tags, where each added component is assigned a unique DNA barcode.

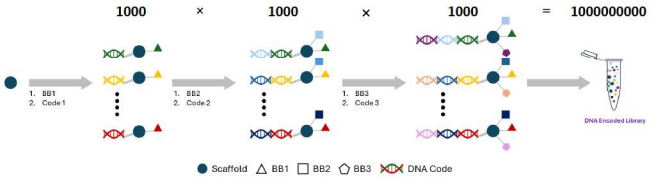

Figure 1 – Split and pool strategy for the synthesis of DEL

The DEL mixture is incubated with the target, and unbound molecules are removed. For the retained molecules, the DNA barcodes are PCR-amplified and decoded to identify hits; with higher counts indicating stronger binders. DEL screening is applicable to a diverse range of targets.[3,4,5] To screen all these targets the on-DNA chemistry also need to be diverse to maximise the chemical space (Figure 2).

Reactions used to make DEL

- Indole C3 alkylation
- **Suzuki**
- Photoredox
- Sonogashira
- **Acylation / Amidation**
- **Reductive amination**
- Buchwald-Hartwig
- **S_NAr**
- **Sulfonylation**
- Thioether oxidation
- Halide to amine, azide, nitrile, acid
- Nitro to amine
- Alkene to aldehyde
- Aldehyde to alkyne
- Triazole / Benzotriazole
- Benzimidazole
- Pyridone
- Imidazolidinone / Isoindolinone...

Figure 2 – Overview of the on-DNA synthesis; highlighted in blue is the most common chemistry

After hit identification, resynthesis "off-DNA" is required for two reasons. First, there is no direct analytical validation of the on-DNA hits. Secondly, nmol scale yields for on-DNA products are insufficient for determining affinity in orthogonal assays such as surface plasmon resonance (SPR) or protein crystallography.

On-DNA compounds are attached to DNA *via* linkers that, although designed to be solvent exposed, can still interact with the target. Therefore a 'scar' motif must also be considered in off-DNA synthesis (Figure 3). As DEL screening becomes mainstream approach in medicinal chemistry, rapid synthesis of off-DNA hits is becoming more important for rapid validation of targets.

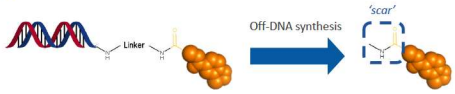

Figure 3 – Representation of the off-DNA DEL hits with 'scar' group incorporation

2. PAC-FragmentDEL synthesis

Building upon Vernalis' recognised expertise in fragment-based drug discovery (FBDD), we have developed a DEL approach suitable for finding fragment hits. Fragments are molecules with 16 or fewer heavy atoms; and are expected to show weak affinity in standard DEL screens. To address this issue, a photo-activated-capture (PAC) fragment library[6] was developed, allowing weak binders to covalently bind to the target through a UV crosslinking step (Figure 4).

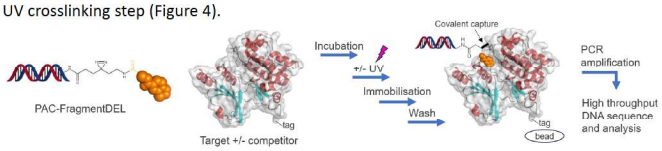

Figure 4 – Covalent capture of DEL fragments via photoactivated capture

After identifying fragment hits, we amplify our hit list using machine learning methods to explore a broader chemical space. As synthesising all candidates immediately is impractical, we apply automated approaches (KNIME workflows) to prioritise compounds based on chemical diversity, commercial availability and synthetic accessibility (Figure 5).

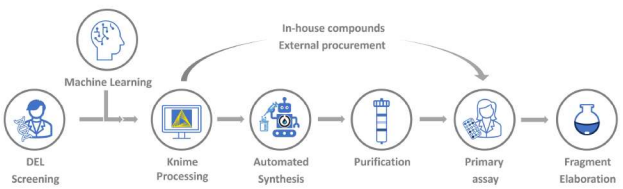

Figure 5 – Overview of the PAC-fragment DEL hit synthesis

Once triaged, most fragments only require one step for synthesis; enabling parallelisation and automation. We use a programmable liquid handler (OT-2), enhanced with 3D printed racks for flexible reaction size (Figure 6). These racks can be transferred for workup or put straight onto automated mass directed chromatography for purification (Waters).

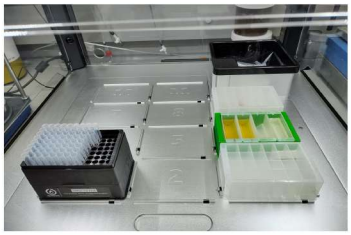

Figure 6 – Opentrons set-up with 3D printed rack (green)

3. Solid phase synthesis in continuous flow

Most DELs are assembled using multi-step synthesis. Batch synthesis of off-DNA DEL hits requires multiple steps with multiple purifications and is slower than the DEL synthesis. In DELs, DNA acts as a water-soluble support, enabling purification via trituration. However, DNA tags are costly and difficult to scale, prompting us to look at solid phase synthesis for cleaner reactions with only a single final purification. Using our Syrris Asia flow system (Figure 7) we can automate the solid phase synthesis in continuous flow. This uses segmented flow to deliver serial reactants and reagents, and the carrier solvent acts as the washes between steps (Figure 8).

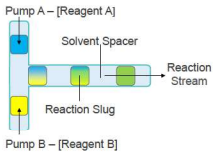

Figure 8 – Segmented flow schematic

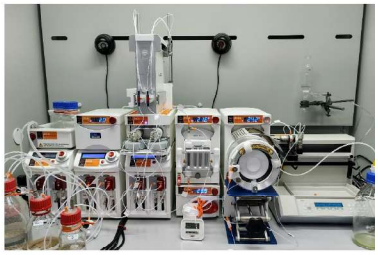

Figure 7 – Vernalis' Syrris Asia flow setup

We have been using a commercial polystyrene support, to which we added an azide linker to allow for a click reaction to attach the substrate. To hold the reaction, we used a RediSep™ cartridge with 3D printed caps and connectors that are compatible with our flow chemistry equipment (Figure 9).

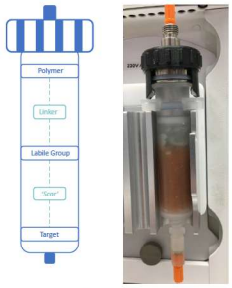

Figure 9 – Concept vs final product for SPS-CF

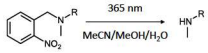

Figure 10 – Photo-deprotection of a nitrobenzyl group

Based on the solid phase work of Seeberger[7], a nitrobenzyl group was chosen as our cleavable group. This group tolerates standard synthetic conditions and can be mildly released with UV light (Figure 10). Two modifications were made to the nitrobenzyl group; first a reductive amination was used to attach the amine with the 'scar' group. Second an alkyne tether was introduced to enable click chemistry to link to the polymer (Figure 11).

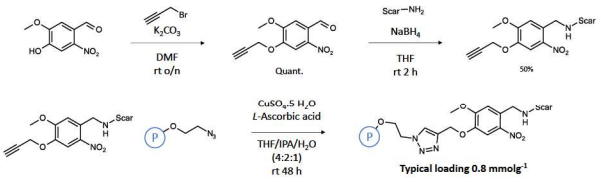

Figure 11 – Method for attaching the photolabile group onto the polymer (P)

The RediSep™ cartridges allow for light permeability and therefore can be placed directly from the flow machine into the a photoreactor for deprotection (Figure 12). Although the photolabile deprotection step is mild, a standard 365 nm LED (18 W) is not powerful enough to penetrate the polymer and achieves only 20% deprotection. The Lucent 360™'s 365 nm LEDs (54 W) are significantly stronger and achieve full deprotection.

The high loading of the polymer is also a major consideration; this reduces the reaction volume, which means shorter residence time and high concentration. High concentration can be an issue in flow due to solubility. To address this issue, we mix the support with glass beads to increase the reaction volume.

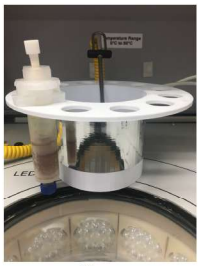

Figure 12 – RediSep™ in Lucent 360™ ready for deprotection

Figure 13 shows an example of a multistep synthesis comprising of amide coupling, Suzuki coupling, and photocleavage. Using our solid phase continuous flow setup, we can achieve a final compound within 5 hours (with purification); significantly faster than the multiday batch synthesis. Using this automated flow process, we can setup the synthesis of a range of molecules, only having to replace the cartridge in-between targets.

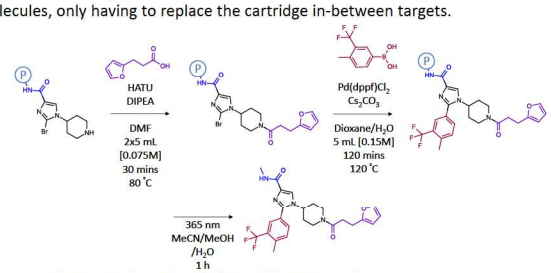

Figure 13 – Method for the amide coupling/Suzuki coupling DEL Hit examples

4. Future prospects

We have shown that our solid phase synthesis in continuous flow is a rapid way to synthesise off-DNA hits. There are, however, still some limitations. We are investigating a 'stop-flow' paradigm to improve catalytic turnover in cross-coupling reactions. One issue with 'stop-flow' is maintaining pressure, and this is currently being addressed with additional flow streams. We are also evaluating alternative cartridges to increase the working temperature range for chemistries of interest.

References

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