## Required DNA Components

The SPINE/DIMPLE algorithm will generate a list of synthetic oligos representing the diversity that will be encoded in the library, a list of primers for amplifying individual subpools from the oligopool, and a list of primers for amplifying the rest of the plasmid with BsmBI adapters for each subpool.

Before use, the oligopool should be suspended in elution buffer (10 mM Tris-Cl, pH 8.5) to a concentration of 20 ng/µl. Make every effort to minimize freeze-thaw cycles.

## Sublibrary Preparation

Each sublibrary includes two pieces: a diversified insert which must be amplified from a synthetic oligopool, and a fixed backbone fragment comprising the remainder of the plasmid.

### For the backbone:

Begin with a high-quality template plasmid (prepared via silica-resin column purification and isopropanol precipitation; not just a miniprep). This should be stored undiluted at -20C. Before use, dilute to the desired concentration range and quantitate using Qubit High-Sensitivity assay. Minimize the amount of template DNA used in inverse PCR reactions, as any template carried over to subsequent steps may result in unwanted wild-type virus contamination. For these inverse PCR reactions, 100 pg per reaction is generally sufficient.

Inverse PCR is performed using Q5 high-fidelity polymerase (New England Biolabs). Use 50 µl reactions.

Set up reactions as follows:

|  |  |  |
| --- | --- | --- |
| **Component** | **Per 50 µl Reaction** | **Final Concentration** |
| 5x Q5 reaction buffer | 10 µl | (1x) |
| dNTPs (10 mM) | 1 µl | 200 µM |
| Forward primer (10 µM) | 2.5 µl | 0.5 µM |
| Reverse primer (10 µM) | 2.5 µl | 05 µM |
| Template DNA | 100 pg | 2 pg/µl |
| Q5 DNA polymerase | .5 µl | 0.02 U/µl |
| Nuclease-free water | To 50 µl |  |

Run reactions as follows:

|  |  |  |  |
| --- | --- | --- | --- |
| **Step** | | **Temperature** | **Time** |
| Initial denaturation | | 90°C | 1 min. |
| 35 cycles | Denature  Anneal  Extend | 98°C | 10 sec. |
| 65°C | 30 sec. |
| 72°C | 10 min. |
| Final extension | | 72°C | 10 min. |
| Hold | | 10°C | Until use |

After PCR, reactions should be cleaned up using NEB Monarch PCR & DNA Cleanup kit (5 µg). Since this is a large amplicon, use a 2:1 ratio of binding buffer to PCR reaction.

### For the insert: