# Bird of Prey™ QUICKSTART: Mono-Effector Multigene

Bird of Prey genes in the form of CloneCards or CloneTabs can be stored at room temperature (15-25ºC) for up to 24 months.

For more information, please refer to the Bird of Prey Handbook, which can be found at www.ospreybio.com. For technical assistance, please contact us at clonecard@ospreybio.com

**Notes before starting**

- It is recommended to perform a plasmid prep for each gene involved in your vector design to ensure enough DNA is available for subcloning.
- We recommend 1µl of enzyme in restriction digests regardless of units/µl to ensure consistency, efficiency, and proper enzyme activity.
- Never use the same Transcription Unit (TU) or IRES element in a single vector construct. This will lead to vector recombination.

**Materials Needed:**

- BoP Vectors: Effectors & Controllers
- Restriction enzymes: PvuI, KasI, XhoI, NotI, SalI
- T4 DNA Ligase and buffer
- Quick CIP
- Gel electrophoresis equipment
- DNA fragment purification kit
- Competent E. coli cells

## *Step-by-Step Procedure:*

**1. Design**

- Choose desired BoP Transcription Units and Effector vectors.

**2. Digest**

- Set up two separate digestion reactions:
  a) Controller Vector: PvuI + KasI
  b) Insert Vector (Effector/ORF): PvuI + KasI
- Incubate for 2 hours at 37°C
- Add CIP and incubate at 37°C for 1 hour

| | Volume (µL) | | | | | | |
|---|---|---|---|---|---|---|---|
| **Sample ID** | **Buffer** | **Vector Acceptor (pDNA Backbone)** | **Insert Donor (DNA)** | **PvuI** | **KasI** | **CIP** | **$H_20$** |
| **TU-1** | 4 | 20 | - | 1 | 1 | 1 | 13 |
| **TU-2** | 4 | 20 | - | 1 | 1 | 1 | 13 |
| **E1** | 4 | - | 20 | 1 | 1 | - | 14 |
| **E2** | 4 | - | 20 | 1 | 1 | - | 14 |

**3. Gel Electrophoresis**

- Run digested samples on a 1% agarose gel using the above table volumes (in µl).
- Visualize bands under UV light.

**4. Fragment Purification**

- Excise desired bands from gel (try using OspreyBio's gel band cutters for faster and more accurate results).
- Purify DNA using gel extraction kit (we recommend Zymo's "Zymoclean Kit" – Cat #D400 series).

**5. Ligation**

- Mix purified Controller and Insert fragments in individual and labeled tubes.
- Add T4 DNA Ligase and buffer (see below table for values).
- Incubate at room temperature overnight.
- After 12-24 hours, heat shock at 37ºC to stop reaction

| | Volume (µL) | | | | |
|---|---|---|---|---|---|
| **Sample ID** | **Buffer** | **Vector Acceptor (pDNA Backbone)** | **Insert** | **Ligase** | **$H_20$** |
| **TU-1 (control)** | 10 | 5 | - | 1 | 84 |
| **TU-1 + E1** | 10 | 5 | 10 | 1 | 74 |
| **TU-2 (control)** | 10 | 5 | - | 1 | 84 |
| **TU-2 + E2** | 10 | 5 | 10 | 1 | 74 |

**6. Transformation**

- Transform ligation mixture into competent E. coli
- Plate on Kanamycin antibiotic-containing agar
- Incubate overnight at 37°C

**7. Pick Colonies & perform miniprep**

- Pick two colonies from incubated plates
- Add each to a culture tube of 3ml kanamycin LB broth
- Place in a shaker incubator at 37°C overnight
- Perform miniprep to isolate plasmid DNA
- Verify construct via DNA sequencing

**8. Digest II**

| | Volume (µL) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Sample ID** | **Buffer** | **Vector Acceptor** | **Insert Donor** | **Enzyme I: XhoI** | **Enzyme II: NotI** | **Enzyme III: SalI** | **CIP** | **$H_20$** |
| **TU1-E1** | 4 | 20 | - | 1 | 1 | - | 1 | 13 |
| **TU2-E2** | 4 | - | 20 | - | 1 | 1 | 0 | 14 |

**Digest**:

- Incubate at 37°C for 2 hours
- Add CIP and incubate at 37°C for 1 hour

**Gel Electrophoresis**: Perform in the same manner as above.

**Fragment Purification**: Perform in the same manner as above.

**9. Ligation II**

| | Volume (µL) | | | | |
|---|---|---|---|---|---|
| **Sample ID** | **Buffer** | **Vector Acceptor** | **Insert** | **Ligase** | **$H_20$** |
| **TU-1 (control)** | 10 | 5 | - | 1 | 84 |
| **TU-1 + TU-2** | 10 | 5 | 10 | 1 | 74 |

**10. Sequence Verification**

Run transformation protocol, pick colonies, & perform minipreps to verify sequence (steps on Page 2).

Remember to always refer to the full Bird of Prey handbook for detailed protocols and troubleshooting advice. This QuickStart guide is intended as a concise reference for experienced users.