Bird of Prey™ QUICKSTART: Mono-Effector Monogene

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.

Materials Needed:

- BoP Vectors: Effectors & Controllers
- Restriction enzymes: PvuI & KasI
- 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 Unit(s) and Effector vector(s).

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
E1	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

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
- Proceed with downstream applications (e.g., protein expression)

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.