Bird of Prey™ QUICKSTART: Multi-Effector Monogene

Bird of Prey genes in the form of CloneCards or CloneTabs can be stored at room temperature (15-25ºC) for up to 18 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.
- If a 3rd Effector is desired, please refer to the Optional section beginning on Page 4. Note that you must still start at Step 1 below.

Materials Needed:

- BoP Vectors: Effectors, IRES elements, Transcription Units
- Restriction enzymes: EcoRV, PacI, PvuI, KasI, ScaI
- T4 DNA Ligase and buffer
- Gel electrophoresis equipment
- DNA purification kit
- Competent E. coli cells

Step-by-Step Procedure:

1. Design

- Create a table with chosen design combinations

2. Digest I – First Effector

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

	Volume (µL)							
Sample ID	**Buffer**	**Vector Acceptor (pDNA Backbone)**	**Insert Donor (DNA)**	**Enzyme I: PacI**	**Enzyme II: KasI**	**Enzyme III: PvuI**	**CIP**	**H_20**
IRES-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
- 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**
IRES-1 (control)	10	5	-	1	84
IRES-1 + E1	10	5	10	1	74

6. Digest II – Second Effector

	Volume (µL)							
Sample ID	**Buffer**	**Vector Acceptor (pDNA Backbone)**	**Insert Donor (DNA)**	**Enzyme I: EcoRV**	**Enzyme II: PvuI**	**Enzyme III: ScaI**	**CIP**	**H_20**
IRES-1-E1	4	20	-	1	1	-	1	13
E2	4	-	20	-	1	1	-	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.

7. Ligation II

	Volume (µL)				
Sample ID	**Buffer**	**Vector Acceptor**	**Insert**	**Ligase**	**H_20**
***IRES-1-E1 (control)**	10	5	-	1	84
E2 + IRES-1-E1	10	5	10	1	74

8. Transformation

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

9. 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

10. Digest III (IV if 3-Effectors) – Multi-Effector into Transcription Unit

	Volume (µL)						
Sample ID	**Buffer**	**Vector Acceptor**	**Insert Donor**	**Enzyme I: PvuI**	**Enzyme II: KasI**	**CIP**	**H_20**
TU-1	4	20	-	1	1	1	13
E2-IRES-1-E1	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.

11. Ligation III (IV if 3-Effectors)

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

12. 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.

OPTIONAL: Three-Effector Vectors
If a third ORF is desired, a second IRES must be used. We recommend limiting each Transcription unit to three ORFs to mitigate against poor expression levels.

If after designing the construct you wish to create, a third ORF is needed, we recommend performing the restriction digest of the desired Effectors and second IRES at the same time as the first IRES and its first ORF to save time.

Digest I – Simultaneous IRES-1 & IRES-2
Please note that the enzymes will be the same for both sets of IRESes.

a. IRESes: PacI (E_1) and KasI (E_2).
b. Effectors/ORFs: PvuI (E_1) and KasI (E_2)

	Volume (µL)						
Sample ID	**Buffer**	**Vector Acceptor**	**Enzyme I: PacI**	**Enzyme II: KasI**	**Enzyme III: PvuI**	**CIP**	**H_20**
IRES-1	4	20	1	1	-	1	13
E1	4	20	-	1	1	0	14
IRES-2	4	20	1	1	-	1	13
E3	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.

Ligation I

	Volume (µL)				
Sample ID	**Buffer**	**Vector Acceptor**	**Insert**	**Ligase**	**H_20**
IRES-1 (control)	10	5	-	1	84
IRES-1 + E1	10	5	10	1	74
IRES-2 (control)	10	5	-	1	84
IRES-2 + E3	10	5	10	1	74

Digest & Ligation II
Proceed with second Digest & Ligation for the first IRES to make your 2-Effector IRES (Steps 6 & 7).

Digest III

	Volume (µL)							
Sample ID	**Buffer**	**Vector Acceptor**	**Insert Donor**	**Enzyme I: ScaI**	**Enzyme II: KasI**	**Enzyme III: EcoRV**	**CIP**	**H_20**
E2-IRES-1-E1	4	20	-	1	1	-	1	13
IRES-2-E3	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.

Ligation III

	Volume (µL)				
Sample ID	**Buffer**	**Vector Acceptor**	**Insert**	**Ligase**	**H_20**
E2-IRES-1-E1 (control)	10	5	-	1	84
E1-IRES-1-E2+IRES-2-E3	10	5	10	1	74

Sequence Verification
Run transformation protocol, pick colonies, & perform minipreps to verify sequence (steps on Page 3).

Digest IV & Ligation IV
Proceed to Step 10 to subclone your 3-Effector vector into your Transcription Unit of choice and proceed with the rest of the protocol.