

OMTM, Volume 17

Supplemental Information

AAV-Mediated CRISPR/Cas9 Gene

Editing in Murine Phenylketonuria

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Supplementary Figure 1: Guide 1 Validation.

A. Surveyor Assay. Gel electrophoresis of Surveyor Assay performed with Guide 1, 2, and 3, as indicated, with control uncut 800bp PCR target and flanking 100bp ladder. Guide 1 was the only guide showing any visible cutting of the target, as indicated. **B. Takara Guide-It Assay.** Gel electrophoresis of *in vitro* Guide-It Takara Assay performed with Guide 1, with 50bp ladder on the left and uncut 800bp substrate on the right. Again, RNP-Guide1 showed visible cutting of the target PCR substrate.

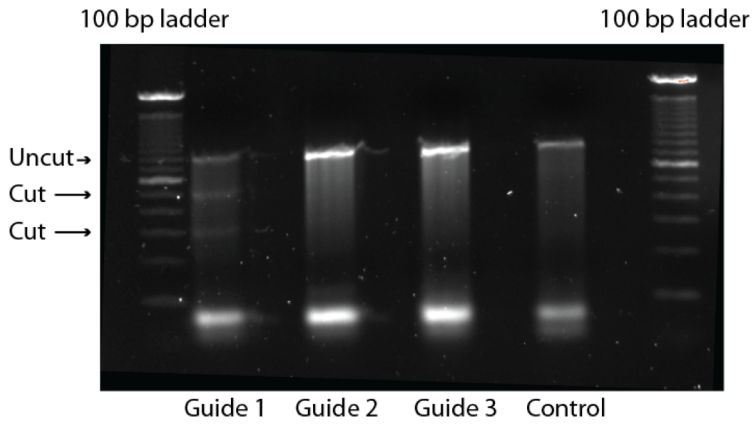
Supplementary Figure 2: NGS Selection and Validation.

A. SpCas9-Guide1 Target Sites. Table of all COSMID produced *SpCas9*-Guide1 potential cutting sites. Yellow is on-target site, orange is off-target sites selected for further investigation. **B. NGS Read Uniformity.** Display of NGS read depth at every location investigated in every animal, indicating all samples were represented and no samples were dropped.

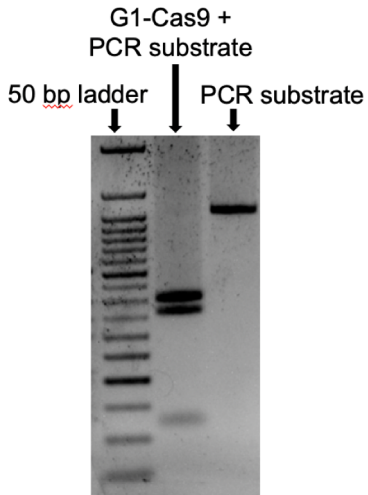
Supplementary Figure 3: dAAV+Van Breeders.

A. dAAV+Van Breeder Nesting. Photograph of dAAV+Van breeder pair nesting. **B. *Pah*^{enu2}/+ Breeder Nesting** *Pah*^{enu2}/+ Breeder Nesting Photograph of heterozygous breeder pair nesting. **C. Female dAAV+Van Breeder.** Female dAAV+Van breeder animal at 65 weeks, with a dark fur coat and healthy mass of 29.2 grams. **D. Male dAAV+Van Breeder.** Male dAAV+Van breeder animal at 65 weeks, with a dark fur coat and obese mass of 40.7 grams.

A. Surveyor Assay



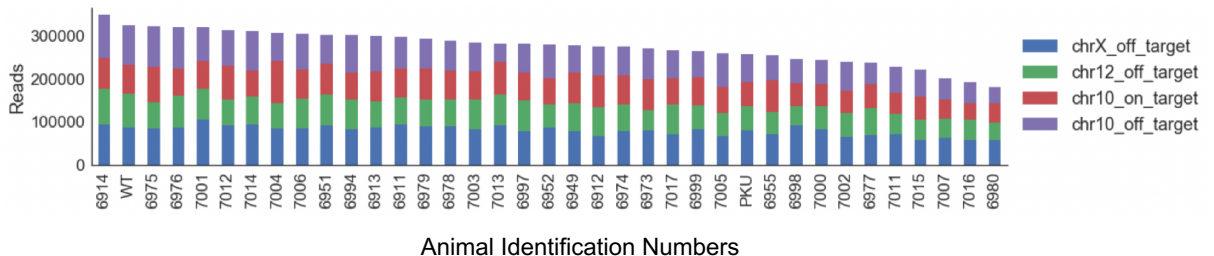
B. Takara Guide-It Assay



A. COSMID Output

Chr Position	Strand	Cut site	Score
Chr10:87570336-87570358	-	87570342	0
Chr3:62532887-62532909	+	62532903	5.42
ChrX:140587182-14058720	-	140587188	2.93
Chr17:78827214-78827236	+	78827230	22.45
ChrX:52428425-52428447	+	52428441	11.19
Chr10:116187078-1161871	+	116187094	0.71
Chr11:107380762-1073807	-	107380768	9.27
Chr1:193399721-19339974	-	193399727	6.6
Chr10:87570336-87570357	-	87570342	0.63
Chr10:116187079-1161871	+	116187094	1.34
Chr3:62532888-62532909	+	62532903	5.93
Chr10:87570336-87570357	-	87570342	0.79
Chr10:87570336-87570357	-	87570342	0.96
Chr7:71689783-71689804	+	71689798	23.04
Chr16:26312076-26312097	+	26312091	8.78
Chr18:59943710-59943731	+	59943725	23.86
Chr12:60323904-60323925	+	60323919	1.33
Chr4:69351258-69351279	+	69351273	7.11
Chr13:48943433-48943454	-	48943439	5.6
Chr18:52791504-52791525	-	52791510	7.11
Chr12:99734046-99734067	-	99734052	11.41
Chr19:32989811-32989832	+	32989826	5.93
Chr10:11174367-11174388	-	11174373	6.16
Chr11:81943011-81943032	+	81943026	7.08
Chr5:93684753-93684774	+	93684768	10.01
Chr5:93948467-93948488	-	93948473	10.01
Chr5:94140055-94140076	-	94140061	10.01
Chr5:94457664-94457685	-	94457670	10.01
Chr5:95614290-95614311	-	95614296	10.01
Chr5:95755669-95755690	-	95755675	10.01
Chr10:87570337-87570358	-	87570343	30.51
Chr10:87570337-87570358	-	87570343	26.51
Chr10:87570337-87570358	-	87570343	40.51
Chr10:87570337-87570358	-	87570343	20.51
Chr10:116187078-1161870	+	116187093	21.22
Chr10:87570336-87570359	-	87570342	0.95
Chr10:87570336-87570359	-	87570342	0.97
Chr10:87570336-87570359	-	87570342	1.14
Chr13:37361079-37361102	+	37361096	3.89
Chr12:57512651-57512674	-	57512657	3.67
Chr7:5484550-5484573	-	5484556	7.23
Chr14:72236511-72236534	+	72236528	7.72
Chr10:87570335-87570358	-	87570341	32.7
Chr17:78827214-78827237	+	78827231	23.15
Chr10:87570335-87570358	-	87570341	40.7
Chr10:87570335-87570358	-	87570341	40.7

B. NGS Read Uniformity



A. dAAV+Van Breeder Nesting



B. *Pah^{enu2/+}* Breeder Nesting



C. Female breeder at 65 weeks



D. Male breeder at 65 weeks

